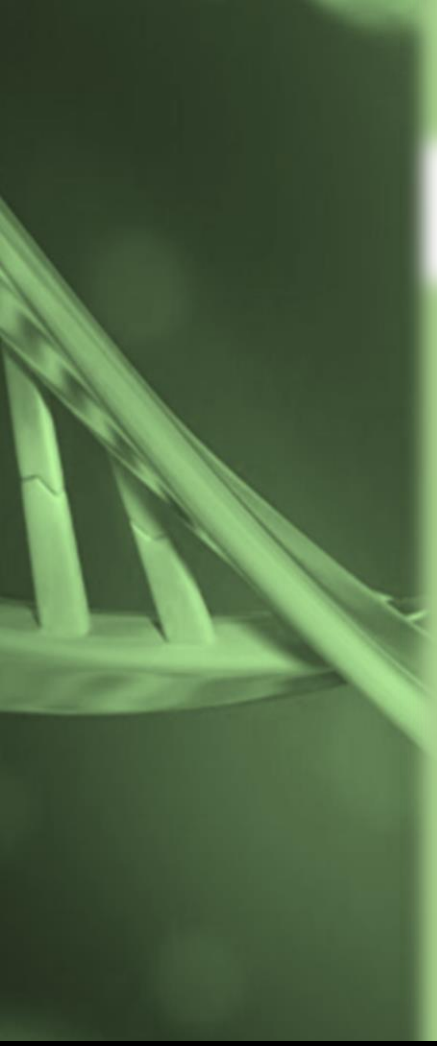

- Natural resources and natural products
- Aquatic biotechnology
- Renewable energy
- Solid waste, wastewater, and hazardous waste treatment
- Restoration ecology
- Marine and freshwater ecology

Current Trends in Biotechnological Research for Environmental Sustainability

Surabaya, August 24-26, 2016

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PROCEEDING

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PREFACE

It is a pleasure of the Organizing Committee to publish this Proceeding as an accomplishment of the successful International Postgraduate Conference on Biotechnology (IPCB) 2016, which was held on 24-26 August 2016 in Surabaya City, Indonesia. With a theme of *Current Trends in Biotechnological Research for Environmental Sustainability*, a total of 7 keynote and 6 invited papers were presented by outstanding speakers during the conference. Seventy-two scientific papers were presented by the participants during oral and poster presentation sessions. Attendees of this conference were from 9 countries, namely Japan, Malaysia, Indonesia, Korea, Taiwan, Nigeria, Pakistan, Belgium, and Spain.

This is the first Proceeding, which is published by IPCB Organizing Committee. Former IPCB events did not publish conference proceedings, as the main objectives of these conferences were for introducing on-going research activities by postgraduate students from the participating universities. Therefore, only limited numbers of articles were sent by the participants to be published in this Proceeding.

The Organizing Committee would like to thank the participants who sent their articles for this Proceeding. We hope that this Proceeding would provide beneficial scientific information on most recent biotechnological research for academicians.

Surabaya, 21 November 2016

IPCB 2016 Organizing Committee

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The Current Development of Biotechnology in Indonesia

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Abstract

As the largest archipelagic country in the world, Indonesia consists of approximately 13,466 islands that spread along 5,110 km from the West to the East. This extensive area provides abundant of natural resources with huge varieties of biodiversity that enable the development of biotechnology in the future. Biotechnology, in general, is a branch of sciences that studies the employment of living organisms and its derivatives to produce a new product or a new processing system.

The utilization of biotechnology has been growing rapidly in the world for several reasons, especially since it is acknowledged as an environmentally-friendly technology. The United States has mapped the four pillars of biotechnology that will dominate the world in the future, namely: health cares, agricultures, industries (i.e., energy, catalysts), and the environment. In term of the environmental aspect, it was started in the 19th century following the escalation of concern on the increase of environmental pollution, such as the contamination of materials that are non-biodegradables, both on land and in the river. For example, the contamination of soil by heavy metals, contamination from petroleum, as well as contamination of pollutants containing halogenated organic compounds. It is believed that the employment of biotechnology can resolve the contamination of these pollutants, for instance, by bioremediation. In addition, the use of biotechnology for the environmental issues has been increasing continuously, especially for wastewater treatment, soil treatment by means of bioremediation, nitrification and denitrification, and toxicity reduction. Moreover, biotechnology has been used to convert the potential pollutant gas into sources of energy in order to minimize its impact to global warming.

The numerous environmental problems that have been growth in many decades have been dealt by biotechnological approaches. Wastewater treatment has been developed to harness microorganisms and immobilization of enzymes for treating highly toxic waste. The use of plants for remediation of the polluted environment has also been widely used in Indonesia, by exploiting local crops. ITS is one of the leading universities in Indonesia that has been involved to develop biotechnology in various fields, including the environmental, biological, chemical and maritime sector. Although, in general, the development of environmental biotechnology in Indonesia still requires further attention when compared with other countries, however, the abundant of biodiversity in this country has a potential to be developed in order to improve the implementation of biotechnology in various fields. The development of several studies in ITS, such as phytoremediation, bioremediation and some other researches have led to the application of biotechnology as environmentally-friendly methods. The support from the government, in cooperation with various stakeholders both within and outside the country, is believed can accelerate the development of biotechnology in Indonesia.

Keywords: **biotechnology, bioremediation, environment, treatment.**

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Innovation, Technological Progress and Economic Growth

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Abstract

Today, it is generally accepted that innovation and technical progress play an important role in economic growth. The purpose of this study is to explore both how innovation is analyzed and economic growth due to technical progress is measured by referring to two economists, Joseph Schumpeter (1883-1950) and Robert M. Solow (1924~), respectively. Schumpeter suggested that innovation by the entrepreneur was the driving force of capitalism, and the fundamental cause of long-term economic growth. He proposed the following types of innovations: (i) Introduction of new goods, (ii) Introduction of new production methods, (iii) Opening of new markets, (iv) Acquisition of new supply sources of raw materials or half-manufactured goods, and (v) Implementation of new organization of any industry. He was among the first to emphasize the vital role of innovation by entrepreneurs in business cycles. Solow has also made contributions by presenting a model showing technological progress is the engine of long-term economic growth, where economic growth can be quantified by technological progress. In the Solow growth model, real GDP growth rate is determined by technical progress, if we assume continuous technical progress. Solow's other contribution was to lay the foundations for growth accounting by which empirical growth analysis becomes possible. In a simple model, economic growth due to technical progress can be measured as the difference between the total output of growth and the output of growth by capital and labor. This difference is frequently called "Solow's residuals". Lastly, the current state of innovation activities of the Organization for Economic Co-operation and Development (OECD) countries will be explored. The OECD has collected science, technology and innovation (STI) activities data and national STI policy information from its members' countries and important non-member countries to review key trends in STI. The OECD biennially publishes the "OECD Science, Technology and Industry Outlook". Based on its most recent issue (2014), university and public research STI policy profiles are reviewed in the following areas: (i) missions and orientation, (ii) financing public research, (iii) commercialization of public research.

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Regional Cooperation in Biotechnology Research for Environmental Sustainability

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Abstract

Sustainable management of marine biological resources has become an emerging interest among the scientific communities in the last two decades. The challenging issues to balance the socio-economic needs to harvest the natural resources with responsibility to sustain livelihoods, while protecting the environment and conservation of the finite resources have become the global agenda. In 2015, the United Nations Sustainable Development Summit has adopted the 2030 target for Sustainable Development Goals (SDGs) with the aim to eliminate poverty, fight inequality and injustice and tackle climate change. Ten out of seventeen goals are linked to the silent stakeholder- the marine environment.

Maritime nations in the Western Pacific have been advocating for regional scientific cooperation through the UNESCO Intergovernmental Oceanographic Commission in the Western Pacific (WESTPAC) and other platforms such as PEMSEA, JSPS and recently, SIMSEA. Apart from the Ocean Circulation and Ocean Dynamics, the topics on Marine Biodiversity, Harmful Algal Blooms, Marine Renewable Energy, Large Marine Ecosystems and Marine Spatial Planning and so forth, the network requires extensive collaborative scientific research, sharing of data and information as well as transfer of knowledge and technology.

The author will share the unique efforts of the Coral Triangle Initiative-Fisheries and Food Security (CTI-CFF) in capacity building for sustainable management of marine resources in the global epicenter of marine biological diversity that covers 1.6% of the planet ocean, contributed to 76% of coral diversity and more than 37% of the world reef fishes. The scientific boundary spans within 6 countries, sustains trade and livelihood of more than 363 million people and at the same time are facing the multiple challenging threats to sustainability. Ecosystems approach to fisheries management and promotion of responsible ecosystem services to the empowered communities whilst tremendous efforts in the development and application of technologies to build the baseline data on bio-geographies and other opportune development of new commercial products based on natural resources have been on the increase.

Social innovation in the context of the changing climate requires aspects of education and training, policy and governance, research and conservation with responsibility, participatory and equitable benefit by all the stakeholders, Federal and Local Government, Scientific Communities and NGOs. CTI-CFF regional Plan of Action and the establishment of Technical Working Groups by CT6 with the Development Partners provide a glimpse of hope to sustain the productivities of the marine environment that focus on biotechnology post-graduate research priorities for creativity and innovations towards the development of alternative resources, instead of continuously exploiting and harvesting precious natural resources from the wild.

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KEYNOTE SPEECH

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Creation of a Recycling Society

-Novel Lake Ecosystem Management by Sustainable Utilization of Aquatic Weed Biomass-

We introduce a lake ecosystem management project supported by the Ministry of Environment, Japan since 2014 that effectively utilizes harvested aquatic weed biomass. The excessive growth of freshwater aquatic weeds has become a major source of various environmental problems in shallow lakes and rivers throughout the world. In the South Basin of Lake Biwa (the largest lake in Japan), aquatic weeds continue to cover more than 90% of the lake surface, causing socio-economic problems such as fishery disturbance, foul odor and deterioration of tourism attractiveness. On the other hand, maintaining a moderate amount of aquatic weeds is essential for a healthy lake ecosystem, since aquatic weeds play a significant role in water purification and habitat for fishes and invertebrates. Historically, aquatic macrophytes were harvested and recycled for fertilization of agriculture in Japan. However, aquatic macrophyte resources are no longer viable because chemical fertilizers are better for growth, easier to manage and more economically profitable. In order to effectively manage and resolve the increasing issues related to the excess aquatic macrophytes, it is imperative to re-establish a new recycling society again. The proposed lake ecosystem management project includes four research themes as follows; 1) clarify the required amount of macrophytes to harvest annually for sustainable utilization and maintenance of a healthy lake ecosystem; 2) evaluate the effects of various aquatic weed removal methods on the biological community, water quality and sediment quality by establishing several experimental mesocosms in the lake; 3) develop effective treatment technology for the macrophyte biomass using anaerobic digestion (AD); 4) develop mass-culture techniques for high-value micro-algae production using effluents derived from the AD process. While most AD process studies have focused only on biogas recovery from the carbon fraction of the waste biomass, the current project establishes essential technology and methods for effective bio-refinery of aquatic weed biomass, enhancing not only biomethane recovery, but also acquiring crucial nutrients from the digestate that can be harnessed for microalgae biomass production.

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Challenges in Developing Marine Bio-products

Marine bio-products are facing great challenges in term of sustaining their resources as well as maintaining the quality of the products. Many companies producing marine bio-products are not in favor of developing new drugs due to their complex molecules structures and the availability of advances technologies. Although the first marine drugs have successfully made it to the shelf, there are still others in different stages of clinical trials. It is interesting to note that four US FDA-approved marine or marine-derived drugs are registered in the EU [1]. The first FDA-approved marine-derived drugs, Cytarabine (Ara-C) and vidarabine (Ara-A) are synthetic pyrimidine and purine nucleosides, originally isolated from the Caribbean sponge *Tethya crypta.* Cytarabine was approved as anticancer drug in 1969, while FDA approved vidarabine as antiviral agent in 1976. Ziconotide (Prialt ®) for the management of severe chronic pain obtained its approval from FDA in 2004. It was a synthetic equivalent of a naturally occurring peptide isolated from the venom of cone snail *Conus magus.* It was stated that more than 1000 new compounds with different biological activities have been reported each year for the past couple of years [2]. It is often found that the symbiotic derived from bacteria happened to be the one that are responsible to produce the compounds or secondary metabolites. One example is the symbiotic interaction between *Prochloron* spp. bacteria and its ascidian animal host. The symbiotic bacteria provides the host with photosynthate and defensive chemicals, and obtain waste nitrogen in return [3]. The clear examples are Patellamides that are cytotoxic secondary metabolite, which isolated from marine tunicate Lissoclinum patella but later was proven to be produced by the associated bacterium [4]. The same thing was also noted in bacterial symbiont *Candidatus Endobugula sertula* that is known as the source for cytotoxic bryostatins that were discovered from the marine bryozoan *Bugula neritina* [5,6].

Actually, when we defined marine bio-products, we could not run away from marine natural products. Natural products are usually small molecules, with molecular weight below 3000 Da, which are produced by biological source such as plants, animals and microorganism [7]. The natural products are often called secondary metabolites due to the fact that they are not biosynthesized by the general metabolic pathways and have no primary functions directly in the normal growth, development of reproduction of an organism. They usually used by the organism to control ecological relationship that involve defense against predation, competition for space or food or quorum sensing. From over 33 animal phyla described to date, 32 are represented in the aquatic environment, with 15 being exclusively marine [8]. Vast exploration of the ocean ecosystems only started in the mid 1970's with the emergence of modern snorkeling, and then the introduction of scuba and remotely operated vehicles (ROVs) [9]. Due to the technical limitations, exploitation of marine organisms started with the collection of large creatures such as red algae, sponges and soft coral, which have shown large variety of compounds with unique structures [10]. These are among the reasons why pharmaceutical companies or industry would invest in the search for interesting high-

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value ingredients for anti-tumors, anti-microtubule, anti-proliferative, photoprotective, antibiotic or anti-infective from the marine microorganisms [11,12].

Microalgae are also important resources that possessed wide range of biotechnology applications. Both Spirulina and Chlorella are being mass cultured for health supplement for general health food. It has also been used as bioremediation of aquaculture wastewater, biological tool for assessment and monitoring of environmental toxicants such as heavy metals, pesticides and pharmaceutical. In Malaysia, the researchers have been tapping into the microalgae resources for high-valued products and applications in wastewater treatment and assessment of environmental toxicants for the past 30 years. A culture collection of microalgae has been established in University Malaya Algae Culture Collection (UMACC) that holds more than 150 microalgal isolates in Malaysia. But the research are more focus into the fundamental studies in phycology which resulted in publications of several checklist and monographs that documented the diversity of microalgae in Malaysia [13]. In another study, marine microalgae grown in POME were suggested to be aquaculture feed due to their high content of PUFA. Cultures aerated with 5% CO_2 are found to produce highest yields of biomass and EPA. Several microalgae especially *Spirulina platensis* have also shown high antioxidant activity based on chemical assay.

This paper will also highlight few marine bio-products that have been recognized and developed for the industrial purposes. Most importantly, some of these products have attracted the SMEs to license the rights in order to bring them to the shelf. The products that will be highlighted are Scalogel from the fish scales, adjuvant from the microalgae and HEME-1, a marine additives produced to culture the marine bacteria from the oceans.

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Phytotechnology for Bioremediation of Contaminated Soils – The Essential Conditions for Mixed Plants

Introduction

Phytotechnology has evolved as a method of utilizing plants and their root microbes to recover the polluted environment. It was an important solution for the complexity of problem in a multimedia environment: air, water, soil. Plants could directly localize and absorb pollutants from the soil through the process of transpiration. Furthermore, the plants supplied the oxygen to the air through the process of photosynthesis, in addition to other benefits such as aesthetics. In line with the plant processes, root microbes also could degrade some pollutants from the soil. Contributions of plant processes could not be met by a single remediation process: the microbiological, physical or chemical. However, phytotechnology has limitations, such as slow process [1] to follow the plant growth. Plant growth rate was different from each other. Single plant might also be able to handle only specific pollutants [2]. Meanwhile, the soil could be enriched by various pollutants. Therefore, the use of mixed plants was important to address the variety of pollutants in the soil. This presentation described the essential conditions for plants and soil, aiming to obtain optimal phytoremediation process.

Mixed plants

The basic principle of phytoremediation was to ensure that the plant must be kept alive during the remediation process. Thus the range finding test of mixed plants has to be carried out to ensure each plant species alive at the maximum concentration of one or some pollutants.

Consumptive plant species quite widely applied in the remediation [3]. However, it was necessary to perform phytotoxicity test to identify the translocation of pollutants in plants, as well as the phytoaccumulation factor of pollutants. As a safety factor for consumers and shorten the procedure, then it was advised to use non-consumptive plants species, such as vetiver.

Mixed plants have to be capable of specifically the uptake of pollutants, as an example of cadmium associated with plant species, but zinc was associated with the soil [4]. Similar results were obtained in the remediation of polluted soil with some heavy metals and oil [5]. In addition, each plant must be able to work in synergy, including with root bacteria [6,7]. The facts directed attention to perform the specification test of pollutants that could be removed by mixed plants.

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Soil treatment

The use of soil amendments such as compost improved the efficiency of phytoremediation. Researchers have confirmed that inorganic amendments (sugar beet lime) and/or organic (compost) were able to increase the level of phytoremediation of soil polluted with some heavy metals (As, Cd, Cu, Zn and Pb) [8]. Similar results were shown in the applications of chelate, such as EDTA and EDGA that were also able to improve the remediation of soil polluted with oil [9]. The use of amendments was able to improve soil physical properties, such as increased porosity, water content and soil aggregation, which supported the plant processes and rhizodegradation [10]. However, the use of metal chelate EDTA might increase the leaching of metal, and therefore required good management in the operation of phytoremediation [11].

Saturation of soil, which was carried out by watering the ground would determine the status of the soil conditions as well as remediation performance. Soil saturated with water would produce anaerobic soil conditions. Soil remediation anaerobically produced a more effective and efficient for endosulfan degradation [12]. Water saturation of the soil to produce anaerobic conditions might not be applicable for all pollutants, contained in the soil. Pollutants that were biodegradable certainly be reduced more under aerobic conditions than anaerobic conditions. The aerobic conditions required soil aeration, by turning the soil or through the aeration system for contaminated soil on a regular basis. Thus, it was necessary scheduling saturation of soil and soil aeration, which was intended to be able to reduce various types of pollutants.

Conclusion

Conditioning of the plant species and the treatment of polluted soil were essential for improving the effectiveness and efficiency of remediation. Each type of plant should be mutually synergistic and specific in remediation of pollutants. Soil treatment must be appropriate to the type of soil pollutants.

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Biotechnology for Renewable Energy Production from Waste Treatment

The demand of clean and renewable biofuels has rapidly increased nowadays due to several global issues such as fast growing of human population, fossil fuel depletion, and global climate change. First generation biofuels using sugar, starch or certain food crops raised the issue of competition between food/feed and energy production, especially under the unexpected weather events including droughts and floods. Because waste reduction and renewable energy production can be achieved at the same time, biogas production such as hydrogen and methane from organic waste fermentation has been investigated with many source materials, including municipal waste fractions, cellulosic materials, domestic wastewater, lipid-extracted microalgae, dairy wastewater, and food wastes. The varied compositions and material characteristics in organic wastes and wastewaters pose challenges in investigation because a variety of substrates may be utilized by different species of microorganisms.

This presentation first introduces recovery of H_2 and CH_4 from treating bioethanol fermentation residues using a lab-scale two-stage bioprocess. In the hydrogen fermentation bioreactor (HFB), carbohydrate removal efficiency was maintained at 82-93% and the highest hydrogen yield was 8.24 mL/g COD at volumetric loading rate (VLR) of 80 kg COD/m^3/day. The results indicated a positive correlation between hydrogen yield and butyrate-to-acetate ratio, which might be due to the mechanisms of lactate/acetate utilization for hydrogen production and acetogenesis occurring in the HFB. Remaining volatile fatty acids and alcohols in the HFB effluent were further utilized for methane production in methane fermentation bioreactor (MFB), in which the highest methane yield of 345.2 mL/g COD was attained at VLR of 2.5 kg COD/m3/day. Overall, the two-stage bioprocess achieved a maximum COD removal of 81% from bagasse BEFR, and converted 0.3% and 72.8% of COD in the forms of H_2 and CH_4, respectively.

Secondly, algae-based biodiesel is considered a promising alternative energy; therefore, the treatment of microalgae residues would be necessary. Anaerobic processes can be used for treating oil extracted microalgae residues (OMR) and at the same time for recovering bioenergy. In this presentation, anaerobic batch experiments were conducted to evaluate the potential of recovering bioenergy, in the forms of butanol, H2, or CH4, from pretreated OMR. Using pretreated OMR as the only substrate, a butanol yield of 0.086 g/g-carbohydrate was obtained at carbohydrate of 40 g/L. With supplemented butyrate, a highest butanol yield of 0.192 g/g-carbohydrate was achieved at pretreated OMR containing 25 g/L of carbohydrate with 15 g/L of butyrate addition, attaining the highest energy yield of 3.92 kJ/g-OMR and energy generation rate of 0.65 kJ/g-OMR/day. CH4 production from pretreated OMR attained an energy yield of 8.83 kJ/g-

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OMR, but energy generation rate required further improvement. H2 production alone from pretreated OMR might not be attractive regarding energy yield, but it attained a superb energy generation rate of 0.68 kJ/g-OMR/day by combining H2 production from pretreated OMR and butanol production from pretreated OMR with supplementary butyrate from H2 fermentation supernatant.

Lastly, the amount of pollutants produced during manufacturing processes of TFT-LCD (Thin-film transistor liquid crystal display) substantially increases due to an increasing production of the opto-electronic industry in Taiwan. The total amount of wastewater from TFT-LCD manufacturing plants is expected to exceed 200,000 CMD in the near future. By investigating 3 full-scale wastewater treatment plant, we demonstrates that the UASB sludge under methanogenic conditions would be favored over the aerobic ones for TMAH treatment due to its superb ability of handling high strength of TMAH-containing wastewaters. Inhibitory chemicals present in TFT-LCD wastewater such as surfactants and sulfate should be avoided to secure stable methanogenic TMAH degradation. Based on the results of terminal restriction fragment length polymorphism (T-RFLP) and quantitative PCR analyses targeting on the methyl coenzyme M reductase alpha subunit (*mcr*A) genes retrieved from the 3 full-scale bioreactors, *Methanomethylovorans* and *Methanosarcina* were the dominant methanogens involving in methanogenic degradation of TMAH in full-scale bioreactors. Furthermore, batch experiments were conducted to evaluate *mcrA* mRNA expression during methanogenic TMAH degradation and the results indicated that a higher level of TMAH favored *mcrA* mRNA expression by *Methansarcina*, while *Methanomethylovorans* could only express considerable amount of *mcrA* mRNA at a lower level of TMAH. These results suggest that *Methansarcina* is responsible for methanogenic TMAH degradation at higher TMAH concentrations, while *Methanomethylovorans* may be important at a lower TMAH condition.

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Developments in the Moving World of Environmental Biotechnology

Some 40 years, for the first time in history, a computer model developed at MIT in the USA was used by the scientists and politicians of the so-called Club of Rome to predict the environmental issues of our planet. The picture was rather gloomy. Around this period that we now live in, resources were predicted to become limited and plenty of perturbations in terms of energy demand and water availability would be occurring. In the mean time, we have indeed experienced that the world has become energy hungry as never before. Moreover we need more and more metal resources, for instance in our communication technology. We have become very fragile in terms of our supplies of fresh clean water. Most of all, we have provoked a world wide phenomenon of Climate Change, particularly by diffusive production of gases such as carbon dioxyde, methane, nitrous oxide. Part of the latter gases are directly linked to environmental technologies such as activated sludge and composting, uncontrolled anaerobic digestion and the bioconversion of mineral nitrogen by nitrification and denitrification. The public wants that scientists and politicians deal with Climate Change; we must evolve towards a bio-economy and food supply with low environmental footprint. It is important to be pragmatic and address the most urgent issues such as the focus on lower emissions of Green House Gases and the focus on a new type of Urban Metabolism for the Cities of the Future. A strategy towards these goals is to develop Environmental Biotechnology contributing to Resource Recovery and Closed Cycle Economy.

Environmental Biotechnology depends on the use of teams of micro-organisms to bring forward clean and effective processes. Recent years have revealed that such teams are not haphazard mixes of micro-organisms. On the contrary, they are well organized just as our human society: they have generalists and specialists, they have division of labor, they have 3-dimentional structures to provide efficient cooperation, they trade among themselves, they appear even to have species which specialize in multi-connecting with all partners (=microbial managers), they have mechanisms to maintain the stability of the team and to exclude invaders. Clearly, we can now, much more than before, engineer such teams, which we prefer to call "microbiomes".

There are a whole series of Environmental Biotechnology processes which can help us to abate Climate Change. A series of 7 of such most promising lines of technology are discussed subsequently.

Starting with the production processes of drinking water, we should learn to re-use valuable side products such as calcium carbonate and iron oxide, as currently demonstrated by the Reststoffenunie in the Netherlands.

When it comes to make us less dependent on the use of fossil fuel, the production of biogas by means of anaerobic digestion from crops and particularly from wastes is a 'cleantech' with ever increasing importance all over the world. A novel feature hereby is that one can upgrade the biogas to plain biomethane, distribute the latter over the gas-net and thus have all over the country a possibility to use green gas for transport and mobility as prioritized recently in Sweden.

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Environmental Biotech should turn away from processes such as conventional composting and activated sludge which directly biologically burn organics in to CO2. We advocate strongly to opt for a technology in which the organics upon arrival in the waste treatment plant are harvested and subjected to anaerobic digestion coupled to a subsequent 'biorefinery' in which a variety of recovery techniques and products are additionally programmed.

Recently, in the domain of resource recovery, a good deal of novelty has been introduced in terms of bio-electrochemistry allowing to make products at the site of the cathode thereby using microbial biosynthesis driven by recovered respectively renewable energy. This line of microbial based production of organic molecules can with time connect to the current petro-chemically based supply system of chemicals and products.

There are now good biotech processes available on the market to recover sulfur, phosphorous and metals and they will gradually become of more importance as these resources become exhausted in the coming century. The message is: yes we have proper tools available already.

Of special interest is the concept of New Urban Metabolism. More and more mega-cities are developing worldwide and we need to make sure that they no longer massively import water and food and massively export wastes to be destroyed without capturing the intrinsic value they represent. The cities of the future must install a totally new type of internal recycling of water and nutrients thus becoming less dependent on the supply from nature and from conventional agro-tech business.

As a seventh and last example in this context, the issue of nitrogen is discussed. The production of chemical nitrogen fertilizers at present consumes 2% or more of the total world fossil fuel budget. Yet, of this fertilizer nitrogen that goes to the fields, only some 15% reaches our plate in the form of protein rich food. The nitrogen present in the latter is, when consumed and discharged as human wastes, subjected to 'destruction' by techniques such as nitrification and denitrification or anammox, without implementing the potentials of recovery or upgrading. It must be emphasized more clearly that nitrogen has a major detrimental effect on the global environment and on Climate Change. We need a totally new approach in which we opt to capture the used nitrogen and re-use it without returning to the conventional agro-system. We must upgrade it within the context of the mega city to microbial protein usuable as feed and food. We demonstrate that both the route of production of microbial protein by means of organotrophic micro-organisms but also the route of using green energy to generate hydrogen and oxygen by electrolysis of water and using hydrogenotrophic micro-organisms, offer new and most challenging perspectives to positively adjust the feed and food supply of the planet.

To strive for a cyclic economy in order to abate climate change is very much a matter of mental attitude and cultural tradition. Hence, to produce reclaimed water and new protein from recovered nitrogen requires that the whole of our society is interested in the products thus generated. There are major challenges to be faced in terms of informing the public so that we all understand and accept these new potentialities as beneficial for us and our future. We need to install quality regulations and garantees all along the recovery processes and the production chains. We need to fully explore these various perspectives which Environmental Biotechnology offers so that they fit in a well functioning market economy. Clearly, as indicated 40 years ago by the Club of Rome, the world scene in terms of environmental quality is very much in evolution. However, the potentials which are appearing in the domain of 'microbiomes ' for environmental clean up and resource recovery in general combined with the commitment and goodwill of the citizens in particular, make us believe that the future certainly can be bright. Let us all think and strive along these lines for a sustainable and enjoyable 'moving' world for us and the generations to come.

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Innovative Wastewater Treatment by Algae and Bacterial Systems

The treatment of domestic and low-strength industrial wastewater under continental climate conditions is currently based on the action of aerobic-anoxic heterotrophic and nitrifying bacteria supported by mechanical aeration in a series of interconnected aerobic, anoxic and anaerobic bioreactors. This technology, mainly implemented in the so called activated sludge processes, has consistently provided satisfactory levels of carbon, nitrogen and phosphorous removal at the expenses of high energy consumptions and environmental impacts (high CO_2 footprint and nutrient loss). On the other hand, despite anaerobic digestion has played a key role on the sanitation of both domestic and industrial wastewaters in tropical areas, its low nutrient removal performance often requires additional (and costly) nitrogen or phosphorous post-treatment steps that entail a poor nutrient recovery. Likewise, the recent advances undertaken in the field of water sanitation by bacterial granulation, anammox-based N removal and membrane biomass retention have not significantly enhanced the sustainability of wastewater treatment in this XXI century (although they have resulted in more compact plant configurations). In this context, the technology envisaged to carry out wastewater treatment in this XXI century should be able to maximize both nutrient recovery and energy production from wastewater at a minimum CO_2 footprint while providing a consistent carbon, nutrients and pathogen removal from wastewater. Hence, the intensification of algal-bacterial symbiosis in engineered photobioreactors has emerged as a promising platform to support a low-cost and sustainable wastewater treatment based on solar irradiation. Algal-bacterial processes support a cost-effective and sustainable wastewater treatment based on their solar-driven oxygenation (mediated by photosynthesis), enhanced nutrient assimilation (as a result of their combined heterotrophic and autotrophic growth) and efficient pathogen removal (due to the high pH and O_2 concentrations mediated by photosynthesis). This technology has experienced significant advances in the past decade as a result of the microalgae boom originated from the chimera of microalgae biodiesel and the potential of microalgae for the mitigation of CO_2 emissions. The combination of microalgae-based wastewater treatment with flue gas treatment or biogas upgrading, and the development of conventional activated sludge schemes based on photosynthetic oxygenation and microalgae retention (via membrane or settling-recycling strategies), have boosted the potential of conventional high rate algal ponds (HRAPs) for wastewater treatment. Finally, both the energy valorisation via anaerobic digestion of the algal-bacterial biomass generated during wastewater treatment and nutrient recovery via algal-bacterial biomass concentration and drying will enhance the environmental sustainability of the process. The presence of phytohormones in fresh microalgae biomass is being recently exploited for the production of organic microalgae-based fertilizer, which are experiencing a good market acceptance. This keynote will present and critically discuss the fundamentals, potential and limitations of this promising green-biotechnology based on the recent advances carried out over the past 10 years in the field of applied phycology and wastewater treatment.

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Production of Biodegradable Polymers (Polyhydroxyalkanoate - PHA) through Biological Processes: Challenges and Opportunities

Polyhydroxyalkanoate (PHA) belongs to the polyester group with physicochemical properties similar to various plastics made from petroleum. However, it is biodegradable and can be produced biologically using various substrates including organic wastewater (Du *et al.*, 2001). This makes PHA an alternative to petroleum-based plastics used today. Currently, the commercial PHA branded with different trade names, such as Biopol, Metabolix, Nodax, etc. produced from glucose using different species (Magdouli et al., 2015). It was developed as early as 1976 by ICI, however the production was terminated in 1998 for a number of reasons (Koller et al., 2016). Recently, on July 6, 2016, Newlight Technology (USA) and Paques Holding bv (the Netherlands) have come to an agreement that will allow Paques to manufacture, process and sell bioplastics (PHA) based on Newlight's biocatalyst process to convert greenhouse gases (methane) to PHA (named AirCarbon) at a rate of up to 1.3 million metric tons/year.

However, the drawback is that the PHA production cost is still much higher than that of petroleum plastics, due to the cost of the substrate (Bengtsson *et al.*, 2008). On the other hand to use the pure culture also involves the high operational costs due to media sterilization and reactor maintenance (Reddy and Mohan, 2012a). The potential reduction in cost is using organic material from agriculture and food industry wastewater as much as possible and mixed cultures system as the microbial agent (Din et al., 2012; Reddy and Mohan, 2012b).

In this paper, an example of PHA production from tapioca industrial wastewater using mixed culture from an activated sludge done in our laboratory is presented. The aims of our study were treating tapioca- processing wastewater to produce PHA and remove COD. We carried out experiments using a SBR under four conditions of aerobic-anaerobic period combination. The content of PHAs produced and removal efficiency of COD were observed (Setyawati et al., 2012). And other example of PHA production from VFA (volatile fatty acids) using a pure culture of is also discussed. This study is focused on the production of PHA by *Ralstonia eutropha* JMP 134 in bioreactors with different operation modes by utilizing volatile fatty acids (VFAs) from palm oil mill effluent (POME) as precursors (Setiadi et al., 2015).

Although there is an increase research on this topic in the last twenty years, however the industrialization of this product is still a challenge. The sustainability of PHA production in the near future will be depend on several factors, such as strain selection, feedstock

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selection, bioreactor cultivation mode, downstream processing and product processing development. This challenges and opportunities will be discussed in this presentation.

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PLENARY LECTURES

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Strain Development by using Genome Editing Technology to Improve Algal Mass Cultivation

Background:

Microalgae are responsible for half of the global primary productivity; they convert solar energy to organic energy and fix carbon dioxide, which makes them important for the mitigation of greenhouse gases. Microalgal biotechnology began to develop in the middle of the last century. Today, there are numerous commercial applications of microalgae in areas such as nutrition, pharmaceuticals, and biofuels (1). Overproduction of specific high-value biochemicals requires the modification of metabolic pathways. However, metabolic engineering in microalgae has been limited because specific transformation tools need to be developed for each microalgal species. Recent development of tools for precise editing of microalgal nuclear genes has enabled metabolic engineering of microalgae (2). Recently, the DNA-free CRISPR-Cas9 method rather than plasmids that encode Cas9 and guide RNAs was employed for specific manipulation of genes in other organisms (3, 4). In our study, one-step transformation of microalgae by using the DNA-free CRISPR-Cas9 method was carried out to generate a commercially desirable strain for mass cultivation. This strain produces a high-value pigment without any induction and its truncated antenna increases productivity of mass culture under bright sunlight.

Results:

DNA-free RNA-guided engineered nucleases (RGENs) derived from the type II CRISPR-Cas9 system were applied to the microalga *Chlamydomonas reinhardtii*. We first generated a specific knockout of the *CpFTSY* gene; knocking out this gene results in truncated chlorophyll antennae of the photosystems (5). Δ*CpFTSY* mutants showed small insertions and deletions (indels) in *CpFTSY* gene as detected by targeted deep sequencing. We next applied RGEN-ribonucleoproteins (RNPs) to block zeaxanthin (Zea) epoxidation and thereby to accumulate this xanthophyll in microalgae. We generated three Δ*ZEP* mutants; the mutations were confirmed by Sanger sequencing and by measuring the amounts of Zea by HPLC: indel patterns were found at the expected positions and Zea content was significantly increased (more than 10 times) in Δ*ZEP* mutants compared to the wild type, even under low-light growth conditions.

To enhance productivity and to accumulate Zea in mass culture under bright sunlight conditions, we combined the two genotypes by transforming one of the Δ*ZEP* mutants with RGEN-RNPs targeting *CpFTSY* and thus obtained double knockout mutants. We compared the photosynthetic productivity of the Δ*ZEP*/Δ*CpFTSY* mutant with that of the Δ*ZEP* single mutant. Quantum yields of photosynthesis of Δ*ZEP*/Δ*CpFTSY*, Δ*ZEP* and wild type were essentially the same, indicating that this parameter was not affected by the single or double mutation. Growth of the Δ*ZEP*/Δ*CpFTSY* mutant under high-light conditions was dramatically greater than that of the wild type and the Δ*ZEP* mutant.

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Conclusion:

We achieved DNA-free targeted gene editing in *C. reinhardtii*. Sequential *CpFTSY* and *ZEP* gene knockout resulted in generation of a strain constitutively producing Zea and showing improved photosynthetic productivity. This simple RGEN RNP method could be applied to other microalgae without the need for a laborious cloning step. Moreover, the resulting transformants would be exempt from GMO regulation, facilitating applications of microalgae in the production of pharmaceuticals, nutraceuticals, food and animal feed. Our recent achievement in applying this genome editing method to improve mass cultivation for pigment production will make the use of microalgae more attractive and lucrative.

Keywords: Algal Mass Cultivation, CRISPR-CAS9 System, Biomass, Pigment, Antenna Size

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Remediation of Contaminated Soil in Indonesia

From the perspective of regulation and technology development

Soil has just received national attention much later than water and air environments. This could be understood as pollution in soil is not easily visible as in water or is not as mobile as it is in air. Although it is believed that soil pollution in Indonesia has been happening for quite long ago, however it started to attract concerns when oil sludge contaminated soil discovered in the middle of 90's. It was oil and gas upstream industries who was at that time declared releasing oil sludge and then it was suspected that such practice has been in operations since the beginning of the industries running. Since then effort of improving of oil sludge management has been developed in term of technology, application as well as regulation. Even though it was not exactly related to soil contamination, the ministry of environment regulation no 128/KEPMENLH/2003 has marked the beginning of the great concern of government toward soil contamination. This regulation was about method and conditions of bioremediation of oil sludge. Since the regulation was released, many initiative of treating crude oil contaminated soil were undertaken by oil and gas companies operated in Indonesia. This activity was also driven by PROPER program of Ministry of Environment. Thus remediation of soil environment in Indonesia was still limited to the case of oil and gas industry.

Realizing that the hazardous waste entering soil environment is not only coming from oil and gas industry, the government through ministry of environment declared another regulation expressed in the no 33/PERMENLH/2009 regulating the method of remediating soil contamination by hazardous waste in general. In this regulation, site assessment planning and remediation action planning became very important elements in the soil remediation activity. Since this regulation was in effect, many remediation activity with different kind of hazardous waste as pollutant were carried out. Government enforcement through PROPER program was effective in encouraging industry to clean or remediate their soil environment. It was also for the first time the approach of soil reference quality used as the end point criteria of every remediation activity. Soil remediation in this context is comprised into two elements. First one is to remove the contaminant from the soil. Contaminant must then be either treated or disposed off in a proper way. Second elements is that the remaining soil must be cured.

Regulation and great concerns of government toward soil remediation has driven the improvement of remediation technology. Not only bioremediation which has been the first technology introduced to remediate crude oil contaminated soil, but also physic and chemistry based principle for remediation technology are being considered to be implemented. In the bioremediation for example the practice in Indonesia has moved from intrinsic type bioremediation, to stimulated and then to augmentation type bioremediation. Research for finding good bacteria capable of degrading crude oil were carried out by many researcher although not all such research were published. Involvement of bacteria producing bio surfactant has also been considered both as cell and also as cell's product (bio surfactant itself). The latter initiative specifically being

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considered for remediation of heavy crude oil contaminated soil. The relatively recent regulation that was introduced by the government, PP no 101 year 2014 concerning hazardous waste management gives more comprehensive understanding on how soil contamination should be managed. Such regulation introduced new approach of determination of end point criteria and also post treatment action which was lacking in the previous regulation.

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Zooplankton Ecology Studies in the Strait of Malacca

Introduction

The Strait of Malacca located between the east coast of Pulau Sumatra in Indonesia and the west coast of Peninsular Malaysia is one of the most important trade routes linking the Indian Ocean with the Pacific Ocean via the Andaman Sea and the South China Sea. It is relatively shallow and narrow and the widest section of the Strait is found at its northwest entrance, narrowing gradually to 12 km wide near the southeast entrance. It is a unique tropical environment rich in renewable and non-renewable natural resources such as seafood products, tin, oil and gas (Burbridge 1988). The Strait is an important fishing ground (Razali 2000) and almost 60% of total fish landings in Malaysia originate from the Strait of Malacca. Due to its economic and political importance, the Strait has always been an interest for biological oceanographic studies.

Zooplankton in the Strait of Malacca has historically been examined with early investigations focusing primarily on taxonomic listing of zooplankton species (Sewell 1933, Wickstead 1961). More recent studies concentrate on the zoogeography of zooplankton (Chua and Chong 1975, Othman et al. 1990, Johan et al. 2002, Rezai et al. 2004).

Spatial and temporal distribution study

While studies on taxonomic records and diversity were common in the 1980s-90s, research has advanced into the investigation of ecology and distribution of zooplankton. A general feature of the tropical zooplankton is the lack or absence of seasonality in their biomass (Parsons et al. 1984). However, recent studies in some tropical areas have revealed that zooplankton biomass and species composition indicate seasonal variation (Smith et al. 1998). The seasonal variation in plankton communities is very apparent in boreal to temperate waters due to distinct hydrographic variation. Compared with boreal and temperate regions, the Strait of Malacca does not experience such seasonal climate variations. However, seasonality can also be observed in tropical waters and is most evident during the monsoon seasons. The weather in Malaysia is characterised by two monsoon seasons, namely, the SW Monsoon from late May to September, and the NE monsoon from early November to March.

Leachate Treatment Using Advanced Oxidation Process with Zeolite as a Catalyst

MR Sururi, SA Siti, OP Safria - IPCB 2016, 2016 - researchgate.net

Leachate of three years old active landfill was identified to have a very low biodegrability (low BOD/COD). Ozone-based on Advanced Oxidation Process (AOP) techniques have been currently developed by utilizing heterogeneous catalysts. This study treated leachate from an active landfill with the O3/H2O2 with addition of natural zeolites as catalysts. It was done to show some effect of natural zeolites mass to accelerate the formation of $OH^{\cdot}$; the response of leachate matrices; and the effectiveness of the process. The study was …

Bioprospecting of Fungi Associated with Cladiella sp. as Antibacterial-MDR against Acinetobacter baumannii from Panjang Island Vicinity

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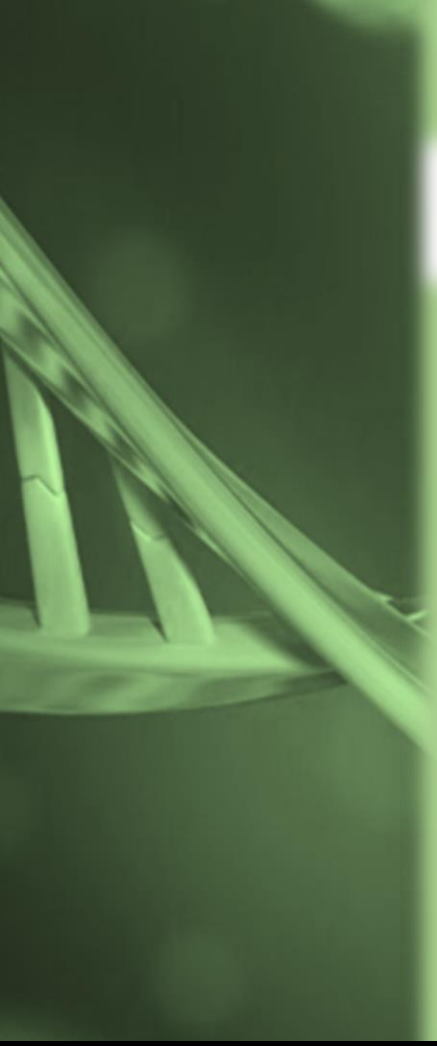

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Current Trends in Biotechnological Research for Environmental Sustainability

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PROCEEDING

3rd INTERNATIONAL POSTGRADUATE CONFERENCE ON BIOTECHNOLOGY

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PREFACE

It is a pleasure of the Organizing Committee to publish this Proceeding as an accomplishment of the successful International Postgraduate Conference on Biotechnology (IPCB) 2016, which was held on 24-26 August 2016 in Surabaya City, Indonesia. With a theme of *Current Trends in Biotechnological Research for Environmental Sustainability*, a total of 7 keynote and 6 invited papers were presented by outstanding speakers during the conference. Seventy-two scientific papers were presented by the participants during oral and poster presentation sessions. Attendees of this conference were from 9 countries, namely Japan, Malaysia, Indonesia, Korea, Taiwan, Nigeria, Pakistan, Belgium, and Spain.

This is the first Proceeding, which is published by IPCB Organizing Committee. Former IPCB events did not publish conference proceedings, as the main objectives of these conferences were for introducing on-going research activities by postgraduate students from the participating universities. Therefore, only limited numbers of articles were sent by the participants to be published in this Proceeding.

The Organizing Committee would like to thank the participants who sent their articles for this Proceeding. We hope that this Proceeding would provide beneficial scientific information on most recent biotechnological research for academicians.

Surabaya, 21 November 2016

IPCB 2016 Organizing Committee

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INSTITUT TEKNOLOGI SEPULUH NOPEMBER

It is a great pleasure for me to congratulate the Department of Environmental Engineering, Faculty of Civil Engineering and Planning on conducting the 3rd International Postgraduate Conference on Biotechnology (IPCB) 2016. I am really grateful to get the opportunity to deliver a keynote message in the proceedings. On behalf of Institut Teknologi Sepuluh Nopember (ITS), I would like to extend my very warm welcome to the honorable fellow guests, President of Soka University, Vice Chancellor of Universiti Malaysia Terengganu, all distinguished keynote and invited speakers, participants from many countries, and the organizing committees from Soka University and Universiti Malaysia Terengganu. I do hope that this collaborative academic activity will continue to develop for years to come in order to strengthen our good networking.

The theme of the conference, "Current Trends in Biotechnological Research for Environmental Sustainability" is indeed an important topic to discuss as biotechnological research is currently playing a key role in dealing with more complex and challenging environmental problems across the world. For years, environmental biotechnology itself has generated remarkable products with less wasteful use of materials and energy, and has been a promising technology for environmental clean-up processes. Recent and novel biotechnology processes, combined with physical and/or chemical processes, have been conducted not only for environmental remediation, but also for renewable energy production and resource recovery as the added values, which are become more important in the coming century. It should be noted that new and innovative biotechnology approaches are required in this century in order to sustaining liveable and balanced environments. Therefore, I am confident that this conference will create a good opportunity for young intellectuals to showcase their groundbreaking research and engage with their fellows who have similar interests in biotechnology as well as take part in knowledge transfer with more experienced experts for the sustainability of the environment.

ITS has mission, as a higher educational institution, to commit in the development of science, technology, and art that can improve the welfare of community through educational activities, research, community service, and management system based Information and Communication Technology (ICT). Having said that, biotechnology as one of the rapidly growing technologies is in line with this mission. Furthermore, this collaborative international conference is a type of implementations of ITS vision, "to become an internationally reputable university in science, technology, and art, especially to support an environmentally conscious industrial and marine development". Therefore, I strongly believe this will strengthen our linkages in the near future, not only in Asia, but also possibly create a new linkage intercontinentally.

Lastly, I would like to sincerely wish all participants of this 3rd IPCB conference every success in your work, have pleasant interaction to exchange fruitful scientific ideas, and enjoy every moment together with your fellows. I would also like to send my sincere gratitude to all the contibutors for the productive and successful conference.

Thank you.

Prof. Ir. Joni Hermana, MSc.ES., PhD

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PRESIDENT
SOKA UNIVERSITY

Congratulations on conducting the 3rd International Postgraduate Conference on Biotechnology hosted by the Institut Teknologi Sepuluh Nopember (ITS) Surabaya, Indonesia. On behalf of Soka University, I would like to congratulate the conference gathering honorable participants from across the globe. I would also like to appreciate the sincere efforts of the ITS committee members and the international supporting institutions in Indonesia, Malaysia, and Japan.

The early years of a researcher's career are the most critical towards fostering a solid foundation in conviction, diligence, patience and resilience. We live in a time of unprecedented information, technology and material convenience which has paradoxically provided both positive advances for humanity, as well as some unfavorable effects to the environment. The IPCB conference this year titled, "Current Trends in Biotechnological Research for Enviromental Sustainability", is a great opportunity for young minds to come together to create value through information exchange, scientific debate and cultural understanding. I am confidentthe conference will be a wonderful opportunity to ponder innovative ways to contribute to a sustainable society, while nurturing the qualities of humanistic values, particularly for young scientists.

The mission of Soka University is to foster and develop individuals capable of creating value in all endeavors towards a global community dedicated to peace and happiness off all humanity, as suggested by our founder Dr. Daisaku Ikeda. In order to secure peace and happiness of all individuals, the resources we depend on will have to be effectively utilized and managed. I am confident this conference, in concert with all of the institutions represented, will allow all of us, together, to create the harmony necessary for a new history in Asia.

It is my sincere wish that all participants of the 3rd IPCB conference enjoy warm exchange and experience cheerful dialogue towards a new era of harmonious scientific advances that will revolutionize humanities outlook towards sustainability. I would also like to acknowledge and offer my sincere gratitude to the countless individuals who continue to work tirelessly in the shadows to make the conference a great success.

Prof. Dr. Yoshihisa Baba,

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VICE CHANCELLOR

UNIVERSITI MALAYSIA TERENGGANU

It is indeed a great honour for me to pen down a few words in the programme book of the 3rd International Postgraduate Conference on Biotechnology (IPCB 2016). The 3rd IPCB 2016 held in Institut Teknologi Sepuluh Nopember, Surabaya this year is a manifestation of increasing strength in research collaboration between Malaysia, Japan and Indonesia. The collaborative outputs between these institutions have been progressing very well since the first IPCB 2011 which was hosted by Universiti Malaysia Terengganu (UMT), Malaysia. I am confident that the cooperation will continue to flourish for years to come.

Global current scenarios in Higher Education have transformed knowledge building approaches into flexible education. Concerted efforts are needed from various stakeholders with regards to cross-border education, human resource ability and joint research development. Researches and students attending this conference would be able to share peers their diverse multicultural and multidisciplinary background which could promote and enhance research cooperation among this region. Via this conference with the theme on application of biotechnology in scientific research management, findings from research conducted from different parts of the world can be shared. We believe, new ideas and knowledge in biotechnology that can unlock the potential of biodiversity resources towards sustainable development will benefit and fulfill the objective of this conference.

The biggest asset of the future research communities and changing landscape is none other than the present postgraduates. IPCB is an ideal platform in disseminating information among future scientists under the guidance of prominent senior experts across regions, vis-a-vis addressing specific issues of the national and regional contexts as part of a wider globalized society.

Biotechnology can lead to new knowledge for Bio-Economy that can improve livelihoods of the islands and peripheral communities. Fundamental to delivering such impacts is imperative to continue to generate and build upon knowledge from all levels of marine and related areas of scientific research that would be anticipated at the end of this conference.

UMT's focus is to drive research in oceanography and marine science towards sustainable management of aquatic resources in the region. Our involvement in Corals Triangle Initiative Fisheries and Food Security (CTI-CFF), IOC-WESTPAC on Ocean Acidification, UNESCO/IOC Ocean Teacher program, Asean Fisheries Education Network (ASEAN-FEN) are among our commitment at international level towards a better tomorrow. In 2015, UMT had successfully secure more than RM10.4 million of research grants, 35% of the funding is mainly for capacity building and human resource development. We believe in the role of passionate, talented and competence indigenous workforce in sustaining the wellbeing of natural resources. In order to deliver the outcomes of research in a systematic manner, UMT has established 5 research groups in marine science and oceanography; tropical fisheries and aquaculture, coastal and island communities, marine biotechnology and materials, maritime technology and management, lake ecosystem and tropical biodiversity. Special Interest Groups (SIG) are established to facilitate interdisciplinary and multidisciplinary research towards ocean of discoveries for global sustainability which is in line with the national policy in Higher Education and National Economic Transformation Programme that meet the millennium development goals.

I take this opportunity to congratulate the organizing committee on their efforts in organizing this important meeting and I wish you all have an exciting scientific bonding and fruitful series of idea exchange in the IPCB.

Prof. Dato' Dr. Nor Aieni Haji Mokhtar

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Chairperson,

3rd International Postgraduate Conference on Biotechnology

It is a great pleasure of the Organizing Committee to host the 3rd International Postgraduate Conference on Biotechnology (IPCB) in ITS Campus. It took a long way of planning, discussion, and preparation to organize this conference, after the successful implementations of the first and second conferences, which were hosted in Universiti Malaysia Terengganu (UMT) in 2011 and Soka University (SU) in 2014. It should be emphasized that this joined Conference can be implemented only because of the strong mutual commitment and good collaboration among the ITS, SU, and UMT committee members.

The Organizing Committee is very honoured with the attendance of President of SU, the Vice Chancellor of UMT, the Rector of ITS, and the President of Watanabe Oyster Laboratory to this conference. These distinguished guests will enrich this conference with their invaluable keynote addresses, which highlight the importance of science and technology development and regional collaboration for the field of biotechnology in particular.

This conference involves outstanding keynote and invited speakers from various European and Asian universities and industries. With a theme of *Current Trends in Biotechnological Research for Environmental Sustainability*, most recent research finding and innovations covering the sub-themes of natural resources, aquatic biotechnology, renewable energy, waste treatment technologies, restoration ecology, and marine and freshwater ecology will be presented and discussed during the conference. About 90 scientific papers will be presented by the participants in oral and poster presentation sessions. The registered attendees of this conference are from 9 countries.

It is expected that this conference could provide a good opportunity for postgraduate students, academicians, and biotechnology users from industries to meet, interact, and share knowledge and experiences for building better research and innovation capacities. It is also expected that collaboration opportunities in research and education among SU, UMT, ITS, and other participating universities and industries can be initiated and strengthened.

This conference cannot be implemented without the supports from many institutes and individuals. For this reason, we would like to express our most sincere gratitude to the President of SU, The Vice Chancellor of UMT, the Rector of ITS, and the Director of Watanabe Oyster Laboratory for their generous supports. None the less, we convey our special gratitude and appreciations to PT Pertamina EP, PT Newmont Nusa Tenggara, Bank Negara Indonesia, PT Total E & P Indonesie, the Local Government of Surabaya City, Bank Mandiri, and ITS alumni, for their generous contributions to this conference.

Lastly, but very importantly, we would like to take this opportunity to gratefully acknowledge the keynote and invited speakers, participants, and every single organizing committee member for the great contribution, support, commitment, and hard work. Also we gratefully thank to all unseen supports and efforts for making the conference this year another success.

We wish you a fruitful conference and enjoyable stay in Surabaya City.

On behalf of the Organizing Committee,

Prof. Dr. Yulinah Trihadiningrum, MAppSc

Department of Environmental Engineering of ITS

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KEYNOTE ADDRESS

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The Current Development of Biotechnology in Indonesia

Prof. Ir. Joni Hermana, MScES, PhD

Rector

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Abstract

As the largest archipelagic country in the world, Indonesia consists of approximately 13,466 islands that spread along 5,110 km from the West to the East. This extensive area provides abundant of natural resources with huge varieties of biodiversity that enable the development of biotechnology in the future. Biotechnology, in general, is a branch of sciences that studies the employment of living organisms and its derivatives to produce a new product or a new processing system.

The utilization of biotechnology has been growing rapidly in the world for several reasons, especially since it is acknowledged as an environmentally-friendly technology. The United States has mapped the four pillars of biotechnology that will dominate the world in the future, namely: health cares, agricultures, industries (i.e., energy, catalysts), and the environment. In term of the environmental aspect, it was started in the 19th century following the escalation of concern on the increase of environmental pollution, such as the contamination of materials that are non-biodegradables, both on land and in the river. For example, the contamination of soil by heavy metals, contamination from petroleum, as well as contamination of pollutants containing halogenated organic compounds. It is believed that the employment of biotechnology can resolve the contamination of these pollutants, for instance, by bioremediation. In addition, the use of biotechnology for the environmental issues has been increasing continuously, especially for wastewater treatment, soil treatment by means of bioremediation, nitrification and denitrification, and toxicity reduction. Moreover, biotechnology has been used to convert the potential pollutant gas into sources of energy in order to minimize its impact to global warming.

The numerous environmental problems that have been growth in many decades have been dealt by biotechnological approaches. Wastewater treatment has been developed to harness microorganisms and immobilization of enzymes for treating highly toxic waste. The use of plants for remediation of the polluted environment has also been widely used in Indonesia, by exploiting local crops. ITS is one of the leading universities in Indonesia that has been involved to develop biotechnology in various fields, including the environmental, biological, chemical and maritime sector. Although, in general, the development of environmental biotechnology in Indonesia still requires further attention when compared with other countries, however, the abundant of biodiversity in this country has a potential to be developed in order to improve the implementation of biotechnology in various fields. The development of several studies in ITS, such as phytoremediation, bioremediation and some other researches have led to the application of biotechnology as environmentally-friendly methods. The support from the government, in cooperation with various stakeholders both within and outside the country, is believed can accelerate the development of biotechnology in Indonesia.

Keywords: **biotechnology, bioremediation, environment, treatment.**

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Innovation, Technological Progress and Economic Growth

Prof. Dr. Yoshihisa Baba

President,

Soka University

Abstract

Today, it is generally accepted that innovation and technical progress play an important role in economic growth. The purpose of this study is to explore both how innovation is analyzed and economic growth due to technical progress is measured by referring to two economists, Joseph Schumpeter (1883-1950) and Robert M. Solow (1924~), respectively. Schumpeter suggested that innovation by the entrepreneur was the driving force of capitalism, and the fundamental cause of long-term economic growth. He proposed the following types of innovations: (i) Introduction of new goods, (ii) Introduction of new production methods, (iii) Opening of new markets, (iv) Acquisition of new supply sources of raw materials or half-manufactured goods, and (v) Implementation of new organization of any industry. He was among the first to emphasize the vital role of innovation by entrepreneurs in business cycles. Solow has also made contributions by presenting a model showing technological progress is the engine of long-term economic growth, where economic growth can be quantified by technological progress. In the Solow growth model, real GDP growth rate is determined by technical progress, if we assume continuous technical progress. Solow's other contribution was to lay the foundations for growth accounting by which empirical growth analysis becomes possible. In a simple model, economic growth due to technical progress can be measured as the difference between the total output of growth and the output of growth by capital and labor. This difference is frequently called "Solow's residuals". Lastly, the current state of innovation activities of the Organization for Economic Co-operation and Development (OECD) countries will be explored. The OECD has collected science, technology and innovation (STI) activities data and national STI policy information from its members' countries and important non-member countries to review key trends in STI. The OECD biennially publishes the "OECD Science, Technology and Industry Outlook". Based on its most recent issue (2014), university and public research STI policy profiles are reviewed in the following areas: (i) missions and orientation, (ii) financing public research, (iii) commercialization of public research.

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Regional Cooperation in Biotechnology Research for Environmental Sustainability

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Abstract

Sustainable management of marine biological resources has become an emerging interest among the scientific communities in the last two decades. The challenging issues to balance the socio-economic needs to harvest the natural resources with responsibility to sustain livelihoods, while protecting the environment and conservation of the finite resources have become the global agenda. In 2015, the United Nations Sustainable Development Summit has adopted the 2030 target for Sustainable Development Goals (SDGs) with the aim to eliminate poverty, fight inequality and injustice and tackle climate change. Ten out of seventeen goals are linked to the silent stakeholder- the marine environment.

Maritime nations in the Western Pacific have been advocating for regional scientific cooperation through the UNESCO Intergovernmental Oceanographic Commission in the Western Pacific (WESTPAC) and other platforms such as PEMSEA, JSPS and recently, SIMSEA. Apart from the Ocean Circulation and Ocean Dynamics, the topics on Marine Biodiversity, Harmful Algal Blooms, Marine Renewable Energy, Large Marine Ecosystems and Marine Spatial Planning and so forth, the network requires extensive collaborative scientific research, sharing of data and information as well as transfer of knowledge and technology.

The author will share the unique efforts of the Coral Triangle Initiative-Fisheries and Food Security (CTI-CFF) in capacity building for sustainable management of marine resources in the global epicenter of marine biological diversity that covers 1.6% of the planet ocean, contributed to 76% of coral diversity and more than 37% of the world reef fishes. The scientific boundary spans within 6 countries, sustains trade and livelihood of more than 363 million people and at the same time are facing the multiple challenging threats to sustainability. Ecosystems approach to fisheries management and promotion of responsible ecosystem services to the empowered communities whilst tremendous efforts in the development and application of technologies to build the baseline data on bio-geographies and other opportune development of new commercial products based on natural resources have been on the increase.

Social innovation in the context of the changing climate requires aspects of education and training, policy and governance, research and conservation with responsibility, participatory and equitable benefit by all the stakeholders, Federal and Local Government, Scientific Communities and NGOs. CTI-CFF regional Plan of Action and the establishment of Technical Working Groups by CT6 with the Development Partners provide a glimpse of hope to sustain the productivities of the marine environment that focus on biotechnology post-graduate research priorities for creativity and innovations towards the development of alternative resources, instead of continuously exploiting and harvesting precious natural resources from the wild.

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KEYNOTE SPEECH

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Creation of a Recycling Society

-Novel Lake Ecosystem Management by Sustainable Utilization of Aquatic Weed Biomass-

We introduce a lake ecosystem management project supported by the Ministry of Environment, Japan since 2014 that effectively utilizes harvested aquatic weed biomass. The excessive growth of freshwater aquatic weeds has become a major source of various environmental problems in shallow lakes and rivers throughout the world. In the South Basin of Lake Biwa (the largest lake in Japan), aquatic weeds continue to cover more than 90% of the lake surface, causing socio-economic problems such as fishery disturbance, foul odor and deterioration of tourism attractiveness. On the other hand, maintaining a moderate amount of aquatic weeds is essential for a healthy lake ecosystem, since aquatic weeds play a significant role in water purification and habitat for fishes and invertebrates. Historically, aquatic macrophytes were harvested and recycled for fertilization of agriculture in Japan. However, aquatic macrophyte resources are no longer viable because chemical fertilizers are better for growth, easier to manage and more economically profitable. In order to effectively manage and resolve the increasing issues related to the excess aquatic macrophytes, it is imperative to re-establish a new recycling society again. The proposed lake ecosystem management project includes four research themes as follows; 1) clarify the required amount of macrophytes to harvest annually for sustainable utilization and maintenance of a healthy lake ecosystem; 2) evaluate the effects of various aquatic weed removal methods on the biological community, water quality and sediment quality by establishing several experimental mesocosms in the lake; 3) develop effective treatment technology for the macrophyte biomass using anaerobic digestion (AD); 4) develop mass-culture techniques for high-value micro-algae production using effluents derived from the AD process. While most AD process studies have focused only on biogas recovery from the carbon fraction of the waste biomass, the current project establishes essential technology and methods for effective bio-refinery of aquatic weed biomass, enhancing not only biomethane recovery, but also acquiring crucial nutrients from the digestate that can be harnessed for microalgae biomass production.

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Challenges in Developing Marine Bio-products

Marine bio-products are facing great challenges in term of sustaining their resources as well as maintaining the quality of the products. Many companies producing marine bio-products are not in favor of developing new drugs due to their complex molecules structures and the availability of advances technologies. Although the first marine drugs have successfully made it to the shelf, there are still others in different stages of clinical trials. It is interesting to note that four US FDA-approved marine or marine-derived drugs are registered in the EU [1]. The first FDA-approved marine-derived drugs, Cytarabine (Ara-C) and vidarabine (Ara-A) are synthetic pyrimidine and purine nucleosides, originally isolated from the Caribbean sponge *Tethya crypta.* Cytarabine was approved as anticancer drug in 1969, while FDA approved vidarabine as antiviral agent in 1976. Ziconotide (Prialt ®) for the management of severe chronic pain obtained its approval from FDA in 2004. It was a synthetic equivalent of a naturally occurring peptide isolated from the venom of cone snail *Conus magus.* It was stated that more than 1000 new compounds with different biological activities have been reported each year for the past couple of years [2]. It is often found that the symbiotic derived from bacteria happened to be the one that are responsible to produce the compounds or secondary metabolites. One example is the symbiotic interaction between *Prochloron* spp. bacteria and its ascidian animal host. The symbiotic bacteria provides the host with photosynthate and defensive chemicals, and obtain waste nitrogen in return [3]. The clear examples are Patellamides that are cytotoxic secondary metabolite, which isolated from marine tunicate Lissoclinum patella but later was proven to be produced by the associated bacterium [4]. The same thing was also noted in bacterial symbiont *Candidatus Endobugula sertula* that is known as the source for cytotoxic bryostatins that were discovered from the marine bryozoan *Bugula neritina* [5,6].

Actually, when we defined marine bio-products, we could not run away from marine natural products. Natural products are usually small molecules, with molecular weight below 3000 Da, which are produced by biological source such as plants, animals and microorganism [7]. The natural products are often called secondary metabolites due to the fact that they are not biosynthesized by the general metabolic pathways and have no primary functions directly in the normal growth, development of reproduction of an organism. They usually used by the organism to control ecological relationship that involve defense against predation, competition for space or food or quorum sensing. From over 33 animal phyla described to date, 32 are represented in the aquatic environment, with 15 being exclusively marine [8]. Vast exploration of the ocean ecosystems only started in the mid 1970's with the emergence of modern snorkeling, and then the introduction of scuba and remotely operated vehicles (ROVs) [9]. Due to the technical limitations, exploitation of marine organisms started with the collection of large creatures such as red algae, sponges and soft coral, which have shown large variety of compounds with unique structures [10]. These are among the reasons why pharmaceutical companies or industry would invest in the search for interesting high-

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value ingredients for anti-tumors, anti-microtubule, anti-proliferative, photoprotective, antibiotic or anti-infective from the marine microorganisms [11,12].

Microalgae are also important resources that possessed wide range of biotechnology applications. Both Spirulina and Chlorella are being mass cultured for health supplement for general health food. It has also been used as bioremediation of aquaculture wastewater, biological tool for assessment and monitoring of environmental toxicants such as heavy metals, pesticides and pharmaceutical. In Malaysia, the researchers have been tapping into the microalgae resources for high-valued products and applications in wastewater treatment and assessment of environmental toxicants for the past 30 years. A culture collection of microalgae has been established in University Malaya Algae Culture Collection (UMACC) that holds more than 150 microalgal isolates in Malaysia. But the research are more focus into the fundamental studies in phycology which resulted in publications of several checklist and monographs that documented the diversity of microalgae in Malaysia [13]. In another study, marine microalgae grown in POME were suggested to be aquaculture feed due to their high content of PUFA. Cultures aerated with 5% CO_2 are found to produce highest yields of biomass and EPA. Several microalgae especially *Spirulina platensis* have also shown high antioxidant activity based on chemical assay.

This paper will also highlight few marine bio-products that have been recognized and developed for the industrial purposes. Most importantly, some of these products have attracted the SMEs to license the rights in order to bring them to the shelf. The products that will be highlighted are Scalogel from the fish scales, adjuvant from the microalgae and HEME-1, a marine additives produced to culture the marine bacteria from the oceans.

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Phytotechnology for Bioremediation of Contaminated Soils – The Essential Conditions for Mixed Plants

Introduction

Phytotechnology has evolved as a method of utilizing plants and their root microbes to recover the polluted environment. It was an important solution for the complexity of problem in a multimedia environment: air, water, soil. Plants could directly localize and absorb pollutants from the soil through the process of transpiration. Furthermore, the plants supplied the oxygen to the air through the process of photosynthesis, in addition to other benefits such as aesthetics. In line with the plant processes, root microbes also could degrade some pollutants from the soil. Contributions of plant processes could not be met by a single remediation process: the microbiological, physical or chemical. However, phytotechnology has limitations, such as slow process [1] to follow the plant growth. Plant growth rate was different from each other. Single plant might also be able to handle only specific pollutants [2]. Meanwhile, the soil could be enriched by various pollutants. Therefore, the use of mixed plants was important to address the variety of pollutants in the soil. This presentation described the essential conditions for plants and soil, aiming to obtain optimal phytoremediation process.

Mixed plants

The basic principle of phytoremediation was to ensure that the plant must be kept alive during the remediation process. Thus the range finding test of mixed plants has to be carried out to ensure each plant species alive at the maximum concentration of one or some pollutants.

Consumptive plant species quite widely applied in the remediation [3]. However, it was necessary to perform phytotoxicity test to identify the translocation of pollutants in plants, as well as the phytoaccumulation factor of pollutants. As a safety factor for consumers and shorten the procedure, then it was advised to use non-consumptive plants species, such as vetiver.

Mixed plants have to be capable of specifically the uptake of pollutants, as an example of cadmium associated with plant species, but zinc was associated with the soil [4]. Similar results were obtained in the remediation of polluted soil with some heavy metals and oil [5]. In addition, each plant must be able to work in synergy, including with root bacteria [6,7]. The facts directed attention to perform the specification test of pollutants that could be removed by mixed plants.

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Soil treatment

The use of soil amendments such as compost improved the efficiency of phytoremediation. Researchers have confirmed that inorganic amendments (sugar beet lime) and/or organic (compost) were able to increase the level of phytoremediation of soil polluted with some heavy metals (As, Cd, Cu, Zn and Pb) [8]. Similar results were shown in the applications of chelate, such as EDTA and EDGA that were also able to improve the remediation of soil polluted with oil [9]. The use of amendments was able to improve soil physical properties, such as increased porosity, water content and soil aggregation, which supported the plant processes and rhizodegradation [10]. However, the use of metal chelate EDTA might increase the leaching of metal, and therefore required good management in the operation of phytoremediation [11].

Saturation of soil, which was carried out by watering the ground would determine the status of the soil conditions as well as remediation performance. Soil saturated with water would produce anaerobic soil conditions. Soil remediation anaerobically produced a more effective and efficient for endosulfan degradation [12]. Water saturation of the soil to produce anaerobic conditions might not be applicable for all pollutants, contained in the soil. Pollutants that were biodegradable certainly be reduced more under aerobic conditions than anaerobic conditions. The aerobic conditions required soil aeration, by turning the soil or through the aeration system for contaminated soil on a regular basis. Thus, it was necessary scheduling saturation of soil and soil aeration, which was intended to be able to reduce various types of pollutants.

Conclusion

Conditioning of the plant species and the treatment of polluted soil were essential for improving the effectiveness and efficiency of remediation. Each type of plant should be mutually synergistic and specific in remediation of pollutants. Soil treatment must be appropriate to the type of soil pollutants.

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Biotechnology for Renewable Energy Production from Waste Treatment

The demand of clean and renewable biofuels has rapidly increased nowadays due to several global issues such as fast growing of human population, fossil fuel depletion, and global climate change. First generation biofuels using sugar, starch or certain food crops raised the issue of competition between food/feed and energy production, especially under the unexpected weather events including droughts and floods. Because waste reduction and renewable energy production can be achieved at the same time, biogas production such as hydrogen and methane from organic waste fermentation has been investigated with many source materials, including municipal waste fractions, cellulosic materials, domestic wastewater, lipid-extracted microalgae, dairy wastewater, and food wastes. The varied compositions and material characteristics in organic wastes and wastewaters pose challenges in investigation because a variety of substrates may be utilized by different species of microorganisms.

This presentation first introduces recovery of H_2 and CH_4 from treating bioethanol fermentation residues using a lab-scale two-stage bioprocess. In the hydrogen fermentation bioreactor (HFB), carbohydrate removal efficiency was maintained at 82-93% and the highest hydrogen yield was 8.24 mL/g COD at volumetric loading rate (VLR) of 80 kg COD/m^3/day. The results indicated a positive correlation between hydrogen yield and butyrate-to-acetate ratio, which might be due to the mechanisms of lactate/acetate utilization for hydrogen production and acetogenesis occurring in the HFB. Remaining volatile fatty acids and alcohols in the HFB effluent were further utilized for methane production in methane fermentation bioreactor (MFB), in which the highest methane yield of 345.2 mL/g COD was attained at VLR of 2.5 kg COD/m3/day. Overall, the two-stage bioprocess achieved a maximum COD removal of 81% from bagasse BEFR, and converted 0.3% and 72.8% of COD in the forms of H_2 and CH_4, respectively.

Secondly, algae-based biodiesel is considered a promising alternative energy; therefore, the treatment of microalgae residues would be necessary. Anaerobic processes can be used for treating oil extracted microalgae residues (OMR) and at the same time for recovering bioenergy. In this presentation, anaerobic batch experiments were conducted to evaluate the potential of recovering bioenergy, in the forms of butanol, H2, or CH4, from pretreated OMR. Using pretreated OMR as the only substrate, a butanol yield of 0.086 g/g-carbohydrate was obtained at carbohydrate of 40 g/L. With supplemented butyrate, a highest butanol yield of 0.192 g/g-carbohydrate was achieved at pretreated OMR containing 25 g/L of carbohydrate with 15 g/L of butyrate addition, attaining the highest energy yield of 3.92 kJ/g-OMR and energy generation rate of 0.65 kJ/g-OMR/day. CH4 production from pretreated OMR attained an energy yield of 8.83 kJ/g-

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OMR, but energy generation rate required further improvement. H2 production alone from pretreated OMR might not be attractive regarding energy yield, but it attained a superb energy generation rate of 0.68 kJ/g-OMR/day by combining H2 production from pretreated OMR and butanol production from pretreated OMR with supplementary butyrate from H2 fermentation supernatant.

Lastly, the amount of pollutants produced during manufacturing processes of TFT-LCD (Thin-film transistor liquid crystal display) substantially increases due to an increasing production of the opto-electronic industry in Taiwan. The total amount of wastewater from TFT-LCD manufacturing plants is expected to exceed 200,000 CMD in the near future. By investigating 3 full-scale wastewater treatment plant, we demonstrates that the UASB sludge under methanogenic conditions would be favored over the aerobic ones for TMAH treatment due to its superb ability of handling high strength of TMAH-containing wastewaters. Inhibitory chemicals present in TFT-LCD wastewater such as surfactants and sulfate should be avoided to secure stable methanogenic TMAH degradation. Based on the results of terminal restriction fragment length polymorphism (T-RFLP) and quantitative PCR analyses targeting on the methyl coenzyme M reductase alpha subunit (*mcr*A) genes retrieved from the 3 full-scale bioreactors, *Methanomethylovorans* and *Methanosarcina* were the dominant methanogens involving in methanogenic degradation of TMAH in full-scale bioreactors. Furthermore, batch experiments were conducted to evaluate *mcrA* mRNA expression during methanogenic TMAH degradation and the results indicated that a higher level of TMAH favored *mcrA* mRNA expression by *Methansarcina*, while *Methanomethylovorans* could only express considerable amount of *mcrA* mRNA at a lower level of TMAH. These results suggest that *Methansarcina* is responsible for methanogenic TMAH degradation at higher TMAH concentrations, while *Methanomethylovorans* may be important at a lower TMAH condition.

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Developments in the Moving World of Environmental Biotechnology

Some 40 years, for the first time in history, a computer model developed at MIT in the USA was used by the scientists and politicians of the so-called Club of Rome to predict the environmental issues of our planet. The picture was rather gloomy. Around this period that we now live in, resources were predicted to become limited and plenty of perturbations in terms of energy demand and water availability would be occurring. In the mean time, we have indeed experienced that the world has become energy hungry as never before. Moreover we need more and more metal resources, for instance in our communication technology. We have become very fragile in terms of our supplies of fresh clean water. Most of all, we have provoked a world wide phenomenon of Climate Change, particularly by diffusive production of gases such as carbon dioxyde, methane, nitrous oxide. Part of the latter gases are directly linked to environmental technologies such as activated sludge and composting, uncontrolled anaerobic digestion and the bioconversion of mineral nitrogen by nitrification and denitrification. The public wants that scientists and politicians deal with Climate Change; we must evolve towards a bio-economy and food supply with low environmental footprint. It is important to be pragmatic and address the most urgent issues such as the focus on lower emissions of Green House Gases and the focus on a new type of Urban Metabolism for the Cities of the Future. A strategy towards these goals is to develop Environmental Biotechnology contributing to Resource Recovery and Closed Cycle Economy.

Environmental Biotechnology depends on the use of teams of micro-organisms to bring forward clean and effective processes. Recent years have revealed that such teams are not haphazard mixes of micro-organisms. On the contrary, they are well organized just as our human society: they have generalists and specialists, they have division of labor, they have 3-dimentional structures to provide efficient cooperation, they trade among themselves, they appear even to have species which specialize in multi-connecting with all partners (=microbial managers), they have mechanisms to maintain the stability of the team and to exclude invaders. Clearly, we can now, much more than before, engineer such teams, which we prefer to call "microbiomes".

There are a whole series of Environmental Biotechnology processes which can help us to abate Climate Change. A series of 7 of such most promising lines of technology are discussed subsequently.

Starting with the production processes of drinking water, we should learn to re-use valuable side products such as calcium carbonate and iron oxide, as currently demonstrated by the Reststoffenunie in the Netherlands.

When it comes to make us less dependent on the use of fossil fuel, the production of biogas by means of anaerobic digestion from crops and particularly from wastes is a 'cleantech' with ever increasing importance all over the world. A novel feature hereby is that one can upgrade the biogas to plain biomethane, distribute the latter over the gas-net and thus have all over the country a possibility to use green gas for transport and mobility as prioritized recently in Sweden.

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Environmental Biotech should turn away from processes such as conventional composting and activated sludge which directly biologically burn organics in to CO2. We advocate strongly to opt for a technology in which the organics upon arrival in the waste treatment plant are harvested and subjected to anaerobic digestion coupled to a subsequent 'biorefinery' in which a variety of recovery techniques and products are additionally programmed.

Recently, in the domain of resource recovery, a good deal of novelty has been introduced in terms of bio-electrochemistry allowing to make products at the site of the cathode thereby using microbial biosynthesis driven by recovered respectively renewable energy. This line of microbial based production of organic molecules can with time connect to the current petro-chemically based supply system of chemicals and products.

There are now good biotech processes available on the market to recover sulfur, phosphorous and metals and they will gradually become of more importance as these resources become exhausted in the coming century. The message is: yes we have proper tools available already.

Of special interest is the concept of New Urban Metabolism. More and more mega-cities are developing worldwide and we need to make sure that they no longer massively import water and food and massively export wastes to be destroyed without capturing the intrinsic value they represent. The cities of the future must install a totally new type of internal recycling of water and nutrients thus becoming less dependent on the supply from nature and from conventional agro-tech business.

As a seventh and last example in this context, the issue of nitrogen is discussed. The production of chemical nitrogen fertilizers at present consumes 2% or more of the total world fossil fuel budget. Yet, of this fertilizer nitrogen that goes to the fields, only some 15% reaches our plate in the form of protein rich food. The nitrogen present in the latter is, when consumed and discharged as human wastes, subjected to 'destruction' by techniques such as nitrification and denitrification or anammox, without implementing the potentials of recovery or upgrading. It must be emphasized more clearly that nitrogen has a major detrimental effect on the global environment and on Climate Change. We need a totally new approach in which we opt to capture the used nitrogen and re-use it without returning to the conventional agro-system. We must upgrade it within the context of the mega city to microbial protein usuable as feed and food. We demonstrate that both the route of production of microbial protein by means of organotrophic micro-organisms but also the route of using green energy to generate hydrogen and oxygen by electrolysis of water and using hydrogenotrophic micro-organisms, offer new and most challenging perspectives to positively adjust the feed and food supply of the planet.

To strive for a cyclic economy in order to abate climate change is very much a matter of mental attitude and cultural tradition. Hence, to produce reclaimed water and new protein from recovered nitrogen requires that the whole of our society is interested in the products thus generated. There are major challenges to be faced in terms of informing the public so that we all understand and accept these new potentialities as beneficial for us and our future. We need to install quality regulations and garantees all along the recovery processes and the production chains. We need to fully explore these various perspectives which Environmental Biotechnology offers so that they fit in a well functioning market economy. Clearly, as indicated 40 years ago by the Club of Rome, the world scene in terms of environmental quality is very much in evolution. However, the potentials which are appearing in the domain of 'microbiomes ' for environmental clean up and resource recovery in general combined with the commitment and goodwill of the citizens in particular, make us believe that the future certainly can be bright. Let us all think and strive along these lines for a sustainable and enjoyable 'moving' world for us and the generations to come.

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Innovative Wastewater Treatment by Algae and Bacterial Systems

The treatment of domestic and low-strength industrial wastewater under continental climate conditions is currently based on the action of aerobic-anoxic heterotrophic and nitrifying bacteria supported by mechanical aeration in a series of interconnected aerobic, anoxic and anaerobic bioreactors. This technology, mainly implemented in the so called activated sludge processes, has consistently provided satisfactory levels of carbon, nitrogen and phosphorous removal at the expenses of high energy consumptions and environmental impacts (high CO_2 footprint and nutrient loss). On the other hand, despite anaerobic digestion has played a key role on the sanitation of both domestic and industrial wastewaters in tropical areas, its low nutrient removal performance often requires additional (and costly) nitrogen or phosphorous post-treatment steps that entail a poor nutrient recovery. Likewise, the recent advances undertaken in the field of water sanitation by bacterial granulation, anammox-based N removal and membrane biomass retention have not significantly enhanced the sustainability of wastewater treatment in this XXI century (although they have resulted in more compact plant configurations). In this context, the technology envisaged to carry out wastewater treatment in this XXI century should be able to maximize both nutrient recovery and energy production from wastewater at a minimum CO_2 footprint while providing a consistent carbon, nutrients and pathogen removal from wastewater. Hence, the intensification of algal-bacterial symbiosis in engineered photobioreactors has emerged as a promising platform to support a low-cost and sustainable wastewater treatment based on solar irradiation. Algal-bacterial processes support a cost-effective and sustainable wastewater treatment based on their solar-driven oxygenation (mediated by photosynthesis), enhanced nutrient assimilation (as a result of their combined heterotrophic and autotrophic growth) and efficient pathogen removal (due to the high pH and O_2 concentrations mediated by photosynthesis). This technology has experienced significant advances in the past decade as a result of the microalgae boom originated from the chimera of microalgae biodiesel and the potential of microalgae for the mitigation of CO_2 emissions. The combination of microalgae-based wastewater treatment with flue gas treatment or biogas upgrading, and the development of conventional activated sludge schemes based on photosynthetic oxygenation and microalgae retention (via membrane or settling-recycling strategies), have boosted the potential of conventional high rate algal ponds (HRAPs) for wastewater treatment. Finally, both the energy valorisation via anaerobic digestion of the algal-bacterial biomass generated during wastewater treatment and nutrient recovery via algal-bacterial biomass concentration and drying will enhance the environmental sustainability of the process. The presence of phytohormones in fresh microalgae biomass is being recently exploited for the production of organic microalgae-based fertilizer, which are experiencing a good market acceptance. This keynote will present and critically discuss the fundamentals, potential and limitations of this promising green-biotechnology based on the recent advances carried out over the past 10 years in the field of applied phycology and wastewater treatment.

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Production of Biodegradable Polymers (Polyhydroxyalkanoate - PHA) through Biological Processes: Challenges and Opportunities

Polyhydroxyalkanoate (PHA) belongs to the polyester group with physicochemical properties similar to various plastics made from petroleum. However, it is biodegradable and can be produced biologically using various substrates including organic wastewater (Du *et al.*, 2001). This makes PHA an alternative to petroleum-based plastics used today. Currently, the commercial PHA branded with different trade names, such as Biopol, Metabolix, Nodax, etc. produced from glucose using different species (Magdouli et al., 2015). It was developed as early as 1976 by ICI, however the production was terminated in 1998 for a number of reasons (Koller et al., 2016). Recently, on July 6, 2016, Newlight Technology (USA) and Paques Holding bv (the Netherlands) have come to an agreement that will allow Paques to manufacture, process and sell bioplastics (PHA) based on Newlight's biocatalyst process to convert greenhouse gases (methane) to PHA (named AirCarbon) at a rate of up to 1.3 million metric tons/year.

However, the drawback is that the PHA production cost is still much higher than that of petroleum plastics, due to the cost of the substrate (Bengtsson *et al.*, 2008). On the other hand to use the pure culture also involves the high operational costs due to media sterilization and reactor maintenance (Reddy and Mohan, 2012a). The potential reduction in cost is using organic material from agriculture and food industry wastewater as much as possible and mixed cultures system as the microbial agent (Din et al., 2012; Reddy and Mohan, 2012b).

In this paper, an example of PHA production from tapioca industrial wastewater using mixed culture from an activated sludge done in our laboratory is presented. The aims of our study were treating tapioca- processing wastewater to produce PHA and remove COD. We carried out experiments using a SBR under four conditions of aerobic-anaerobic period combination. The content of PHAs produced and removal efficiency of COD were observed (Setyawati et al., 2012). And other example of PHA production from VFA (volatile fatty acids) using a pure culture of is also discussed. This study is focused on the production of PHA by *Ralstonia eutropha* JMP 134 in bioreactors with different operation modes by utilizing volatile fatty acids (VFAs) from palm oil mill effluent (POME) as precursors (Setiadi et al., 2015).

Although there is an increase research on this topic in the last twenty years, however the industrialization of this product is still a challenge. The sustainability of PHA production in the near future will be depend on several factors, such as strain selection, feedstock

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selection, bioreactor cultivation mode, downstream processing and product processing development. This challenges and opportunities will be discussed in this presentation.

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PLENARY LECTURES

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Strain Development by using Genome Editing Technology to Improve Algal Mass Cultivation

Background:

Microalgae are responsible for half of the global primary productivity; they convert solar energy to organic energy and fix carbon dioxide, which makes them important for the mitigation of greenhouse gases. Microalgal biotechnology began to develop in the middle of the last century. Today, there are numerous commercial applications of microalgae in areas such as nutrition, pharmaceuticals, and biofuels (1). Overproduction of specific high-value biochemicals requires the modification of metabolic pathways. However, metabolic engineering in microalgae has been limited because specific transformation tools need to be developed for each microalgal species. Recent development of tools for precise editing of microalgal nuclear genes has enabled metabolic engineering of microalgae (2). Recently, the DNA-free CRISPR-Cas9 method rather than plasmids that encode Cas9 and guide RNAs was employed for specific manipulation of genes in other organisms (3, 4). In our study, one-step transformation of microalgae by using the DNA-free CRISPR-Cas9 method was carried out to generate a commercially desirable strain for mass cultivation. This strain produces a high-value pigment without any induction and its truncated antenna increases productivity of mass culture under bright sunlight.

Results:

DNA-free RNA-guided engineered nucleases (RGENs) derived from the type II CRISPR-Cas9 system were applied to the microalga *Chlamydomonas reinhardtii*. We first generated a specific knockout of the *CpFTSY* gene; knocking out this gene results in truncated chlorophyll antennae of the photosystems (5). Δ*CpFTSY* mutants showed small insertions and deletions (indels) in *CpFTSY* gene as detected by targeted deep sequencing. We next applied RGEN-ribonucleoproteins (RNPs) to block zeaxanthin (Zea) epoxidation and thereby to accumulate this xanthophyll in microalgae. We generated three Δ*ZEP* mutants; the mutations were confirmed by Sanger sequencing and by measuring the amounts of Zea by HPLC: indel patterns were found at the expected positions and Zea content was significantly increased (more than 10 times) in Δ*ZEP* mutants compared to the wild type, even under low-light growth conditions.

To enhance productivity and to accumulate Zea in mass culture under bright sunlight conditions, we combined the two genotypes by transforming one of the Δ*ZEP* mutants with RGEN-RNPs targeting *CpFTSY* and thus obtained double knockout mutants. We compared the photosynthetic productivity of the Δ*ZEP*/Δ*CpFTSY* mutant with that of the Δ*ZEP* single mutant. Quantum yields of photosynthesis of Δ*ZEP*/Δ*CpFTSY*, Δ*ZEP* and wild type were essentially the same, indicating that this parameter was not affected by the single or double mutation. Growth of the Δ*ZEP*/Δ*CpFTSY* mutant under high-light conditions was dramatically greater than that of the wild type and the Δ*ZEP* mutant.

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Conclusion:

We achieved DNA-free targeted gene editing in *C. reinhardtii*. Sequential *CpFTSY* and *ZEP* gene knockout resulted in generation of a strain constitutively producing Zea and showing improved photosynthetic productivity. This simple RGEN RNP method could be applied to other microalgae without the need for a laborious cloning step. Moreover, the resulting transformants would be exempt from GMO regulation, facilitating applications of microalgae in the production of pharmaceuticals, nutraceuticals, food and animal feed. Our recent achievement in applying this genome editing method to improve mass cultivation for pigment production will make the use of microalgae more attractive and lucrative.

Keywords: Algal Mass Cultivation, CRISPR-CAS9 System, Biomass, Pigment, Antenna Size

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Remediation of Contaminated Soil in Indonesia

From the perspective of regulation and technology development

Soil has just received national attention much later than water and air environments. This could be understood as pollution in soil is not easily visible as in water or is not as mobile as it is in air. Although it is believed that soil pollution in Indonesia has been happening for quite long ago, however it started to attract concerns when oil sludge contaminated soil discovered in the middle of 90's. It was oil and gas upstream industries who was at that time declared releasing oil sludge and then it was suspected that such practice has been in operations since the beginning of the industries running. Since then effort of improving of oil sludge management has been developed in term of technology, application as well as regulation. Even though it was not exactly related to soil contamination, the ministry of environment regulation no 128/KEPMENLH/2003 has marked the beginning of the great concern of government toward soil contamination. This regulation was about method and conditions of bioremediation of oil sludge. Since the regulation was released, many initiative of treating crude oil contaminated soil were undertaken by oil and gas companies operated in Indonesia. This activity was also driven by PROPER program of Ministry of Environment. Thus remediation of soil environment in Indonesia was still limited to the case of oil and gas industry.

Realizing that the hazardous waste entering soil environment is not only coming from oil and gas industry, the government through ministry of environment declared another regulation expressed in the no 33/PERMENLH/2009 regulating the method of remediating soil contamination by hazardous waste in general. In this regulation, site assessment planning and remediation action planning became very important elements in the soil remediation activity. Since this regulation was in effect, many remediation activity with different kind of hazardous waste as pollutant were carried out. Government enforcement through PROPER program was effective in encouraging industry to clean or remediate their soil environment. It was also for the first time the approach of soil reference quality used as the end point criteria of every remediation activity. Soil remediation in this context is comprised into two elements. First one is to remove the contaminant from the soil. Contaminant must then be either treated or disposed off in a proper way. Second elements is that the remaining soil must be cured.

Regulation and great concerns of government toward soil remediation has driven the improvement of remediation technology. Not only bioremediation which has been the first technology introduced to remediate crude oil contaminated soil, but also physic and chemistry based principle for remediation technology are being considered to be implemented. In the bioremediation for example the practice in Indonesia has moved from intrinsic type bioremediation, to stimulated and then to augmentation type bioremediation. Research for finding good bacteria capable of degrading crude oil were carried out by many researcher although not all such research were published. Involvement of bacteria producing bio surfactant has also been considered both as cell and also as cell's product (bio surfactant itself). The latter initiative specifically being

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considered for remediation of heavy crude oil contaminated soil. The relatively recent regulation that was introduced by the government, PP no 101 year 2014 concerning hazardous waste management gives more comprehensive understanding on how soil contamination should be managed. Such regulation introduced new approach of determination of end point criteria and also post treatment action which was lacking in the previous regulation.

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Zooplankton Ecology Studies in the Strait of Malacca

Introduction

The Strait of Malacca located between the east coast of Pulau Sumatra in Indonesia and the west coast of Peninsular Malaysia is one of the most important trade routes linking the Indian Ocean with the Pacific Ocean via the Andaman Sea and the South China Sea. It is relatively shallow and narrow and the widest section of the Strait is found at its northwest entrance, narrowing gradually to 12 km wide near the southeast entrance. It is a unique tropical environment rich in renewable and non-renewable natural resources such as seafood products, tin, oil and gas (Burbridge 1988). The Strait is an important fishing ground (Razali 2000) and almost 60% of total fish landings in Malaysia originate from the Strait of Malacca. Due to its economic and political importance, the Strait has always been an interest for biological oceanographic studies.

Zooplankton in the Strait of Malacca has historically been examined with early investigations focusing primarily on taxonomic listing of zooplankton species (Sewell 1933, Wickstead 1961). More recent studies concentrate on the zoogeography of zooplankton (Chua and Chong 1975, Othman et al. 1990, Johan et al. 2002, Rezai et al. 2004).

Spatial and temporal distribution study

While studies on taxonomic records and diversity were common in the 1980s-90s, research has advanced into the investigation of ecology and distribution of zooplankton. A general feature of the tropical zooplankton is the lack or absence of seasonality in their biomass (Parsons et al. 1984). However, recent studies in some tropical areas have revealed that zooplankton biomass and species composition indicate seasonal variation (Smith et al. 1998). The seasonal variation in plankton communities is very apparent in boreal to temperate waters due to distinct hydrographic variation. Compared with boreal and temperate regions, the Strait of Malacca does not experience such seasonal climate variations. However, seasonality can also be observed in tropical waters and is most evident during the monsoon seasons. The weather in Malaysia is characterised by two monsoon seasons, namely, the SW Monsoon from late May to September, and the NE monsoon from early November to March.

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Idris et al. (1999) conducted a 12-month study on the population dynamics of planktonic copepods in the coastal waters off Port Dickson. A total of 36 species were identified. The copepod density showed an upward trend during the NE monsoon but reached its peak during the SW monsoon. In addition, four distinct populations were observed throughout the 12-month period. The members of each group and their occurrence are given in Table 1.

Table 1. Population groups of copepod genera and their occurrence in the waters off Port Dickson.

Group	**Genera**	**Occurrence**
1	*Paracalanus, Euterpina, Acrocalanus* and *Oithona*	high density throughout the year
2	*Acartia* and *Microsetella*	moderately high density
3	*Acrocalanus, Clausocalanus* and *Corycaeus*	low densities throughout the year
4	*Subeucalanus, Labidocera, Candacia* and *Temora*	restricted periods of the year

Another study conducted in the coastal waters off Port Klang, Selangor, identified 35 species of copepods of which the family Paracalanidae was dominant (Johan et al. 2002).

The zooplankton in the Strait has been extensively studied by Rezai et al. (2003, 2004, 2005, 2009). The patterns of zooplankton distribution were investigated using samples collected in vertical hauls (140 um mesh) during four oceanographic cruises in the Strait of Malacca from November 1998 to August 2000 (Rezai et al. 2003). The average zooplankton biomass was higher during the SW monsoon than the NE monsoon. Higher zooplankton biomass occurred in the central region of the Strait although the spatial and temporal variations in biomass were statistically not significant. Rezai et al. (2004) further examined the distribution of copepods and identified 117 species of which 9 species were new records for the Strait of Malacca. Two copepod communities were distinguished. One belongs to the northern area and the other to the southern area of the Strait. The shallow southern area was characterised by a high density of copepods but low species diversity index with the dominance of a few coastal species such as *Euterpina acutifrons, Oithona simplex*, and *Paracalanus parvus*. The deeper waters of the northern area were characterised by low density of copepods but high species diversity index and the presence of epipelagic species belonging to the oceanic communities.

A study investigating the seasonal variation of zooplankton community at a fixed station in the Strait of Malacca was carried out monthly from June 2002 to May 2004 (Yoshida et al. 2006), where sampling was done by using vertical hauls of a 140 um plankton net. The copepods of the genera *Acartia, Acrocalanus, Paracalanus, Euterpina, Corycaeus* and *Oithona* accounted for approximately 90% of the total copepod population throughout the year. This indicated the importance of small species in tropical zooplankton communities. Zooplankton biomass generally peaked at the beginning of each monsoon and gradually decreased toward the inter-monsoon periods and showed a weak correlation with increasing ambient chlorophyll-a concentration. Cluster analysis of zooplankton abundance showed two distinct groups corresponding to the SW and NE monsoons. The populations of *Acartia pacifica* and *A. spinicauda* showed alternating

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peaks throughout the year, with *A. pacffica* appearing primarily during the NE monsoon and *A. spinicauda* during the SW monsoon.

Physiological ecology study

Physiological responses of organisms to the environment dictate tolerance limits and define favourable conditions for the optimal energy balance of the species. Thus research is focused on the biology and physiology of copepods due to their increasing importance as live feed for tropical aquaculture. The effect of environmental parameters on the reproduction and development of a tropical marine harpacticoid copepod *Nitocra affinis* was observed by Matias-Peralta et al. (2005). The highest ($p<0.05$) overall reproduction and fastest development time were achieved by copepods reared under the salinity of 30-35 and the optimum temperature required for the maximum reproduction was 30°C. The overall reproduction was highest ($p<0.05$) and development rate of *N. affinis* was shortest ($p<0.05$) under lowest experimental light intensity (25 umol $m^{-2}s^{-1}$), Although *N. affinis* is tolerant to a wide range of environmental conditions, prolonged exposure to subnormal environments affects its reproduction and development. Thus, the possibility of mass culture for commercial purposes and use in toxicity studies is promising.

The copepod *Apocyclops dengizicus* has the potential to be utilised as live feed (Farhadian et al. 2007, 2009) as they are found to be a suitable food for shrimp larvae *Penaeus monodon* (Farhadian et al. 2007) which preferred *Apocyclops* over *Artemia* as its food. The larvae which fed on *Apocyclops* showed higher dry body weight than those fed on *Artemia*. The survival rate and nutritional composition of the copepods were found to meet the requirements of fish and shrimp larvae. Coupled with the ease for mass culture, *Apocyclops* makes an economically viable replacement of *Artemia* (Farhadian et al. 2009).

Studies on the physiology on *Acartia* spp. were carried out to understand its contribution to zooplankton biomass in the Strait (Yoshida 2012). Prey size and concentration of food particles on the egg production of *A. pacifica* were examined by Eio et al. (2007). Their results revealed the copepods' preference for larger prey size indicated by significantly higher ingestion rates. Egg production, however, was not related to prey size and carbon demand by the copepods could be met by metabolic regulation of carbon transfer to eggs. The vertical distribution of *A. erythrea* was observed by Liong et al. (2007), where underwater light intensity did not show significant relationship with the vertical distribution of copepods in the Strait. This could be characteristic of shallow coastal waters where turbidity levels and vertical mixing are high and variable. Underwater light variation in such conditions may be of little advantage to offset predation risk and acquisition of foods.

Taxonomy and biodiversity study

Sewell (1933) conducted one of the earliest studies of copepods in the Malaysian waters and collected calanoid copepods from the vicinity of Penang and the Sungai Kurau estuary in Perak. He identified 19 species of calanoid copepods. Wickstead (1961) carried out a quantitative and qualitative study of plankton around Peninsula Malaysia. In the early 70's Chong and Chua (1975) studied cyclopoid copepods from the family Oithonidae. They found *O. simplex, O. attenuata, O. plumifera, O. rigida,* and *O. nano* to be common, and *O. rigida* especially abundant in the Strait of Malacca. Othman et al (1990) compared the copepods from the Gulf of Carpentaria, Australia with that of the Strait. In addition, Othman and Toda (2112) published results of a taxonomic research on copepods concentrating on the family Pontellidae, from which *Labidocera jaafari* (Othrnan 1986) was described as new to science.

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Amphipods were recently studied by Azman and Othman (2012), Othman and Morino (2006). Tan et al. (2014) studied the mysids adding new taxonomic records to the growing list of species found in Malaysia and Othman et al (2016) described a new species *Nebalia terazakii* from Pulau Payar, an island in the northern part of the Strait.

Conclusion

The importance of research on tropical zooplankton in understanding processes in the marine ecosystem has increasingly been recognised. Some of the principal issues on future advances in tropical zooplankton research include (l) determination of how environmental variability, instead of mean conditions, affects physiology and behavior; (2) relation of growth rates and production to environmental conditions; (3) determination of nutritional sources and requirements; (4) long term observations of population and community dynamics to see the effects of climate change; (5) need to have a bigger pool of specialists in taxonomy; and (6) development of mathematical models encompassing biological, chemical and physical parameters. Zooplankton research is being conducted by individual investigators working independently with a wide range of perspectives. Continued efforts in multidisciplinary activities and multilateral collaborations in the region are necessary to enhance communication, outline common goals and areas of future study which could accelerate the zooplankton research in Malaysia,

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A Novel Antioxidant Extracted from Oyster (Crassostrea gigas): The Permeability to the Brain and Sleep-Improving Effects in Human

We have carried out research on the antioxidant properties of oyster extract. Oxidative stress is known to accelerate significantly the progression of oxygen-related diseases such as diabetes and neurological diseases. Antioxidation is important for the prevention of these diseases. *In vivo* antioxidant are classified into enzymatic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (Fig. 1, GSH-Px), and the non-enzymatic antioxidants such as vitamin E and vitamin C according to their structure.

A double-blind, placebo-control study was conducted to investigate the antioxidative effects of the oyster extract in patients with type 2 diabetes. The urinary 8-OHdG of the patients was found to decrease as compared with the placebo-control. Since 8-OHdG is known to be a DNA oxidative stress marker, an antioxidant effect was present in the oyster extract.

In order to further investigate the antioxidant effects of oyster extract on the kidneys in a Type II diabetic model mouse was investigated. The results showed that the kidney 8-OHdG was significantly decreased. However, although a significant increase of GSH-Px was observed and only a slight increase in the activity of SOD was observed in the kidneys. Antioxidants can convert free radicals to water. The results, therefore, indicated that the enzymatic antioxidants were not fully active. Moreover, since, the known the non-enzymatic antioxidants that are present in the oyster extract could not be detected due to low concentration. There may be a novel antioxidant.

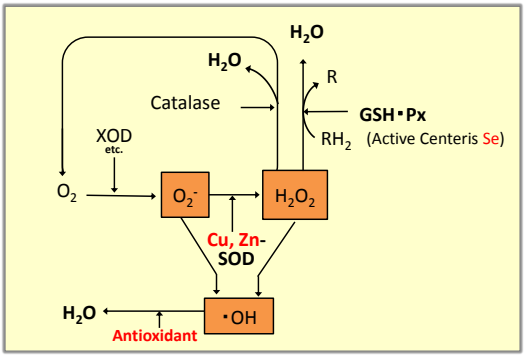

Fig. 1. Metabolism of reactive oxygen by enzymatic antioxidants

To further investigate the novel antioxidant, organic solvent extraction was conducted and the antioxidant capacity was determined using oxygen radical absorbance

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capacity assay (ORAC). The strongest antioxidant activity fraction was further purified by TLC and HPLC. The compound was then identified as 3,5-dihydroxy-4-metoxybenzyl alcohol (tentative name: E6) on the basis of ^{1}H and ^{13}C NMR, HMBC, ESI-MS spectral analyses. E6 is an amphiphilic compound that contains both hydrophilic group and hydrophobic group (Fig. 2).

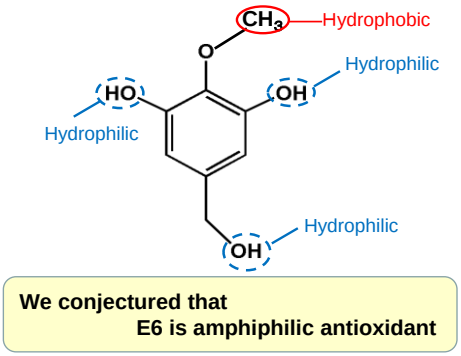

Fig. 2. Chemical structure of 3,5-dihydroxy-4-metoxybenzyl alcohol (E6)

A free radical is an unstable molecule that has one or more unpaired electrons. In order to make the free radical stable, they move to take electrons from other compounds or give away unpaired ones. In contrast, an antioxidant has the ability to pass the electrons to a free radical to prevent oxidation. E6 is an antioxidant that is able to pass its electrons to a free radical (Fig. 3). The antioxidant capacity was found to be 2.4 times higher than both vitamin C and vitamin E (Table 1).

Table 1 ORAC value of antioxidants

Antioxidants	ORAC value (µmol TE/µmol)
E6 (Isolated substance from oyster)	1.24 ± 0.35
Trolox (Reference substance)	1.00
Vitamin C	0.53 ± 0.13
Vitamin E	0.50 ± 0.13

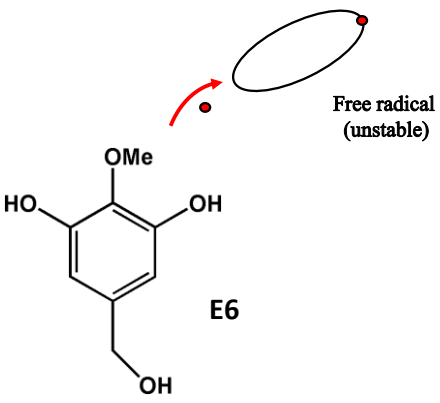

Fig. 3. Schematic of E6 passes electron to free radical

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From the chemical structure (Fig. 3), we conjectured that E6 is an amphiphilic antioxidant. E6 is postulated to penetrate into the brain by its amphiphilicity. A study was conducted to investigate permeability of E6 into the brain after oral administration of E6 in mice. The MRM chromatogram of LC-MS/MS showed the single peak of E6 was detected in the brain (Fig. 4). This result confirmed that E6 has penetrated into the brain. Fig. 5 shows the changes of E6 concentration in the brain after oral administration.

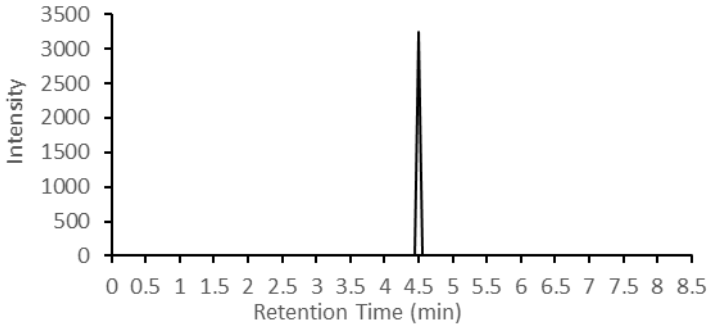

Fig. 4. MRM chromatogram of E6 in the brain after E6 oral administration

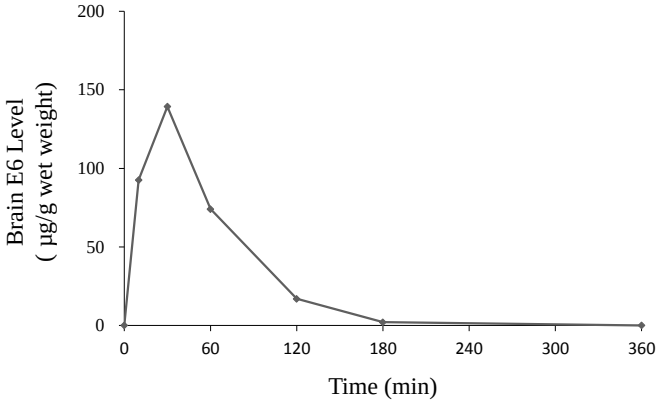

Fig. 5. Changes in brain E6 concentration after oral administration

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Applications of Biotechnology in Color Removal

Color in water and wastewater is a manifestation of the presence of inorganic or organic matters. It affects the aesthetic value of water and can be harmful to human health and the environment depending on the cause of the color. In natural water, color can be caused by dissolved inorganic metals such as iron and manganese or dissolved organics such as tannin and lignin, or due to algae growth. In industrial wastewater, color is mainly caused by the chemical residuals that are not consumed within the manufacturing process or compounds generated from raw materials being processed. Color from inorganic metals is normally removed through chemical precipitation and coagulation-flocculation followed by sedimentation process. Color caused by dissolved organics are typically treated using physico-chemical processes, which include coagulation-flocculation, chemical oxidation (eg. ozonation, chlorination, advanced oxidation), carbon adsorption and membrane separation. While these processes have been successful in treating colored wastewater, they are expensive and complex in terms of operation and maintenance. Unfortunately, due to the non-biodegradable nature of the color causing compounds, conventional biological process, which is a cheaper process, is incapable of treating the colored wastewater. Therefore, there is a need to manipulate the capability of the biological process in treating colored wastewater.

This presentation will discuss and share the findings of the studies that have been carried out to treat textile wastewater using integrated biological system. The system, which is comprised of sequential anaerobic-aerobic process was operated in both separate and hybrid reactors with size ranging from 1.5 L to 2000 L. It is well established that under anaerobic condition, the N=N bond of the azo dyes are cleavaged, leading to the production of colorless byproducts, namely amines; this is followed by complete mineralization under aerobic condition. Different forms of biomass (i.e. flocs, biofilm and biogranules) have been applied in different types of reactor. Synthetic and actual textile wastewaters with COD and color of up to 1000 mg/L and 4500 ADMI, respectively, have been treated. The effects of co-substrate, which include acetate, glucose, sewage and pineapple wastewater were also evaluated. The system was able to obtain maximum COD and color removal of more than 85% and 90%, respectively, within maximum hydraulic retention of 24 hours. The findings of the study indicate the potential of the biological system in treating colored wastewater from the textile industry.

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PROCEEDING

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Biosorption of Cadmium Ion from Aqueous Solution by *Aphanothece sp*: Equilibrium, Kinetic and Thermodynamic Studies

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Abstract

This study evaluates cadmium biosorption removal by dried biomass of *Aphanothece sp*. This microalgae species (from Cyanobacterium phylum) was isolated from a hypereutrophic lake, cultivated and processed into biosorbent in the laboratory. Experiments were carried out to study the effect of various parameters such as initial cadmium concentration, experimental pH, and temperature on the biosorption potential of this biomass. This study shows that the optimum pH for biosorption of cadmium was found to be 8. A maximum sorption capacity of about 17.27 mg of cadmium per gram of this microalgae biomass was observed at pH 8 for 1.03 to 3.63 mg/L solution of cadmium, while in initial cadmium range from 6.48 to 53.38 mg/L, the maximum sorption capacity was 119.05 mg per gram dried biomass. Kinetics of cadmium in this process is better described by pseudo second order kinetic model. The equilibrium isotherm data are very well represented by Langmuir isotherm and Dubinin-Radushkevich isotherm equations which confirmed monolayer coverage of cadmium onto surface of dried microalgae biomass and chemisorption process. According to thermodynamic parameter such as ΔG^0, ΔH^0 and ΔS^0 calculation this biosorption process was exothermic, and driven to the product formation.

Keywords: cadmium, biosorption, Aphanothece sp

1. INTRODUCTION

Various industries such as plastics, mining, textile, pigment, electroplating, and metallurgical discharge heavy metals borne wastewater to the aquatic system. These metals are considered persistent environmental contaminants, since they are very difficult to be degraded. Their existence in aquatic food chain will pose serious problems due to its accumulation and toxic effects not only for biota but also humans as top consumer. Therefore, it is necessary to eliminate heavy metals from industrial wastewater, and also important for economic considerations [1-3]

Cadmium (Cd) is one heavy metal known as a high toxic element. It has adverse effect on human health leading to various diseases, such as renal dysfunction, liver damage, bone degradation and hypertension. USEPA has also classified Cd as group B1carcinogen [4]. Conventional wastewater treatment technologies for heavy metals removal includes chemical precipitation, evaporation, ion exchange and membrane separation. Even though some improvements on these technologies has been done, there are still major disadvantages on their cost and effectiveness. Other challenges includes energy intensive processing, low efficiency, inability to reduce the Cd ion concentration as per environmental regulation in many countries (for example Indonesian environmental ministry stated 0.05 mg Cd/L as permissible Cd level in industrial waste water before being disharged into open waters) and production of toxic chemical sludge

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which require some industries to provide additional treatments. Therefore, it is necessary to develop an economical, effective and safe methods for Cadmium removal [1, 5, 6].

A relatively new emerging technology for removing heavy metals ions from dilute solutions is biosorption. This technology receive significant attention since it is proved as an efficient, clean and inexpensive way to treat wastewater containing heavy metals [6, 7]

In line with trend in biotechnology, microalgae were reported to be one of the usable bio-sorbents due to their low cost, relatively large surface area, high binding affinity and high metal recovery. *Aphanothece sp* as biosorbent is one proper choice primarily due to its high growth and high abundance in metal polluted and hyper-eutrophic lake [8, 9].

*Aphanothece s*p is a member of cyanobacter or blue-green algae. It belongs to the family of *choroococaceae* and forming blue green colonies adapted for floating. Their cells are spherical to ovate and loosely embedded in mucilage. Its cells size are 1-4µm wide and 2-8 µm long. It is commonly found on the side of lakes and ponds [10]. Microalgae are rich of polysaccharides, lipids and protein. These compounds contain varieties of functional group such as carboxyl, hydroxyl, sulphate and other charged groups which are beneficial for metal binding [1, 11]. Even biosorption technology has received much attention, actual interactions between metal ions and the functional groups on the cell, kinetic, and thermal properties of biosorption have not been well defined [12, 13]. Many studies in which microorganisms have been used as biosorbents for heavy metals are useful for development of a low cost industrial wastewater treatment. Mainly, those studies were focused on to find rapid removal of several heavy metals [14-16].

Most studies were focusing on removal of Pb^{2+}, Cd^{2+} and Ni^{2+} ion from wastewater using various bio-sorbents, such as aquatics macrophytes (*Spirodela intermedia, Lemna minor* and *Pistia stratiotes*) which were reported to be more efficient in removing Pb^{2+} and Cd^{2+} [17]. However, microalgae has different cell surfaces characteristics compared to aquatics macrophytes. Biosorption by using freshwater microalgae such as *Oscillatoria limnetica, Anabaena spiroides, Eudorina elegans* and *Chlorella vulgaris* have shown immense sorption capacity on these metals especially Pb^{2+} [18]. *Spirulina platensis* also demonstrated an effective biosorption capability on Pb^{2+}, Cd^{2+} and Ni^{2+} [19]

In this study, the optimum of operational condition of biosorption process, isotherm equilibrium, kinetics and thermodynamics aspects of biosorption of Cd^{2+} by *Aphanothece s*p were investigated.

2. MATERIAL and METHODS

2.1. Preparation of biomass

The cyanobacter *Aphanothece s*p was obtained from the culture collection of Laboratory of Bioprocess Engineering, Dept. Of Chemical Engineering-ITB. It was grown in BG-11 medium under constant light of 3000 lux, aeration flows 3L/minutes of atmospheric carbondioxyde and temperature 24-28°C. After 14 days cultivation the cultures was then centrifuged at 6000 rpm for 15 minutes at 25 °C, decantated, and washed with deionized water to remove spent medium. Harvested biomass was then dried in oven at 60 °C for 7x 24 hours, crushed, and then sieved into particle size of 354 µm. Dried biomass was then characterized (elemental analysis, moisture content, optical and SEM images) and stored in polyethilen bottles for biosorption experiments.

2.2. Preparation of synthetic cadmium solution

Stock cadmium solution of 1000 mg/L was prepared by dissolving 2.0318 gram of $CdCl_2.2.5H_2O$ in 1 L of deionized water added with 20 mL of 1:1 HCL solution.

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Cadmium solution of different concentration was prepared by suitable dilution of the stock solution to known volumes.

2.3. Batch Biosorption Procedure

Initial concentration of Cd (mg/L) in this study were designed in two range group of data, namely low range (1.028; 1.986; 3.63; 6.477; 7.767) and high range (5.41; 9.58; 19.45; 53.38; 99.92). For effect of pH on biosorption study, test solutions were adjusted at pH 3 (buffer KH-phtalate and 0.1 M HCl); pH 5 (buffer KH-phtalate and 0.1 M NaOH); pH 7 and pH 8 (buffer KH_2PO_4 and 0.1 M NaOH); pH 9 (buffer Na-tetraborate and 0.1 M HCl); and pH 11 (buffer Na-bicarbonate and 0.1 M NaOH). Then, 1 g/L of biosorbent was added in a series of bottles, containing 25 mL of Cd solution with initial concentration 10 mg/L in each pH value. This bottle is then shaked at 120 rpm and temperature of 30°C for 120 minutes. The effect of initial Cd concentration on equilibrium uptake was estimated by contacting 1.0 g/L biosorbent with 25 mL of different initial of Cd concentration ranging from 1.099 to 7.77 mg/L (low range) and 1.03-83.17 mg/L (high range). This experiment also conducted in shaker at 120 rpm and 30 °C for 120 minutes. The effect of biosorbent dosage on cadmium biosorption capacity was obtained by agitation at 120 rpm and 30 °C of a series of weighed biomass ranging from 0.1 to 2.0 g/L with initial Cd concentration of 7.77 mg/L and pH 8. The concentration of Cd in solution before and after adsorption was determined using Flame Atomic Absorption Spectrophotometer Shimadzu AA-7000 at 226.502 nm wavelength.

2.4. Biosorption Kinetics and Equilibrium Studies

Biosorption kinetic experiments were carried out by shaking 25 mL of Cd solution of known initial concentration with 1.0 g/L biosorbent at 30°C and 120 rpm and andoptimum pH value (8.0) for desired contact time. The time required for reaching equilibrium condition was estimated by drawing samples at regular time interval until reaching equilibrium. Samples were then filtered and analyzed using flame AAS. The experiments were repeated at 25, 27,32,35,40, 42 and 50 °C. Analysis were repeated twice and the results were averaged. The amount of Cd adsorbed onto biosorbent at certain contact time was calculated from the mass balance of the equation given below:

$$q_t = (C_0 - C_t)\frac{V}{w} \quad (1)$$

Where C_0 and C_t are the initial and concentration of solution at time t (mg/L), q_t is concentration of Cd on biosorbent (mg/g), V is the volume of Cd solution (L) and W is the mass of the biosorbent used (g).

The percent biosorption of the metal was calculated as follow

$$Biosorption\ (\%) = \left(\frac{C_0 - C_f}{C_0}\right) \times 100 \quad (2)$$

Where C_0 and C_f are the initial and final metal concentrations.

The linearized form of PSO-1 is given below:

$$\ln(q_e - q_t) = lnq_e - k_1 t \quad (3)$$

where q_t and q_e (mg/g) are the amounts of sorbed ion at equilibrium (mg/g) and t (minute), k_1 is the rate constant of the biosorption (min^{-1}). Plotting of ln (qe-qt) versus t must be done to find k_1. Experimental data were also tested by the PSO-2 which given in the following linearized form:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right) t \quad (4)$$

where k_2 (g/mg.min) is the rate constant of PSO-2, q_t (mg/g) is the amount of biosorption at time t (minute) and q_e is amount of biosorption equilibrium (mg/g).

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The Langmuir isotherm model assumes that biosorption occurs at specific homogenous sites on the biosorbent and is used successfully in many monolayer biosorption process. This model can be written in linear form as:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \quad (5)$$

Where q_e is the equilibrium metal ion concentration on the biosorbent (mg/g), C_e is the equilibrium metal ion concentration in the solution (mg/L), q_m is the monolayer biosorption capacity (mg/g), and K_L is the Langmuir biosorption constant relating the free energy of biosorption (L/mg). Freundlich isotherm is used for modelling the biosorption on heterogenous surfaces. This isotherm can be explained as linear form as follow:

$$\ln q_e = \ln K_F + \left(\frac{1}{n}\right) \ln C_e \quad (6)$$

Where K_F is a constant relating to the biosorption capacity and 1/n is an empirical parameter relating to biosorption capacity which varies with the heterogeneity of the biomass. The experimental equilibrium data also applied to the D-R isotherm model to determine the nature of biosorption process as physical or chemical. The linear form of D-R isotherm equation as below:

$$\ln q_e = \ln q_m \ -\beta \varepsilon^2 \quad (7)$$

Where q_e is the amount of metal ions adsorbed per unit weight biomass (mol/L), q_m is the maximum biosorption capacity (mol/g), β is the activity coefficient related to mean of biosorption energy (mol^2/J^2) and ε is the Polanyi potential ($\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right)$). The mean biosorption energy (E) gives information about biosorption mechanism, physical or chemical. It is calculated as follow

$$E = \frac{1}{\sqrt{-2\beta}} \quad (8)$$

If E value is between 8 and 16 kJ/mol, the biosorption process is chemisorption, while E less than 8kJ/mol, the biosorption process is physisorption.

Thermodynamic behavior of Cd (II) biosorption onto dried biomass of *Aphanothece sp* was described by using three thermodynamic parameter including of free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0). These parameter were calculated from the following equations:

$$\Delta G^0 = -RT \ln K_d \quad (9)$$

Where, R is the universal gas constant (8.314J/molK), T is temperature (K) and Kd ($=q_e/C_e$) is the distribution coefficient.

Estimation on the enthalpy and entropy of biosorption then conducted by considering following equation from the slope and intercept of the plot of ln Kd versus 1/T yields (Figure 3).

$$\ln K_d = \left(\frac{\Delta S^0}{R}\right) - \left(\frac{\Delta H^0}{RT}\right) \quad (10)$$

3. RESULTS and DISCUSSION

3.1. Elemental and Images analysis

Dried biosorbent used was characterized using elemental analysis (Labconco CHNS Analyzer) which show that *Aphanothece sp* mainly contain of 42,53 %C, 6,43 %H, 7,71 %N and 0,51%S, while moisture content was found to be 93,16 to 99,72%.

Optical image was obtained using a trinocular light microscope (Motic BA310). SEM characterization was carried out using JEOL-JSM6360. Optical and SEM micro

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images is shown in Figure 1. The optical image showed that living *Aphanothece sp* have circular shape with cell radius and size typical for Cyanobacter [10]. Whereas SEM micro image of dried biosorbent confirmed that mainly size of dried biosorbent was less than 500µm with a slight rough surface.

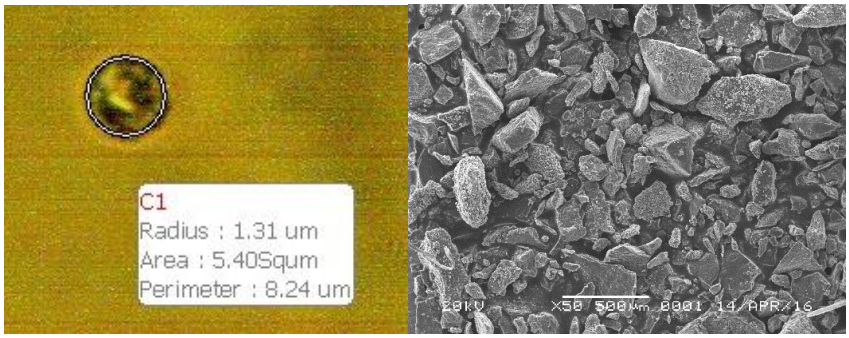

Figure 1. Typical images of Aphanothece sp: (a) obtained with optical microscope (400x) and (b) scanning electron image microscope of dried biosorbent made of Aphanothece sp.

3.2. Effect of pH on Biosorption

Both of biosorption capacity and removal of Cd were low at pH 7 but increased considerably from 0.2 to 0.65 mg/g and 43.35 to 95.8 % as pH increased to 8. It decreased at pH 9 which capacity and Cd removal dropped to 0.25 and 24.83. At pH 11, biosorption capacity Cd removal was increased, but removal of Cd was decreased. Precipitation was also found, indicating fast formation of metal-hydroxyl complex, therefore metal binding by biosorbent was not possible. Subsequent experiments were conducted at initial pH of 8.

The decreased biosorption capacity and removal of Cd at low pH (3.0-5.0) could be attributed to the competition of the binding sites on surface of dried biomass for H^+ ions. At low pH Cadmium is unable to be bound due to protonation of biosorbent surface, giving repulsion to the metal cations. Increased biosorption of Cd at pH above 7 could be attributed to the fact that at higher pH, more negative charges were available, enhancing Cd biosorption [1, 6, 7].

3.3. Effect of Initial Concentration

The equilibrium Cd biosorption capacity of dried *Aphanothece sp* biomass at various initial of Cd concentration is depicted in Figure 2. Cd biosorption was studied in batch experiment (pH 8 at 30°C) using different initial concentration divided in low range (1.090-7.994 mg/L) and high range (9.58-83.07 mg/L) concentration. Biosorption capacity of Cd has been found to be higher at high range than in low range. However removal percentage decreased as initial concentration increased (Figure 2). A similar pattern has also been reported [20]. During biosorption process, mass transfer resistance between solid phase and aqueous phase of metal ion can be overcome by enhancing of initial metal concentration. Metal uptake will be increased with increasing of initial metal concentration due to enhancing mass transfer driving force of metal ion between solid phase and aqueous phase [21]. Since % biosorption is decreased with increasing of initial Cd concentration, it indicate that at higher concentration range, binding sites availabile for adsorption, on the contrary, tend to be limited [22, 23].

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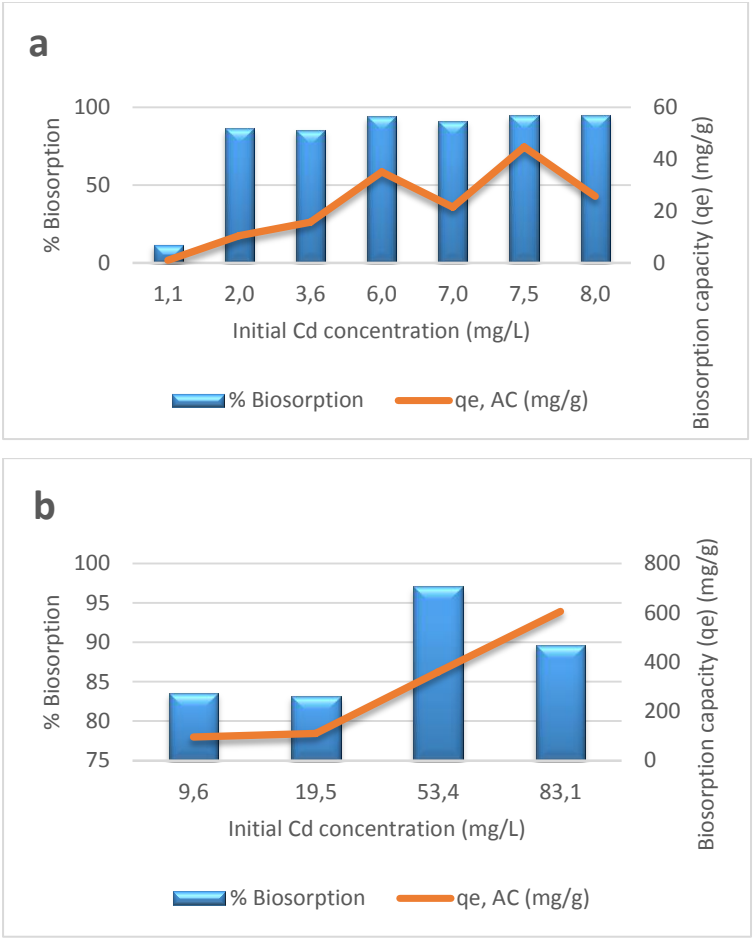

Figure 2. Effect of initial Cd concentration on biosorption of Cd by dried Aphanothece sp biomass: (a) at low range of initial cadmium concentration and (b) at high range of initial cadmium concentration

3.4. Effect of Temperature

Effect of temperature on the equilibrium Cadmiun sorption capacity of *Aphanothece sp* biomass has been investigated in two temperature range groups. Group I (with initial Cd concentration 2 mg/L) was conducted at 25, 30, 35, 40, 45 and 50 °C, whereas Group II (with initial Cd concentration 8 mg/L) was conducted at 27, 32, 37, 42, and 47 °C. Experiments on both groups were conducted at pH 8, 120 rpm, and 60 minutes contact time. A maximum biosorption capacity at 16.52 mg/g was observed at 30 °C in group I, while in group II was 56.23 mg/g at 37 °C. This suggests that cadmium biosorption by *Aphanothece sp* biomass is a chemical interaction process. Increasing temperature leads to decreasing Cd biosorption owing to damage of active binding sites on biomass surface and reducing biosorption capacity [24].

3.5. Biosorption Kinetics

The study on biosorption kinetics is important to find best model suitably represents the experimental data. There are several models provided to comprehend on biosorption behavior, evaluate the controlling mechanism, andassess experimental data. In this study, biosorption equilibrium data were analyzed using two simple kinetics models, pseudo-first-order (PSO-1) and pseudo-second-order (PSO-2) model [25].

The rate constants (k_1 and k_2), coefficients determination (R^2) and q_e values are given in Table 1.

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The correlation coefficients at various initial Cd for both models were determined and then compared with the obtained R^2. It can be observed in Table 1, the value of R^2 of PSO-2 are higher than PSO-1 except of initial concentration 83.2 mg/L. This shows that kinetics of Cd biosorption by *Aphanothece sp* dried biomass is better described by pseudo second order kinetic model. Also, $qe_{(cal)}$ using pseudo second order is closer to that obtained experimentally (qe_{exp}). Similar finding has been reported in case of removal Cd using dried biomass of *Spirulina platensis* [19]

Table 1. Kinetics rate constants for PSO-1 and PSO-2 models at various initial cadmium concentrations

Initial conc. (mg/L)	$qe_{(exp)}$(mg/g)	Pseudo First Order rate constants			Pseudo Second Order rate constants		
		k_1 (min^{-1})	$qe_{(cal)}$(mg/g)	R^2	k_2 (g/mg.min)	$qe_{(cal)}$mg/g)	R^2
Low range							
1.0	3.11	8.20E-03	3.64	0.949	1.18E-01	2.51	0.979
2.0	9.96	2.29E-02	9.45	0.969	5.40E-02	8.67	0.996
3.6	23.46	1.29E-02	23.57	0.949	1.95E-02	18.83	0.996
6.5	43.90	1.25E-02	42.84	0.952	7.88E-02	34.13	0.992
7.8	66.80	1.23E-02	61.93	0.907	3.41E-02	66.80	0.985

Initial conc. (mg/L)	$qe_{(exp)}$(mg/g)	Pseudo First Order rate constants			Pseudo Second Order rate constsnts		
		k_1 (min^{-1})	$qe_{(cal)}$(mg/g)	R^2	k_2 (g/mg.min)	$qe_{(cal)}$mg/g)	R^2
High range							
5.4	53.75	2.91E-01	42.84	0.962	4.95E-03	52.63	0.992
9.8	95.43	1.50E-02	88.2	0.863	5.38E-03	96.15	0.993
19.5	104.50	5.90E-02	129.32	0.945	6.36E-03	107.53	0.996
53.4	388.90	4.30E-02	320.86	0.937	7.18E-04	400.00	0.997
83.2	652.13	2.50E-02	625.34	0.994	3.66E-04	625.00	0.991

3.6. Analysis of Biosorption Isotherms

The capacity of biosorption can be described by equilibrium sorption isotherm, which is characterized by defined constants which express the surface properties and affinity of biosorbent. In this study, three important sorption isotherm models were selected to fit experimental data, namely Langmuir, Freundlich and Dubinin-Radushkevich (D-R) isotherm models. Table 2 present the results of those three isotherm model.

Table 2. Langmuir, Freundlich and Dubinin-Radushkevich isotherm constants for the biosorption of cadmium on dried biomass of Aphanothece sp.

Range of Initial Cd	Langmuir constants			Freundlich constants			Dubinin-Radushkevich constants		
conc. (mg/L)	q_{max} (mg/g)	K_L (L/mg)	R^2	$K_{F\ (mg/g(L/g)^{1/n})}$	n	R^2	q_{max} (mol/g)	E (kJ/mol)	R^2
1.03-3.63	17.27	4.52	0.996	32.69	1.19	0.904	0.183	8.45	0.975
6.48-53.38	119.05	3.23	0.998	137.39	0.75	0.719	0.651	8.45	0.988

Based on the R^2 values in Table 2, the Langmuir model and D-R model exhibited a better fit to the adsorption data of Cd biosorption compared to Freundlich isotherm model. The best fit of equilibrium data in Langmuir isotherm expression confirms monolayer adsorption of Cd onto dried biomass of *Aphanothece sp.* Maximum biosorption capacity of Cd have similar pattern with reported by other study [26] which using dried biomass of *Desmodium pleimorphus* to remove Cd ion in aquous solution. It reported that at initial Cd concentration 5 mg/L there found maximum capacity was 47.1 mg/g [26]. The Langmuir isotherm fits the experimental data very well might be due to homogenous distribution of active sites on the surface of this dried microalgae biomass, since the Langmuir equation assumes that the surface is homogenous. D-R isotherm model well fitted the equilibrium data since R^2 values was found to 0.975 and 0.988. Average biosorption energy (E) was calculated as 8.45 kJ/mol. This result indicated that

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the biosorption of Cd (II) onto dried biomass of *Aphanothece sp* may be carried out as chemisorption process involving valence force through sharing or exchange of electron between sorbent and sorbate.

3.7. Thermodynamic Parameter

Thermodynamic behavior of Cd (II) biosorption onto dried biomass of *Aphanothece sp* was described by using three thermodynamic parameter including of free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0). Estimation of thermodynamic parameter is shown in Figure 3.

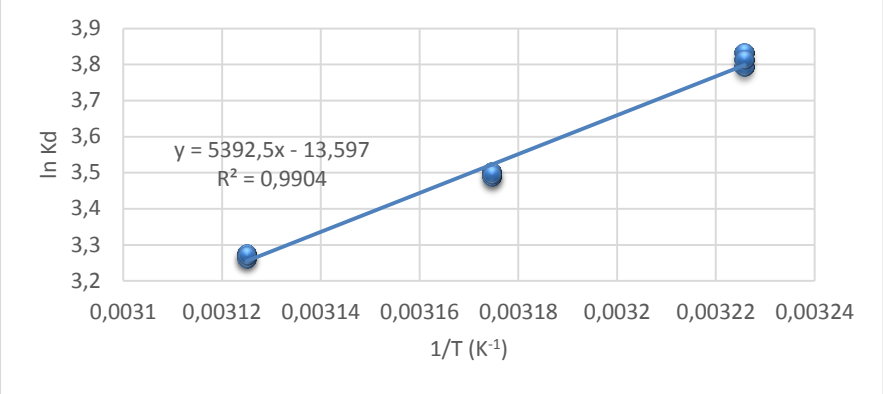

Figure 3. Plot of ln K_d against 1/T for the estimationof thermodynamic parameter for biosorption of Cd (II) onto dried biomass of Aphanothece sp.

The free energy change (ΔG^0) was calculated to be -9.82, -9.15, -8.70 kJ/mol for the biosorption of Cd (II) at 37, 42 and 47 °C, respectively. Negative values indicated thermodynamically feasible and spontaneous nature of biosorption. The decrease in ΔG^0 value with increasing temperature show a decrease in feasibility at higher temperature. The ΔH^0 was found to be -46.24 kJ/mol. The negative ΔH^0 is indicator of exothermic nature of biosorption and its magnitude can give information on the type of biosorption which can be either physical or chemical. In this study, biosorption proceeded in chemisorption process because falls into 20.9-418.4 kJ/mol. The ΔS^0 parameter found to be -141.90 J/mol K for Cd biosorption. The negative ΔS^0 value suggests a decrease in the randomness at a solid/solution interface during the biosorption process.

4. CONCLUSIONS

This study focused on the biosorption of Cd (II) ions onto dried biomass of *Aphanothece sp.* from aqueous solution in batch scale. The operating parameter such as pH of solution, biomass concentration, contact time, initial cadmium concentration, and temperature, gave strong influence on the biosorption capacity and efficiency of Cd (II) ions. Biosorption equilibrium was better described by Langmuir and Dubinin-Radushkevich isotherm compared to Freundlich isotherm. Monolayer biosorption capacity of *Aphanothece sp.* was found to be 17.27 mg/g dried biomass (initial Cd ions concentration 1.03-3.63 mg/L) and 119.05 mg/g dried biomass (initial Cd ions concentration 6.48-53.38 mg/L). From the D-R model, the average energy was determined as 8.45 kJ/mol, indicating that biosorption of Cd (II) ions onto dried biomass of *Aphanothece sp.* from aqueous solution was carried out as chemisorption process. Kinetic examination of the equilibrium data showed that the biosorption of Cd (II) ions onto dried biomass of *Aphanothece sp* suitably followed the pseudo-second order kinetic model. The thermodynamic calculations indicated the feasibility, exothermic and spontaneous nature of biosorption process at 37, 42 and 47 ^{0}C, respectively. Results of this study can be implemented in bioreactor design for large scale batch biosorption system treating Cadmium contaminated wastewater.

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Hybrid Constructed Wetland (HCW) For Domestic Wastewater Treatments on Highly Populated Settlements at Riverbanks

(Case Study: Kejawan Gebang Region of Gebang Putih Surabaya)

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Abstract

Kejawan Gebang, Region of Gebang Putih, Surabaya is one of the highly populated areas at the riverbanks of Surabaya with a headcount of 275 families that do not have waste water treatment plant. In addition, people of Kejawan Gebang dispose grey water directly into the body of Bokor River without any treatment. This will cause a decline in the quality of rivers, such as the occurrence of algal blooms.

Constructed Wetland (CW) is one of the most effective methods in the processing of domestic waste water. On the other hand, the land required is quite large, so the development of CW system is required. In this planning, design of CW is combined and called *Hybrid Constructed Wetland* (HCW), with the aim of effluent released meets quality standards with relatively small land area needed. Kejawan Gebang planning area is divided into three zones, namely Zone 1, Zone 2, and Zone 3.

Based on the calculation, the dimensions of HCW Zone 1 as follows; Stage 1: 35 m x 1.5 m, Stage 2: 35 m x 1.5 m, Stage 3: 40 m x 2 m, so that the total area of land required is 185 m^2. For HCW Zone 2 as follows; Stage 1: 30 m x 3 m, Stage 2: 30 m x 3 m, Stage 3: 35 m x 1.5 m, so that the total area of land required for 232.5 m^2. For Zone 3 as follows: 27 m x 13 m. The cost required to build HCW Zone 1 is Rp201.850.807, - HCW Zone 2 is Rp220.830.131, - SSFCW Zone 3 is Rp251.149.742,

Keywords: domestic waste water, Hybrid Constructed Wetland (HCW), riverbanks, Kejawan Gebang

1. INTRODUCTION

The growth of slums along the river is increasing. Data obtained from BPS said that the growth of slums in the area at the riverbanks in Indonesia reached 1.37% in a year [1]. Settlements at the riverbanks categorized slums due to drainage facilities which are inadequate and the lack of sewerage facilities in the form of feces or domestic waste water.

Kejawan Gebang, Region of Gebang Putih, Surabaya is one of the highly populated areas at the riverbanks with a headcount of 275 families that do not have waste water treatment plant. Meanwhile, according to the Department of Public Works in 2010 that required planning Communal WWTP is planned for areas with a density of 100

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people/ ha. In addition, people of Kejawan Gebang dispose grey water directly into the body of Bokor River without any treatment. Septic tank effluent is also discharged directly into sewers and drainage in front of the house.

Furthermore, the results of research conducted by Jakarta Public Works Department and JICA Team (2010) showed that the overall amount of waste water is estimated at around 1.3 million m^3/day, which more than 80% is coming from domestic waste water, wastewater offices and commercial areas [2]. While the rest of the wastewater generated by industrial waste. Pollution of water bodies by domestic waste water from grey water and septic tanks effluent will cause a decline in the quality of rivers, such as the occurrence of algal blooms due to eutrophication, the death of aquatic biota, until silting up the river.

Therefore, it is required to have an innovative technology that capable of treating domestic waste water by utilizing residential structure riverbanks which are highly populated and has limited area. In this planning, it will be planned how to design *a Hybrid Constructed Wetland* of domestic waste water, which are: grey water and septic tank effluent in the area of Kejawan Gebang, Region of Gebang Putih Surabaya. Planning is done by calculating the DED to the image of the unit, also will count the Bill of Quantity (BOQ) and how much the Budget Plan required to realize each unit in the *Hybrid Constructed Wetland.*

Constructed Wetland concept of a wastewater treatment is the most inexpensive and safe. However, the drawback of this method is in need of extensive land and potentially cause odor and become a den of wild animals. Therefore, required innovative technology that capable of treating domestic waste water by utilizing residential structure river banks which are highly populated and limited.

One type of wetland that is effective in treating wastewater is sub-surface Flow System. In Sub-surface flow (SSF) system, sewage treatment occurs when water flows slowly by the plants grown on porous media, such as gravel, gravel, and soil [3].

The processes occurring in wastewater treatment systems by utilizing water plant wetland system are:

a. Physical processes by sedimentation and filtration removal mechanism.
b. Physical and chemical processes with removal mechanism of adsorption and precipitation of phosphorus and heavy metals.
c. Biochemical processes with removal mechanism: the decline of organic matter, nitrification, denitrification, anaerobic decomposition, the absorption of water plants [4].

2. PLANNING METHODS

2.1. Development of Design Alternatives

In this plan will be designed two types of wetland. The first design is the planning of Subsurface Flow Constructed Wetland (SSFCW), the domestic waste water is collected in a collecting tub than will be supplied into the subsurface flow constructed wetland to be processed, and then the treated water will go into the control tub and then poured into the collector tub. This design is suitable applied to zone 3. The main reason for the application of the design is only suitable for zone 3 because the area of land available in zone 3 is sufficient to plan the constructed wetland, which generally require sizeable land.

The second design is the development of the first design and will be applied for Zone 1 and Zone 2 which has limited land. This design is a combination of a wetland with a two-way flow. Wastewater that has collected in the tub, will be pumped into the first stage of constructed wetland to be titrated using hiperacumulator plants. Then the treated water will be filtered back into the second stage, and so on until the treated water meets quality standards. In this second design, the use of hiperacumulator plants variations and variations in the use of media is very influential in determining the

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efficiency of processing, the number of stages, as well as the dimensions of the wetland itself.

2.2. Planning Activity Series

In the process of making the design of *Hybrid Constructed Wetland*, complete necessary data/information which are related to the design model are needed. Data collected included primary data and secondary data. Primary data includes the quality and quantity of domestic waste water in Kejawan Gebang. Determination of discharge of domestic waste water based on consumption of water per person per day. While the quality of domestic waste water derived from laboratory analysis of samples of domestic waste water of Kejawan Gebang.

Secondary data include domestic waste water quality and type of plants used. The quality standards used as a reference for the planned WWTP effluent is East Java Governor Regulation No. 72 of 2013 on Wastewater Quality Standard and/or Other Business Activities. For this type of crop or weed water used is the type of emergent, i.e. plants that have roots attached in the bottom waters with stems and leaves are above the water surface. In this planning, it is used the variation of the three plants, namely *Cyperus papyrus*, kana, and bamboo water. While BOQ and cost construction calculation on this plan is carried out by calculating the dimensions of domestic waste water treatment plant and Unit Price Cost of Workers (HSPK) in Surabaya in 2015.

3. RESULTS AND DISCUSSION

3.1. Planning Overview

In this planning, the service area for the wastewater treatment unit is divided into three zones, this is the area of land available and adapted to study existing SPAL.

Figure 1. Distribution Zone of Planning Region

Caption: Location of WWTP

Total House Each Zone

- Zone 1 = 103 Houses
- Zone 2 = 91 Houses
- Zone 3 = 81 Houses

Based on the results of the questionnaire, the majority of people Kejawan Gebang has served taps, especially in Zone 2 which has reached 100%, followed by 94% of Zone 1 and Zone 3 is 86%, while others still use wells. Taps for water usage itself varies, in general society Kejawan Gebang use tap water for washing and bathing, for bathing, washing, drinking and for bathing, washing, drinking, cooking. As for the ownership of a septic tank in Zone 2 93% of the people have had septic tanks, followed by 71% Zone 3 and Zone 1 is 56%.

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3.2. Determination of Water Quality and Quantity of Domestic Waste Water

The quality of domestic wastewater from the results of laboratory analysis are presented in Table 1. Here:

Table 1. Domestic Waste water Quality of Kejawan Gebang

No.	Parameter	Value (mg/L)
1	TSS	264
2	BOD	126
3	COD	202
4	total N	136
5	total P	12.4

Determination of discharge of domestic waste water based on consumption of water per person per day. The amount of water used comes from the account of a resident taps in Kejawan Gebang. Average water consumption in Kejawan Gebang is 150 L / people.day. Clean water used is estimated to be as much as 80% becomes domestic waste water [5]. Determination of the quantity of domestic waste water is based on the following formula:

Q_{ave} water = the need of clean water per person per day x number of population (1)

Q_{ave} wastewater = 80% x Q_{ave} water (2)

Q_{peak} wastewater sewage x = Q_{ave} x f_{peak} (3)

Based on equations 1, 2, and 3 Q_{peak} wastewater obtained as follows:

Zone 1 : 101 m3 / day

Zone 2 : 87 m3 / day

Zone 3 : 80 m3 / day.

3.3. Wastewater Treatment Plant

In this planning is used two systems of constructed wetland. Here's an explanation for each system.

1. *Horizontal Systems sub- Surface Flow Constructed Wetland:*

The system is planned to be implemented in three zones which has a land area available is quite large. The following wastewater treatment schemes in zone 3:

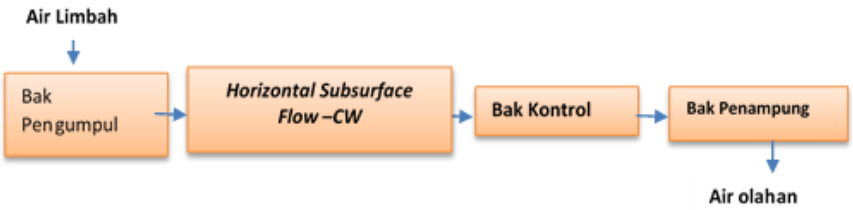

Figure 2. Schematic of Wastewater Treatment in Zone

2. *Hybrid Constructed Wetland* System (HCW):

Different Constructed Wetland can be combined to achieve higher removal efficiency by using the advantages of the individual systems or commonly called *Hybrid System* [6]. *Hybrid Constructed Wetland* (HCW) combines filtration between *Vertical Flow-Sub Surface Constructed Wetland* with *Horizontal-Sub Surface Flow Constructed Wetland*, in order to achieve wastewater effluent that meets the standard [7]. In addition, variations in media usage and combination of plants is also planned, so that the area of HCW can meet the land required in Zone 1 and Zone 2. Waste water treatment scheme in Zone 1 and Zone 2 is shown as follows:

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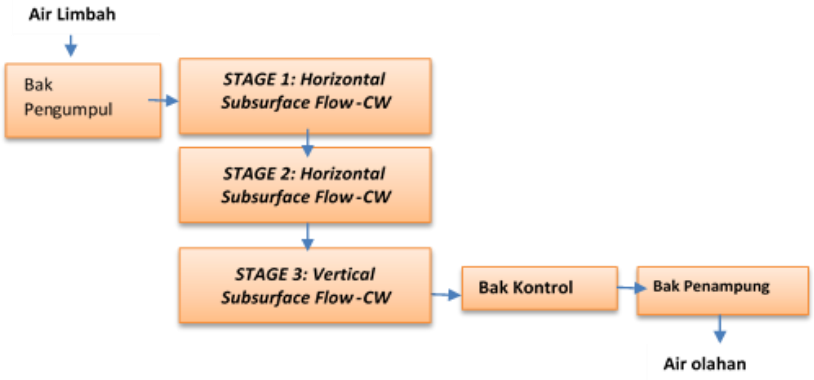

Figure 3. Scheme of Wastewater Treatment Zones 1 and 2

3.4. Dimension Calculation of Hybrid Constructed Wetland

Planning of HCW unit is equipped with one collector unit tub, sump, and tubs indicators for each zone. Bak collector serves to collect domestic waste water before entering the HCW unit. Bath tub serves as an indicator to test the quality of the effluent from HCW with biological indicators. While the tank serves to accommodate the domestic wastewater effluent.

Collector tub is planned 1.8 m in length; width 1 m; and a depth of 1.6 m typical for all zones. While the indicator tub and sump scheduled to have a length of 1 m; width 0.5 m; and a depth of 0.8 m typical for all zones.

The formula used in calculation of HCW are as follows [8]:

$KT = K20 \times (1.1)\ (T-20)$, temperature in ^{o}C (4)

$$t = \frac{\ln C / \ln Co}{kT} \quad (5)$$

$Ac = Q / (k_s . S)$ (6)

$W = Ac / d$ (7)

$L = (t' \times Q) / (W \ XDX\ \alpha)$ (8)

$As = L \times W$ (9)

$Lw = Q / As$ (10)

$LBOD5 = ((Qinf \times BOD\ inf)/As)$ (11)

Information:

Q	= Debit (m^3/day)
t'	= Time detention pore-space (day)
K_{20}	= Coefficient at a temperature of 20^0C (per day)
T	= Temperature (^{0}C)
Ks	= Hydraulic conductivity (m/day)
Air conditioning	= *Cross Sectional Area* (m^2)
W	= Basin width (m)
L	= Basin length (m)
US	= Basin surface area (m^2)
d	= Depth of media (m)
∝	= Porosity of media
C	= Effluent of BOD concentrations (mg/L)
Co	= Concentration of the influent BOD (mg/L)
S	= *Slope* media

Depth of media is tailored to the length of the roots of plants used are stage 1: *Cyperus papyrus*, stage 2: *Canna indica*, stage 3: water bamboo roots ± 0,6m in length as adults [9]. And tilt (slope) 0.01. Here are the details of planning for Zone 1 and Zone 2:

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Table 2. HCW Planning Zones 1 and 2

	PLANNED	ZONE 1	ZONE 2
stage 1	Plant	*Cyperus papyrus*	*Cyperus papyrus*
	Media	*Coarse sand*	*Gravelly sand*
	Ks	480 m^3/ m^2.hari	500 m^3/ m^2.hari
	∝	0.39	0.35
	Co	126 mg / L	126 mg / L
	C	85 mg / L	85 mg / L
	K20	1.34	0.86
stage 2	Plant	*Canna indica L.*	*Canna indica L.*
	Media	*Coarse sand*	*Coarse sand*
	Ks	480 m^3/m^2.hari	480 m^3/m^2.hari
	∝	0.39	0.39
	Co	85 mg / L	85 mg / L
	C	58 mg / L	41 mg / L
	K20	1.34	1.34
stage3	Plant	bamboo Water	bamboo Water
	Media	*medium sand*	*medium sand*
	Ks	420.5 m^3/m^2.hari	420.5 m^3/m^2.hari
	∝	0.42	0.42
	Co	58 mg/L	41 mg/L
	C	26 mg/L	21 mg/L
	K20	1.84	1.84

As for Zone 3, the medium used is gravel with Ks = 500 m3 / m2.hari, ∝ = 0.35, K_{20} = 0.86 [10]. Types of vegetation is Cyperus papyrus, with a depth of 0.6 m.

The effluent from HCW after passing through stage 3 for zones 1 and 2 and after passing through the unit SSFCW for Zone 3 must meet quality standards that are used, the concentration of BOD effluent <30 mg/L, the concentration of COD effluent <50 mg/L, the concentration of TSS effluent < 50 mg/L, the concentration of total N <30 mg/L by Permen LH No. 5 of 2014 (page 81), and the concentration of Total P <1 mg/L under PP 82 of 2001 (annex page 1).

Based on calculations using the equations 4-11, obtained dimensions, HLR, and BOD loading rate as follows:

Table 3. Dimensions of HCW ZONE 1

	STAGE 1	*STAGE 2*	*STAGE 3*
Length	35 m	35 m	40 m
Wide	1,5 m	1,5 m	2 m
Height (d + 0.2 m)	0.8 m	0.8 m	0.8 m
A_S	52.5 m^2	52.5 m^2	80 m^2
hydraulic loading rate (Lw)	193 cm/day	190 cm/day	124 cm / day
BOD5 loading rate	242.4 Kg/m^2.hari	162.34 Kg/m^2.hari	71.77 Kg/m^2.hari

The next step is checking hydraulic loading rate of the entire system (Lw) and the BOD5 loading rate of the entire system, and obtained Lw = 0.55 m^3/m^2/day = 55 cm/day (OK), and LBOD5 = 687.9 Kg/Ha.day (OK).

Allowance for suspended solids (SS) to the system can be calculated using the equation below [11]:

$$Ce = Co\ [0.1058 + 0.0011\ (HLR)] \quad (12)$$

Information:
Ce = effluent TSS, mg/L
Co = influent TSS

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And obtained the Ce to the HCW systems Zone 1 = 6.5 mg / L (meet quality standards).

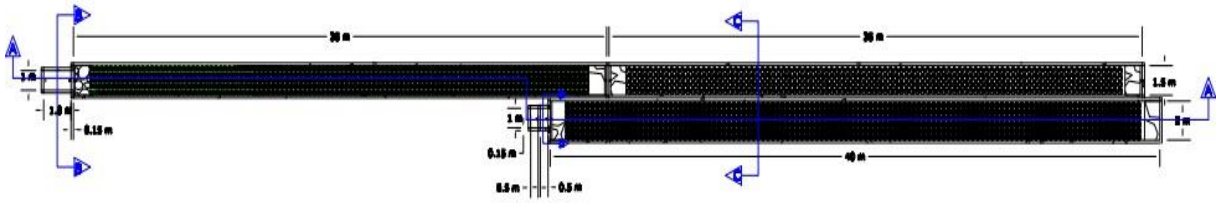

Figure 4. Layout of HCW Zone 1 without scale

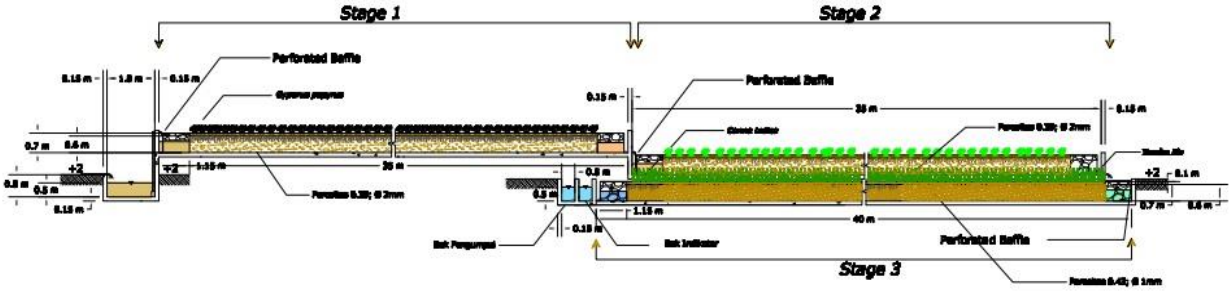

Figure 5. Pieces lengthwise of HCW Zone 1 without scale

Here are the results of the calculation for Zone 2:

Table 4. Dimensions HCW ZONE 2

	STAGE 1	*STAGE 2*	*STAGE 3*
Length	30 m	30 m	35 m
Wide	3 m	3 m	1,5 m
Height (d + 0.2 m)	0.8 m	0.8 m	0.8 m
A_S	90 m^2	90 m^2	52.5 m^2
hydraulic loading rate (Lw)	96 cm/day	94 cm/day	158 cm/day
BOD5 loading rate	121.8 Kg/m^2.hari	81.5 Kg/m^2.hari	66.4 Kg/m^2.hari

The next step is checking hydraulic loading rate of the entire system (Lw) and the BOD5 loading rate of the entire system, and obtained Lw = 0.37 m3 / m2 / day = 37 cm / day (OK), and LBOD5 = 460.6 Kg / Ha .day (OK).

Allowance for suspended solids (SS) for the system for one system obtained Ce HCW Zone 2 = 3.35 mg / L (meet quality standards).

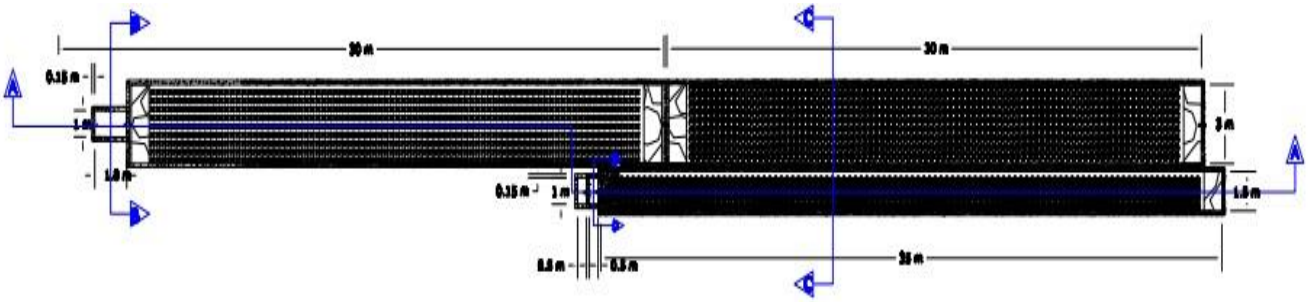

Figure 6. Layout of HCW Zone 2 without scale

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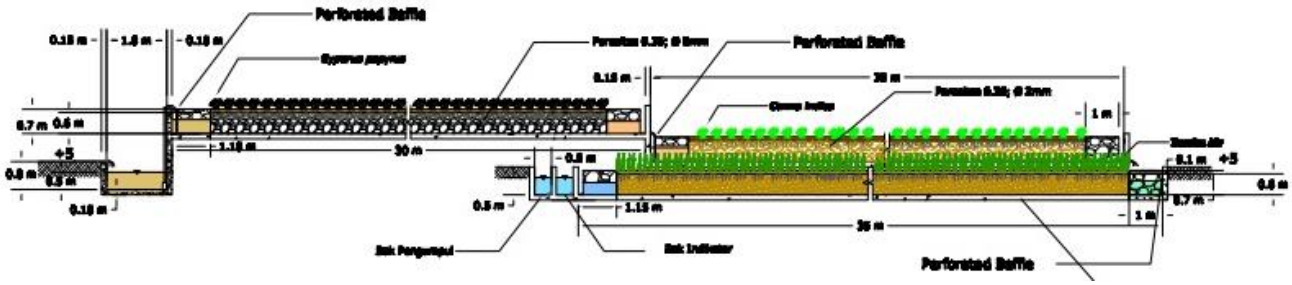

Figure 7. Pieces elongated of HCW Zone 2 without scale

For Zone 3 HCW obtained the following dimensions:

Table 5. Dimensions SSFCW ZONE 3

Length	27 m
Wide	13 m
Height (d + 0.2 m)	0.8 m
US	351 m^2
hydraulic loading rate (Lw)	23 cm/day (OK)
BOD5 loading rate	291 Kg/m^2.hari (OK)

Allowance for suspended solids (SS) for the system for one system obtained Ce SSFCW Zone 3 = 35 mg / L (meet quality standards).

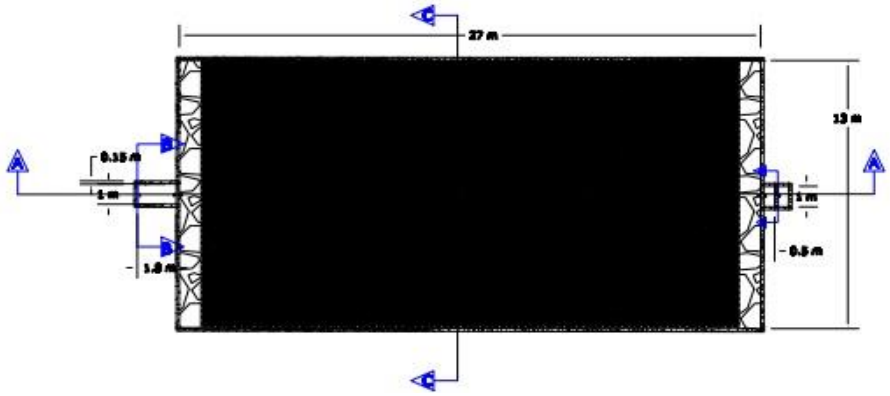

Figure 8. Layout of SSFCW Zone 3 without scale

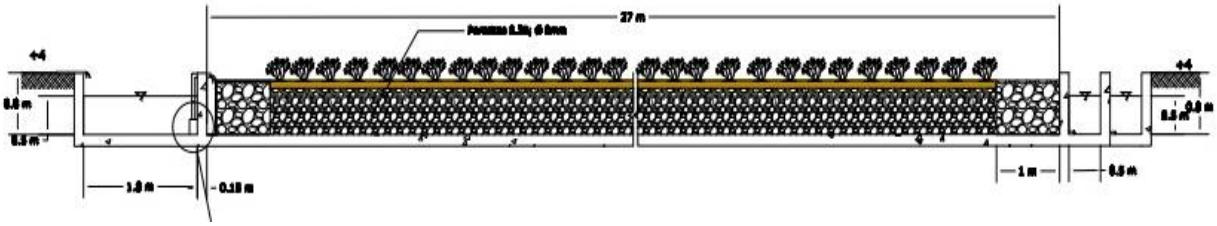

Figure 9. Pieces elongated of SSFCW Zone 3 without scale

The calculation of each parameter based on the percent removal or the removal efficiency of each unit wetland that has been calculated. For Zone 1 are as follows:

Stage 1 = 33%, Stage 2 = 32%, and Stage 3 = 55%

As for Zone 2 is as follows:

Stage 1 = 33%, Stage 2 = 52%, and Stage 3 = 49%

For Zone 3% removal SSFCW unit is 84%.

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3.5. Water Balance

Debit entry (Q_{in}) into the CW will not be the same as the discharge exit (Q_{ef}) on CW. CW effluent discharge is affected by the rate of evapotranspiration (ET), precipitation (P), and infiltration (I) [13].

$$Q_{out} = Q_{in} - ET + EP + P + I \quad (12)$$

Where,

Q_{out} = Debit output of wetland (m^3/day)
Q_{in} = Debit entry (m^3/day)
ET = evapotranspiration of plant (m^3/day)
P = Precipitation (m^3/day)
I = Infiltration (m^3/day)

Constructed Wetland is generally coated with clay, synthetic liner, or made of concrete that are to minimize infiltration, therefore infiltration ignored and considered zero [13].

The value of *Cyperus papyrus* evapotranspiration is 41.9 mm/day [14], the value of *Canna indica* evapotranspiration is equal to 28.55 mm/day [15], and the value of Bamboo Water evapotranspiration is at 19 mm/day [16] , As for the average precipitation is taken from an average rainfall of Surabaya, when the rainy months at the monitoring station in Keputih, Region of East Surabaya is 14.5 mm/day [17].

Here are the results of calculations Water Balance of each stage in each zone:

Table 6. Water Balance of Zone 1

	stage 1	*stage 2*	*stage 3*
ET	2.2 m^3/day	1.5 m^3/day	1.5 m^3/day
P	0.76 m^3/day	0.76 m^3/day	1.16 m^3/day
Q_{in}	101 m^3/day	99.77 m^3/day	99 m^3/day
Q_{out}	99.77 m^3/day	99 m^3/day	98.7 m^3/day

Table 7. Water Balance of Zone 2

	stage 1	*stage 2*	*stage 3*
ET	3.8 m^3/day	2.6 m^3/day	1 m^3/day
P	1.3 m^3/day	1.3 m^3/day	0.76 m^3/day
Q_{in}	87 m^3/day	84.2 m^3/day	83 m^3/day
Q_{out}	84.2 m^3/day	83 m^3/day	82.7 m^3/day

Table 8. Water Balance of Zone 3

ET	15 m^3/day
P	5 m^3/day
Q_{in}	81 m^3/day
Q_{out}	71 m^3/day

3.6. Calculation of Bill of Quantity (BOQ) and Cost Construction

WWTP units planned for zones 1 and 2 cover 1 piece unit tub collectors, 1 unit HCW which consists of three stages CW, 1 tubunit indicator, and 1 sump. As for the WWTP Zone 3 includes 1 piece unit tub collectors, 1 unit SSFCW, 1 tub unit indicator, and 1 sump. And obtained of cost construction Zone 1 is IDR 201,850,807; Total Cost Construction of Zone 2 is IDR 220,830,131; Total Cost Construction of Zone 3 is IDR 251,149,742.

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4. CONCLUSION

From this planning, it can be deduced as follows:

1. Based on the calculation, the dimensions of the WWTP units planned are:

   -Collecting Tub unit typical for each zone with a size of 1.8 m; width 1 m; and a depth of 1.6 m
   - WWTP in forms of HCW consisting of three stages, for HCW Zone 1 is as follows; Stage 1: 35 m x 1.5 m, Stage 2: 35 m x 1.5 m, Stage 3: 40 m x 2 m, so that the total area of land required by 185 m^2. For HCW Zone 2 is as follows; Stage 1: 30 mx 3 m, Stage 2: 30 m x 3 m, Stage 3: 35 m x 1.5 m, so that the total area of land required for 232.5 m^2. For SSFCW Zone 3 are: 27 m x 13 m.
   - Indicator Tub and sump typical unit for all zones, the dimensions are 1 m; width 0.5 m; and a depth of 0.8 m

2. The efficiency of removal of each wetland unit for Zone 1 are as follows: Stage 1 = 33% = 32% Stage 2 and Stage 3 = 55%. As for Zone 2 are as follows: Stage 1 = 33% = 52% Stage 2 and Stage 3 = 49%. For Zone 3% removal SSFCW unit is 84%. Effluent from Constructed Wetland has met the quality standard of domestic waste water in the East Java Governor Regulation No. 7 In 2013, Permen LH No. 5 of 2014 (page 81), and PP 82 of 2001 (annex page 1).

3. The cost required to build HCW Zone 1 is IDR 201.850.807, - HCW Zone 2 is IDR 220.830.131, - SSFCW Zone 3 is IDR 251.149.742, -

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Effect of different hydrolysis time on the properties of freeze dried protein hydrolysate from Yellowstripe Scad (*Selaroides leptolepis*)

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Abstract

Yellowstripe scad belongs to the small pelagic group that is abundantly found in South China Sea and is categorised as low value fishes. The objective of this paper is to determine the effect of different hydrolysis time on the properties of freeze dried protein hydrolysate from Yellowstripe Scad (*Selaroides leptolepis*). Protein hydrolysate was treated using potassium buffer followed by 0.50% of Alcalase at a series of hydrolysis time (1h, 2h, 3h and 4h). The properties of freeze dried protein hydrolysate were evaluated for yield, protein content, degree of hydrolysis, water activity and colour. Results showed that prolonged hydrolysis give increasing yield (14-15%) and protein content (19-30%) among the four hydrolysis time. Degree of hydrolysis of these samples increased from 1h to 2h (60% to 69%) but decreased for the subsequent hours (from 69% to 50%). However, water activity was higher in the second and third hour, 0.259 and 0.258, respectively.

Keywords: fish protein hydrolysate, Alcalase enzyme, yield, protein, degree of hydrolysis

1. INTRODUCTION

Yellowstripe scad belongs to the small pelagic group which is categorised as low value fishes, is one of the plentiful marine source in Vietnam sea area (Bui Tran Nu Thanh Viet, 2014). Yellowstripe scad is a small species fish with a maximum length of 22cm but more commonly less than 15cm (Froese et al., 2012). In Malaysia, this species is considered underutilised, yellowtail scad has been used in fish burger, with acceptable favour (Yu and Siah, 1998).

In order to increase the value and utilization of low value proteinacious fish, processes such as protein hydrolysis via enzymatic hydrolysis is used to produce a more marketable and functional protein hydrolysate (Aspmo et al., 2005). Hydrolysis affects hydrophobicity, polar groups and molecular weight (size) which directly influence the functional properties of hydrolysate as food ingredient (Kristinsson & Rasco, 2000) a. Fish protein hydrolysate produced by controlled enzymatic hydrolysis, is considered to be the best fish protein hydrolysate due to its nutritional properties of well balanced amino acids composition and these hydrolysate is highly digestible by consumers (Kristinsson and Rasco, 2000) b.

Alcalase is a commercially obtainable enzyme which is widely used in protein hydrolysis because of its thermostability (50°C) and high optimal pH (pH8.5) where it can minimise growth of microorganisms along hydrolysis process (Salwanee, 2013). Extraction buffer or solvent plays an important role as the proper concentration, ionic

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strength and pH is required to extract different types of protein such as water soluble or fat-soluble protein (Nison, 2012). Some buffer include mild detergent or any appropriate dissociating agent to increase the efficiency of extraction (Nison, 2012). Hydolysis time is one of the parameters that affect the enzymatic activity on the hydrolysis (Salwaee et al., 2013).

This paper aimed to determine the effect of different hydrolysis time on the properties of freeze dried protein hydrolysate from Yellowstripe Scad (*Selaroides leptolepis*)

2. MATERIALS AND METHOD

2.1 Protein extraction

Edible portion of fish was obtained by removing the head, viscera, tails and fins. The fish was grinded using a blender. Fifty grams of fish meat was deactivated by immersing into water bath at 90°C for 10 min. It was centrifuged at 3500 rpm for 20 minutes for oil separation. Then it was mixed with 100ml of potassium buffer and adjusted to pH 8 using 2.0 M sodium hydroxide. 0.5 % of enzyme concentration was added. The hydrolysis was conducted for 1 h, 2h, 3h and 4h. The solutions were centrifuged at 10000 rpm for 20 min and filtered. The liquid hydrolysate were then dried using freeze dryer.

2.2 Drying methods

The samples were freeze dried using Labconco Freeze Dryer with Stoppering Tray. It was operated at -54°C with vacuum condition of 0.250mbar. The samples were pre-frozen prior to freeze drying at -80°C.

2.3 Yield of powdered hydrolysate

The yield of powdered protein hydrolysate was obtained by weighing the powder collected after spray drying or freeze drying. The yield was recorded as (b). The percentage of yield from liquid hydrolysate was calculated as shown in Equation 1.

$$\text{Yield of powder hydrolysate} = \frac{\text{powder collected after drying (b)}}{\text{liquid protein hydrolysate (a)}} \times 100\%$$

[Equation 1]

2.4 Protein content of liquid protein hydrolysate

Protein content was measured using Kjeldahl method to determine the ammonium compound present in the solution (AOAC, 2000). Briefly, one gram of sample (whole fish and edible portion) was weighed and placed into the digestion tube of the instrument, while powdered protein hydrolysate used was only approximately 0.5 g. Two tablets of Kjeltabs catalyst, Cu 3.5 and 12 ml of the concentrated sulphuric acid was added consecutively. After that, the tubes were connected to the digester (2006 Digester, FOSS, Sweden, 1998). This process of digestion was continued until green or light blue solution was formed. Then distillation was continued using distillation unit (2100 Kjeltec Distillation Unit, FOSS, Sweden, 2002). The values from the titration was calculated using Equation 2 and Equation 3 given below.

$$\text{Percentage of nitrogen (\%)} = \frac{(\text{T}-\text{B})\times\text{N}\times 14.007\times 100}{\text{weight of the sample (mg)}} \quad \text{[Equation 2]}$$

$$\text{Percentage of protein (\%)} = \text{percentage of nitrogen} \times \text{F} \quad \text{[Equation 3]}$$

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Where,
T = Titration volume for the sample (ml)
B = Titration volume for the control (ml)
N = Concentration of hydrochloric acid (HCl)
F = Protein factor (6.25)

2.5 Degree of hydrolysis (DH)

Degree of hydrolysis was estimated according to Hoyle and Merritt (1994). The powdered protein hydrolysate was weighed approximately 0.5g in a centrifuge tube, dissolved using 10ml of buffer solution and 5ml of 10% Trichloroacetic acid (TCA) was added to the sample solution. The solution was held in room temperature for 30 minutes. Then, the samples were centrifuged at 4000rpm for 15 min. The supernatant was filtered directly into the digestive tube using a cellulose filter paper and the following processing steps were similar to protein determination.

Two tablets of Kjeltabs catalyst, Cu 3.5, were added. Then, 12 ml of the concentrated sulphuric acid was added into the tube and shook slowly to wet the sample. After that, the tubes were connected to the digester (2006 Digester, FOSS, Sweden, 1998). The system was stopped until the acid vapour appeared only at the top of the exhaust system. This process of digestion was continued until green or light blue solution was formed, then cooled vertically for 10 to 20 min. Then, 75 ml of distilled water was carefully added into the cold tube and the distillation process was continued. Receiving solution was prepared using 25 ml of 4% Boric acid and 10 drops of Green Bromocresol indicator added into a 250 ml conical flask. The receiving solution was placed into the distillation unit (2100 Kjeltec Distillation Unit, FOSS, Sweden, 2002). 50 ml of 40% NaOH was flowed into the tube and the distillation process was operated for 4 minutes until a light green solution was formed. This solution then titrated with 0.1 N of standard HCl until the colour turns blue or grey and the volume was recorded. The values from the titration are calculated using Equation 3.4 and Equation 3.5.

$$\text{Percentage of nitrogen } (\%) = \frac{(T-B)\times N\times 14.007\times 100}{\text{weight of the sample (mg)}} \quad \text{[Equation 3.4]}$$

$$\text{Percentage of DH } (\%) = \frac{\text{Percentage of nitrogen}}{\text{Total percentage of nitrogen}} \text{ X } 100 \quad \text{[Equation 3.5]}$$

2.6 Colour

The colour of powdered hydrolysate was analysed by using Colorimeter (Chromameter, Konica Minolta) based on the L* a* b* colour system in terms of L (lightness), a (redness and greenness) and b (yellowness and blueness). The dried protein hydrolysate in powder form was evaluated with samples kept in a sealed, clear HDPE plastic bag. Each sample was analysed for three times and the method used was a modification of the original method obtained from Loughrey (2002).

3. RESULTS AND DISCUSSION

3.1 Yield

Yield of powdered hydrolysate showed the efficiency of hydrolysis time against the production of yield. Figure 1 shows that the yield obtained increased from 1h of hydrolysis to 4h of hydrolysis. The result showed that the protein yield increased rapidly from first hour to the second hour but slowly increase from second to forth hour of hydrolysis. The decrease in rate could be decrease in substrate concentration. Liaset et al. (2000) reported that the reaction mechanism of the enzymatic hydrolysis of cod followed first order kinetic processes , at which first process involved initial fast reaction where loosely bound polypeptide chain were cleaved to form insoluble protein particles and in second process, the compact protein were digested. Also reported that, the slow

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reaction at the end of reaction might be due to decrease in enzyme activity, substrate saturation or product inhibition. Gbogouri et al. (2004) and Guerard et al. (2002) observed the same phenomenon using Alcalase enzymein extracting salmon byproduct and tuna waste, respectively.

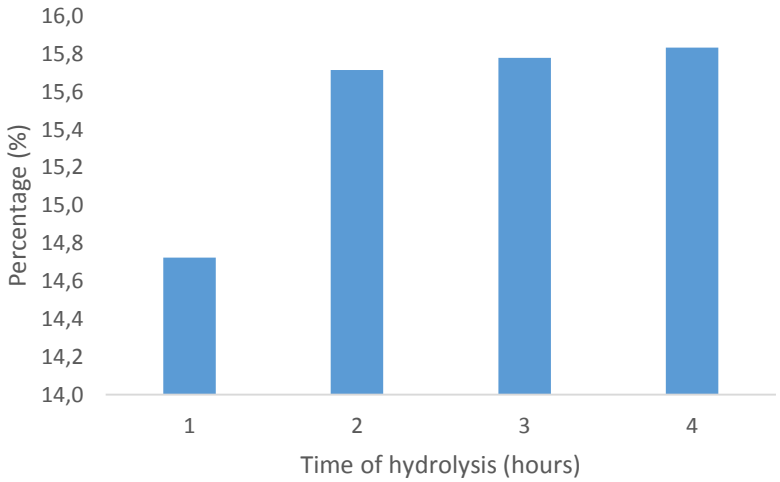

Figure 1: Percentage yield obtained from hydrolysis

3.2 Degree of hydrolysis (DH)

Degree of hydrolysis (DH) plays a vital role in determining important properties of a protein hydrolysate. Figure 2 showed that DH increased from 1 h to 2 h of hydrolysis but decreases in third and forth hour. However, previous studies by Norma et al. (2005) and Guerard et al. (2002) reported that DH increased as incubation time and enzyme-substrate ratio increased on threadfin bream and yellowfin tuna, respectively. The decrease in third and forth hour of hydrolysis might be due to reduction of enzyme activities due to exhaustion the enzyme as substrate as time prolonged. Claver and Huiming (2005) also reported that the decrease in DH could be due to denaturation of protein molecules, subsequently reduces its biological activities. Degree of hydrolysis is also dependent on the availability of susceptible peptide bonds on which primary attack is based and the physical structure of the protein molecule (Kanu et al, 2009).

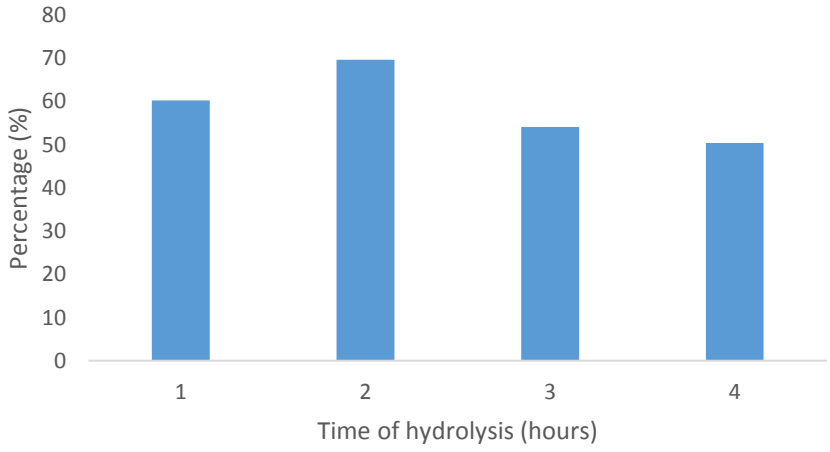

Figure 2. The degree of hydrolysis versus time

3.3 Protein content

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Protein content obtained was mostly referring to the nitrogen compound found in the sample. The nitrogen content reflects the yield of protein that can be recovered from the hydrolysis process. The protein content of the hydrolysates was found to have an increasing trend from 1h to 4 h of hydrolysis as shown in Figure 3. In previous study, the highest protein recovery found at 4 hour of hydrolysis was superior to those reported by Gbogouri et al. (2004), Ovissipour et al. (2009) and Shahidi et al. (1995).

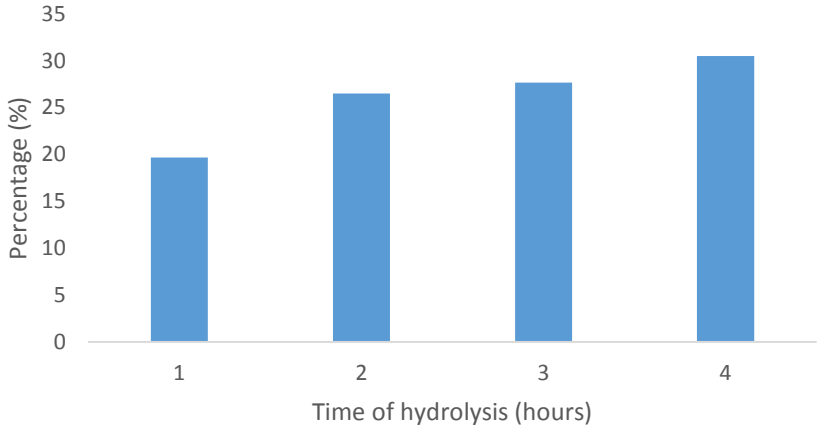

Figure 3. Protein content that can be recovered from hydrolysis

3.4 Colour

The colour of powdered hydrolysate was determined using colorimeter where the value L* indicates the lightness, a* indicates the redness and greenness and b* indicates the yellowness and blueness. Figure 4 elucidates the value for L*, a* and b* which has no drastic increment or reduction. Dark coloured contributed by slight negative a* value might be contributed by oxidation process. The darkening resulted from oxidation of myoglobin and melanin pigment of the raw material (Benjakul & Morrisey 1997). Besides, the process of lipid oxidation upon reaction with basic groups in proteins via Maillard reaction, also produces brown pigments from aldol condensation of carbonyls (Van Boekel, 1998).

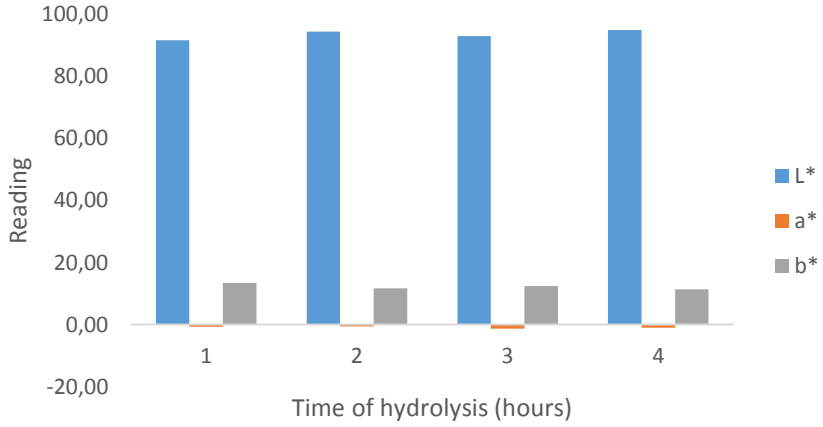

Figure 4. Colour of powdered protein hydrolysate

4. CONCLUSION

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The study clearly shows that 2 h of hydrolysis is more preferable since it has the highest degree of hydrolysis (69%) which gives better functional properties of the protein hydrolysate for further analysis. The yield, protein recovery and colour were among the results with no notable increment or reduction.

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Hydrophytes Bioaccumulation of Heavy Metals in Brantas Kedurus Surabaya, East Java

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Abstract

The aim of this study is for observing the ability of hydrophytes and vegetables toward heavy metals in Kedurus Surabaya, East Java. We use descriptive explorative design with six times replication in the three stations. The result of this study, we found that root, stem, and leaves of eceng gondok (*Eichhornia crassipes*) could accumulate 0.001 g/l of Pb, and Cr, and 0.02 g/l of Cu. Moreover, Zn could be accumulated in root, stem, and leave as much as 0.08 g/l, 0.14g/l, and 0.08 g/l. In addition, we also found that kangkung air (*Ipomoea aquatica*) could accumulate 0.03 g/l, 1.05 g/l, and 0,23 g/l of Cu in root, stem, and leaves, 0.02 g/l of Zn in root, stem, and leaves, and 0.57 g/l, 0.43 g/l, 0.60 g/l of Cd in root, stem and leaves.

Keywords: heavy metals, bioaccumulation, water hyacinth, water spinach

1. INTRODUCTION

Currently, many researchers try to resolve the wastewater accumulating heavy metals with easy methods, cheap, environmentally friendly, effective and efficient [13,14]. The requirement of technology for handling this problem becomes more critical because the increment of industries and the increment of attention from both government and citizen towards the environment [6,2]

Heavy metals ion are entrapped in the cellular structure, and subsequently biosorbed onto the binding site present in the cellular structure. This method of uptake is "biosorption" or "passive uptake". Additionally, heavy metals can also pass into cell, across the cell membrane through the cell metabolic cycle, this mode of metal uptake is referred to as "active uptake". The metal uptake by both active and passive odes can be termed "bioaccumulation" [7].). Hydrophytes can be used for "cleaning" the heavy metals in environment. This can be one of the alternative for managing wastewater with safety, cheap, and easy. [1].

Hydrophyte such as eceng gondok (*Eichhornia crassipes*) has been proven that it could accumulate heavy metals in big amount [3,8]. A previous study has shown that eceng gondok could clean up the plumb from wastewater of paper industry. It concluded that eceng gondok was very efficient for absorbing heavy metals from wastewater [5]. In addition, some previous studies also have shown that eceng gondok and some vegetable plants growing in aquatic habitat cold clean up the domestic wastewater and the waste from tannery industry [9,10]

According to a previous study, Kalimas, the site of Brantas river contented number of heavy metals like Cd, Hg, Pb, Mn, and Zn [11]. However, the concentration of these heavy metals in kangkung (*Ipomea aquatica*) growing in Kalimas was higher than in the water of Kalimas [15,16] reported that the concentration of Hg, Pb, and Mn in Kalimas exceeded the threshold of water drink standard. [17]. Moreover, kangkung growing in Kalimas contented high concentration of Cd, Hg, Pb, Cu, Mn, and Zn, which Hg was the highest concentration. In addition, a previous study has shown that Bader fish (*Barbonymus gonionotus*) living in Kalimas contented high concentration of Hg, Pb, and

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Mn, which these contents exceed the threshold for human consumption assigned by FAO [4].

2. METHODS

The methods of this study used descriptive observation and experimental. The experiment for checking the heavy metals content by UV-vis was conducted in State University of Malang. The results of observation was analyzed with one way ANOVA and BNT test. The result of experiment was analyzed with ANOVA two factors and Tukey test.

3. RESULTS AND DISCUSSIONS

Table 1. Abiotic parameter in Kedurus

Abiotic parameters	First Station	Second Station	Third Station
DO (ppm)	1.42	4.95	7.45
Turbid (NTU)	68	39	41.5
pH	7.9	8	8.6
Conductivity (µS/cm)	270.5	403	395.5
Salinity	0.1	0.2	0.2
SR	18.8	45.8	90.2
O_2	23.82	7,9	18.9
Temperature (°C)	30	27	26.7
Lux	400	230	400

According to the Table 1 above, it shows that DO in station 1, 2 and 3 was 1.42, 4.95 and 7.45. It indicated that oxygen in water still unfulfilled daily needed except station 3, the DO standard for human needed is about 6-10 ppm. The turbidity in each station was 68 mg/l, 39 mg/l, and 41.5 mg/l. These value was in standard range for turbidity 10-50 mg/l, it meant that it could be consumed. The pH in each station was 7.9, 8, and 8.6, these value also meant that it saved to consume for human. However, the value of conductivity in each station was270.5, 403 and 395.5, it exceed from the standard. We assumed that the water contained heavy metals. The salinity of each station was 0.1, 0.2, and 0,2, these value was in low range. The SR for each station 18.8, 45.8 and 90.2, these value exceed from the standard. The temperature in each station was 30^{o} C, 27^{o}C and 26.7^{o}C. These value was in normal range, even though when we measured it the sun shined brightly and the light was about 500 lux.

Table 2. The contents of heavy metals in water hyacinth and water spinach in Kedurus

Heavy Metal	Aquatic Plant	Roots (ppm)	Stems (ppm)	Leaves (ppm)
Plumbum	Eceng gondok	0.001	0.001	0.001
	Kangkung air	0.02	0.02	0.01

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Heavy Metal	Aquatic Plant	Roots (ppm)	Stems (ppm)	Leaves (ppm)
Chrom	Eceng gondok	0.003	0.021	0.005
	Kangkung air	0.05	0.01	0.01
Cadmium	Eceng gondok	0.01	0.01	0.25
	Kangkung air	0.57	0.43	0.60
Cuprum	Eceng gondok	0.02	0.03	0.02
	Kangkung air	0.03	1.05	0.23
Zinc	Eceng gondok	0.08	0.14	0.05
	Kangkung air	0.20	0.20	0.62

According to the Table 2 and Figure 1, it showed that the roots of eceng gondok accumulated plumbum about 0.001 ppm and kangkung air about 0.02 ppm. Nevertheless, the plant variable of P value was up to 0.05, it was 0.35. It indicate that the accumulation was not significant between other plants. The content of plumbum in plant in Kedurus was not real difference, it meant that H_o was rejected and H_1 was accepted.

Moreover, it showed that roots, stems, and leaves of eceng gondok accumulated chromium about 0.003 ppm, 0.021 ppm, and 0.005 ppm. However, based on statistic analyze, it was not significant because the F test of plant variable was 0.003 and P value lower than 0.05, it was 0.003. It meant that H_0 was accepted and H_1 was rejected. The cadmium accumulation by eceng gondok and kangkung air were 0.01 ppm and 0.57 ppm. Based on statistic analyze, it showed that it was real difference because P value was 0.004. The content of cadmium accumulation was up to the average of allowed standard. As the result the kangkung air growing in kedurus was not allowed to consume.

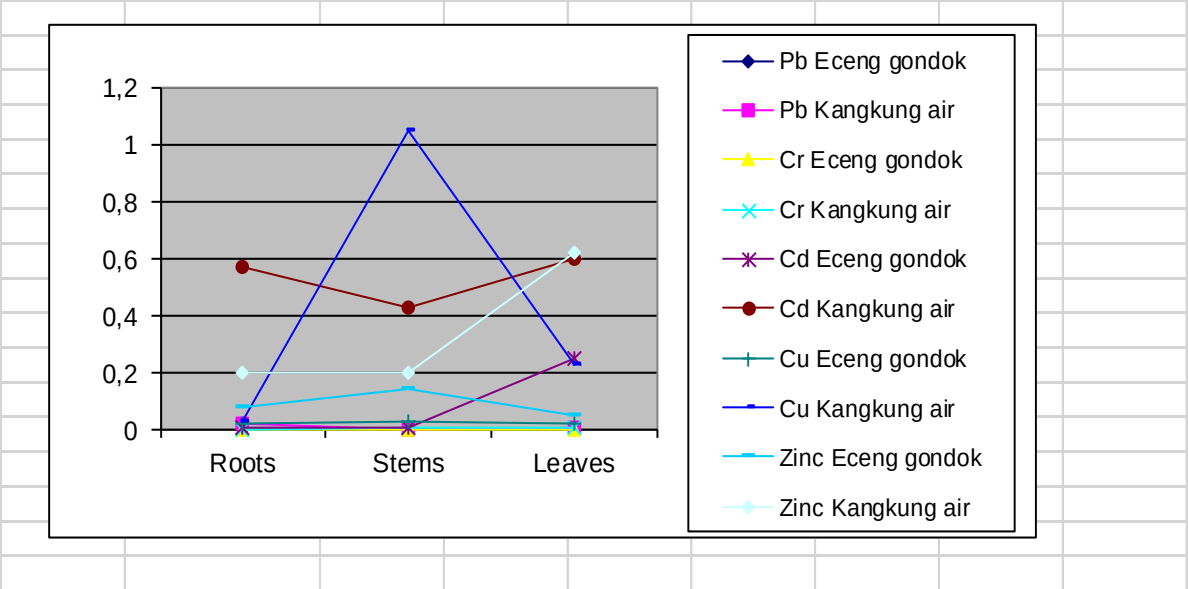

Figure 1. The result of heavy metals analyze in water hyacinth and water spinach organs

According to the result, cuprum was accumulated 0.02 ppm and 0.03 ppm by eceng gondok and kangkung air. It showed real difference between one plant with another plant. This result indicated that eceng gondok was good bioaccumulator of cuprum, it caused the p value was 0.01. Zinc was accumulated 0.08 ppm by eceng gondok and 0.20 ppm by kangkung air. Based on statistic analyze, it showed real difference because the p value was 0.03. It indicated that H_0 was accepted and H_1 was rejected.

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The analyze result of various heavy metals accumulation in eceng gondok and kangkung air in Kedurus has shown in Figure 2 and Table 2. Eceng gondok was accumulated plumbum 0.001 ppm and kangkung air 0.02 ppm. However the plant variable of P value was 0.35, it meant that this accumulation was not significant between a plant with another plant. The accumulation of Plumbum for plants in Kedurus were not real difference, this meant Ho was rejected and H_1 was accepted.

According in Figure 2 and Table 2, it shows that eceng gondok and kangkung air were accumulated cuprum 0.02 ppm and 0.61 ppm. From the statistic result, it was showed that it was real difference between one plant with another plant. It shown that eceng gondok was good bioaccumulator for cuprum. It was because of the the plant variable of P value was 0.001. Zinc was accumulated by eceng gondok and kangkung air about 0.093 ppm and 0.337 ppm. Based on the statistic analyze it shown real difference because the p value 0.03, it meant that H_0 was accepted and H1 was rejected.

Table 3. The accumulation of various heavy metals in three organs of water hyacinth and water spinach in Kedurus

Organ	Plumbum	Chromium	Cadmium	Cuprum	Zinc
Roots	0,006	0,005	0,29	0,02	0,14
Stems	0,006	0,06	0,22	0,35	0,17
Leaves	0,005	0,02	0,37	0,22	0,34

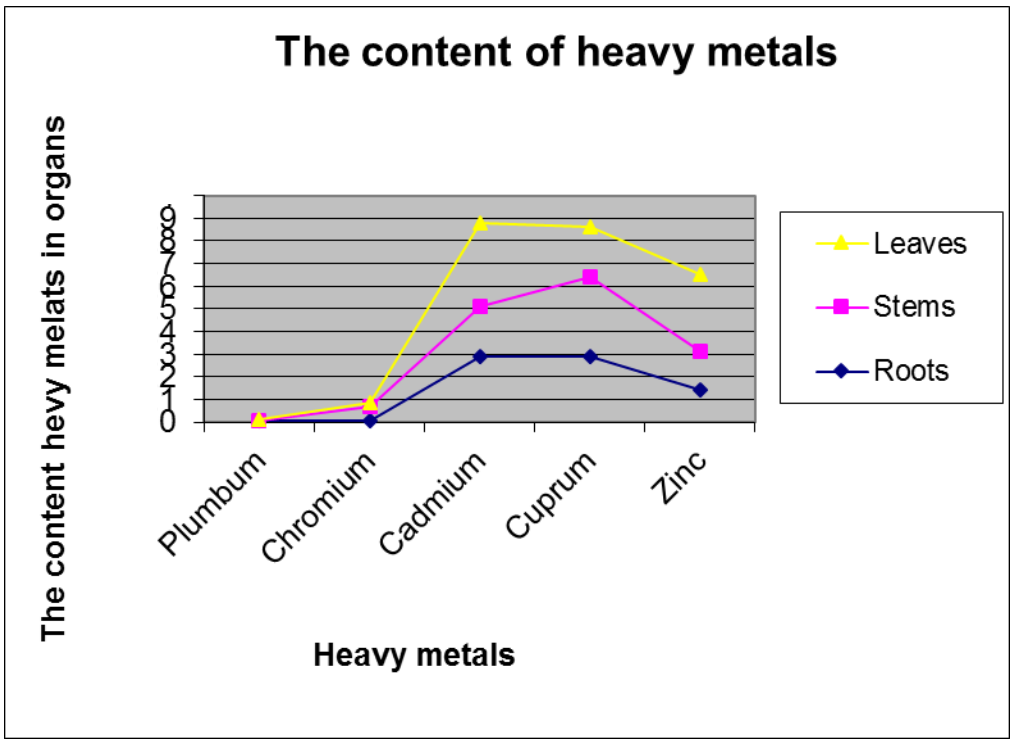

Figure 2. The result of heavy metals analyze in water hyacinth and water spinach organs

According to the analysis result Table 3. It shown that roots and stems and leaves accumulated plumbum. However, the plant variable of P value was above 0.05 that it was 0.34, it meant that this accumulataion was not significant between an organ with another

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organ. The result of the accumulation plumbum in organs in Kedurus was not real difference. It meant that H_o was rejected and H_1 was accepted.

Moreover, from the analysis result Table 3, it shown that the chromium accumulation in stems, leaves and roots was 0.06 ppm, 0.02 ppm and 0.05 ppm. However according to statistic analyze, it was not significant because F test for organs variable was 0.003 and P value < 0.05 that it was 0.003. The accumulation of cadmium in roots, stems, and leaves was 0.29 ppm, 0.22 ppm and 0.37 ppm. According to the statistics analysis it was difference, it was because the organ variable of P was 0.004. This accumulation was not allowed by the government rule. It meant that the vegetable in Kedurus was contaminated.

The cuprum accumulation in stems, leaves, and roots based on Table 3 was 0.02, 0.037, and 0.29. According to statistics analysis there was real difference. It shown that stems was good cuprum accumulator, because organ variable of P value was 0.01, it meant that H_0 was accepted and H_1 was rejected. The leaves could accumulate zinc very well, it could accumulate zinc about 0.034. In addition, stems and roots only could accumulate zinc about 0.15 ppm and 0.14 ppm. Based on statistics analysis it was not difference because P value was 0.09.

Figure 3. Kedurus dam was occurring eutriphication.

4. CONCLUSSION

The organ ability to accumulate heavy metals in Kedurus was not real difference. It meant that H_0 was accepted and H_1 was rejected. The accumulation of Zn was decreased, it was because the agriculture plant was also decreased. Nevertheless, it was changed into industrial area as the result the content of Cd was increased than other heavy metals.

The accumulation of organs, plants, and plant organ in laboratory were real difference, H_0 was accepted and H_1 was rejected. The content of heavy metals accumulation between the plants were different. Kangkung air was more hiperaccumulate than eceng gondok.

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The New Deviation Morphology of *Hydrodictyon sp.* in Malang

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Abstract

Hydrodictyon sp. is one of fresh water algae shaped like water net. It is microscopic, closed, cylindrical or flatted, single layer, formed by several hundred to many thousand cells, and the meshes of net are pentagonal or hexagonal and the angle being formed by the union of three of the elongate cylindrical cells. The aim of this study is for describing the new deviation morphology of *Hydrodictyon sp.* that we found in Malang. In this study, we measured the abiotic factors and analyzed the morphology with microscope. As the result, we found some of deviation morphologies such as *Hydrodictyon sp.* had long around 60 cm, it formed triangle and hexagonal shape of mashes, the angle of meshes was formed by two-five cells, and we found branching of the mature cell in the middle and in the end cell.

Keywords: Hydrodictyon sp., fresh algae, morphology, deviation

1. INTRODUCTION

*Hydrodictyon*is known as water net occurs in fresh water and streams in free floating condition. It is microscopic, closed, cylindrical or flattened, single layer, net-like colonies consist of several hundred to many thousand cells. The meshes of the net are pentagonal or hexagonal and the angles being formed by the union of three of the elongate cylindrical cells [3]. Vegetative reproduction occurs by biflagellate net-forming zooids which are lived in parent cells, forming a daughter coenobium [7].At maturity the coenobium disintegrates and arranges new small daughter. Later it releases from parent cells. Mitosis within cells of daughter net forms coenocytic cells and produces a new mature coenobium. Sexual reproduction occurs by bi-flagellate gamete zooids forming zygospores and finally forming a flat germ net of 100-300 cells [8]. Each of this cells of this alga's germ net can form cylindrical, vegetative, daughter nets.

Hydrodictyon reticulatum is one of the species in *Hydrodictyon* that is widespread all over the world. It is widely distributed in the Northern Hemisphere, while is seldom recorded from the Southern Hemisphere [10]. However, this species is found in South Africa, Australia, and New Zealand [9,2]. So far, these algae had new deviation morphologies that it has never recorded from Indonesia. In this study, the species was collected from rice paddy field in Malang City, East Java. Its morphological characteristic and habitat parameters will describe in detail.

2. MATERIALS AND METHOD

The thalli of *Hydrodictyon* were collected from rice paddy fields in Mojolangu, Malang. The abiotic factors such as pH, DO, turbidity, salinity, conductivity, temperature, and lux were measured. The *Hidrodictyon* samples then were observed in laboratory, the structure of the colonies and the cells were explored by light microscope.

3. RESULTS AND DISCUSSION

We found the algae in Mojolangu, Malang in the rice paddy field. *Hydroductyon* found abundant when the paddy plant were about 2-3 weeks (Figure 1). The abiotic

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factors result have shown in Table 1. It shown that these result was normal for *Hydrodictyon* habitat. *Hydrodictyon sp.*enrichment. Growth of acolony is observed at temperatures of 5–40°C withoptimum – +25°C [4,5].*Hydrodictyonsp*could tolerate a widerange of temperature (10–35°C), light intensity(300–5300 Lx) [11]. The pH in the habitat was 7.5 – 8.6, it because *Hydrodictyon* can produce the increase of water pH [6].

Table 1. The result of abiotic factors measurement

Abiotic factors	Station 1	Station 2	Station 3
DO (mg/L)	6.46	9.95	7.45
Turbid (mg/L)	11	13.3	11.5
pH	7.5	8	8.6
Cond. (µS/cm)	202.5	440	395.5
Salin	0.1	0.2	0.2
SR (%)	79.8	88.8	90.2
Temperature (^{0}C)	29.2	24.2	26.7
Lux	500	10000	4000

Figure 1. Hydodictyon sp. habitat in rice paddy field

According to our observation, we found some deviations of *Hydrodictyon* morphologies that different with some previous studies. The algae have net-like shape, cylindrical coenobium, and 30 cm in length[3]. However, we have found the thalli of *Hydrodictyon* about 60 cm long (Figure 2). We assumed that this deviation was because of the habitat of its alga. Nevertheless, the abiotic factors of this alga was same with other previous studies and the abiotic factors that we measured was normal for *Hydrodictyon*life (Table 1).

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Figure 2. Hydrodictyonsp had about 60 cm long

We found the with triangle, pentagonal and hexagonal meshes of the net (Figure 3). This alga was different with [3] statement that *Hydrodictyon* was formed by hexagonal meshes of the net. The normal the morphology of *Hydrodictyon* generally having three cells for forming the angels. However, we found that its angles were formed by two until five of cylindrical cells (Figure 4).

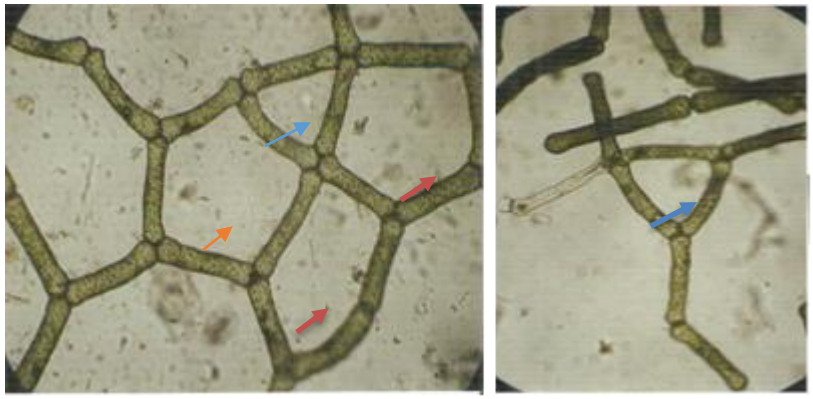

Figure 3. Specimen of Hydrodyctyon formed triangles (blue arrow) and pentagonal (red arrow) shape of mashes

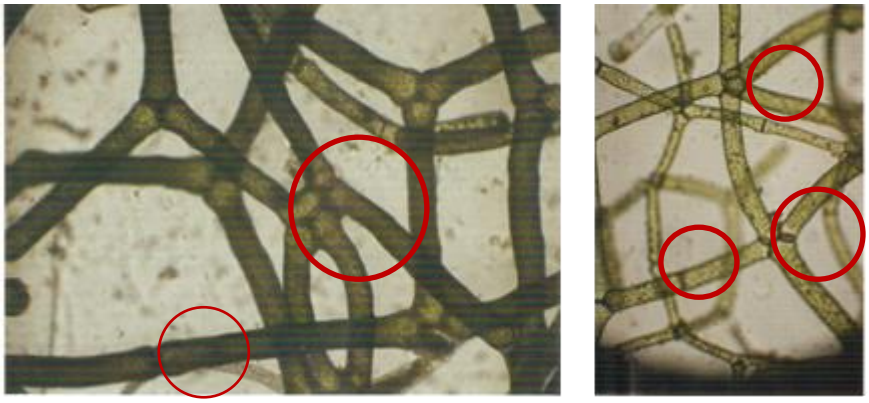

Figure 4. The angle of meshes are formed by two-five cells. (a)itshowed that the angels are formed by two and five cells. (b), the angles are formed by two, four and five cells.

In addition, we found branch in the middle and terminal cells of *Hidridictyon* (Figure 5). It was same with previous study that they found Y shaped *Hidrodictyon* [1]. We also found a bud that had two branches in the angle of the alga (Figure 6). This bud was rare to find in *Hydrodictyon* morphology.

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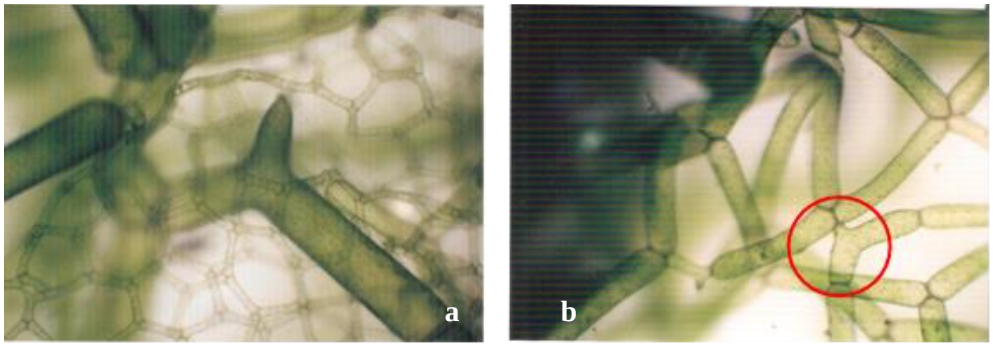

Figure 5. Branching of the mature cell (a) terminal branching (b) middle branching

Figure 6. Budding in Hydrodictyon. The bud had two branches in the terminal of the cell

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NATURAL RESOURCES AND NATURAL PRODUCTS

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Potential of Yeast Isolated from Kerdas Seed (*Archidendron Bubalinum*) as a Leavening Agent in Bread Making

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Abstract

Kerdas (*Archidendron bubalinum*) is a fruit of leguminosae plant in the family of Fabaceae and often used as an *ulam* (traditional vegetable salad). The strong, pungent-smelling seeds make kerdas less favored by the younger generation in particular. This study was conducted to investigate the effect of the yeast isolated from kerdas seeds that can potentially be employed as a leavening agent. The isolation of yeast used different maturation index of kerdas seeds and positive control samples used commercial yeast. The enrichment was carried out by fermenting the samples in medium water at 25°C for 2 days, followed by isolation of respective yeasts. The tested showed the isolates were identified as *S. cerevisiae*, which was verified through morphological tests under microscope, temperature tolerance, and fermentative capacity test. The specific volume of bread showed that the yeast strains *KB4* (2.66±0.410) and *KM8* (2.67±0.250) produced the highest bread volume and had no significant difference ($p>0.05$) on the controlled bread. The number and size of the air pores in the bread showed that almost all bread produced by isolated yeast had no significant difference ($p>0.05$) with the controlled bread. The number and size of air pores was consistent and distributed evenly on breadcrumb structure. The percentage of the moisture content of the bread using yeast strains *KB4*, *KM8* and *RT11* had no significant difference ($p>0.05$) during the storage period of 0 and 7 days. The yeast derived from mature and over-mature kerdas seeds has the potential as a leavening agent.

Keywords: Archidendron bubalinum, *yeast, leavening agent*

1. INTRODUCTION

Yeast especially *S. cerevisiae* strains have been selected for decades for their dough-leavening characteristics. Various efforts are made to diversify resources in the isolation of yeast. This is done indirectly to diversify sources of baking agents in order to replace the functions of commercial yeast. Among the efforts that have been made is the study of the isolated yeast from a local fruit (Malaysia) showed features with better specific volume in the production of bread [1]. Previous studies have reported the isolation of yeast derived from various sources, including Brazilian sugarcane spirit (plant materials) [2], fresh orange fruit and juice [3], and tropical fruits, flowers, and leaves [4-6].

Kerdas (*Archidendron bubalinum*) is a living plant in tropical areas, especially in Southeast Asia and categorized as the family of Leguminosae – Mimosaceae [7]. Kerdas seed has high humidity content, low fat content, high protein content (6%-10%) and chemical compounds with a total of 32% containing valine, methionine, and tyrosine [8].

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The study of the potential of this seed is scarcely available and little attention is given due to limited resources. Nowadays, the nutritious kerdas ulam is growingly forgotten due to its smell unpleasant. In order to avoid that, in this study, kerdas fruit was used as the primary source in yeast isolation. The purpose of this study is to evaluate the potential of yeast isolated from a local natural source (kerdas) as a leavening agent. In addition, this study evaluates and performs physiological tests in order to have a better understanding of the behavior of yeasts in bread making.

2. MATERIAL AND METHOD

2.1 *Material*

Samples of kerdas (*Archidendron bubalinum*) from Maran, Pahang were collected as sources for yeast isolation. The collected samples were placed aseptically in sterile plastic bags, transferred into ice boxes (4°C), and brought to the laboratory for analysis. The samples were then subjected to the following procedures within 24–36 h after collection and transfer to the laboratory. Meanwhile, commercial *S. cerevisiae* strain was obtained (ATCC no. 62418)

2.2 *Maturity Index (Color Measurement)*

Color differences among samples were determined using a Chroma Meter Minolta (CR-300 Trimulus Color Analyzer, Japan). Three values of L, a, and b were measured: L = 100 (white), L = 0 (black); +a = red, - a = green; and +b = yellow, - b = blue.

2.3 *Enrichment Procedures for Yeast Isolation*

The enrichment procedures to detect and isolate fermenting yeast species were carried out by adding 1 mL of the sample solution into yeast fermentation broth (YFB). All of the microfermentations were carried out at 25°C in universal bottles containing 10 mL of pasteurized broth. During fermentation (within 2 days), isolated yeast was transferred on YPD medium (Oxoid, Basingstoke, UK) at 30°C for 2-3 days. Later, the isolated yeast was subcultured in YPD medium (10 g l−1 Bacto yeast extract, 10 g l−1 Bacto peptone and 20 g l−1 glucose) (Oxoid, Basingstoke, UK) added with chloramphenicol to avoid bacterial growth. The plates were incubated at 30°C for 2–4 days. After that, the colonies were counted and selected according to their morphological characteristics [9]. Representative colonies were picked randomly from the plates and pure cultures were subjected to the next identification procedures.

2.4 *Microscope Observation*

A single colony of yeast was mixed in a droplet of sterile distilled water on a glass slide and smeared until the smear dried off. The smear was then stained using diluted methylene blue dye, air dried, and observed under light microscope at 100× magnification.

2.5 *Temperature Tolerance Test*

The ability of the yeast to grow at higher temperatures was verified by plating the yeast isolates onto YPD medium and incubated at 4 different temperatures, i.e. 25, 30, 37 and 45°C for 72 h [10].

2.6 *Fermentative Capacity Test*

The fermentative capacity ability media was prepared and the test was conducted followed by [11]. Before yeast cells grew in yeast fermentation broth (YFB) (Peptone 7.5 g/L, yeast extract 4.5 g/L, and 1 mL of 1.6% (w/v) bromothymol blue as an indicator), 6% (w/v) glucose, sucrose, fructose, maltose, lactose, and galactose were autoclaved

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separately. The YFB was added with respective sugar, then yeast cells were examined on the fermentative ability by using different carbon sources. Durham tubes were placed into the media to trap the carbon dioxide released. The fermented media were green and turned to yellow (acidic) or blue (alkaline) if the yeast cells have the ability to ferment the respective of sugar.

2.7 *Specific Volume of Bread*

The weight and volume of the bread were produced after baking (1 h) after removal of loaves from the oven. According to [12] bread volume was determined by the sesame seed displacement method and specific volume was measured by dividing the volume with bread weight.

2.8 *Number and Size of Air Pores*

The bread was cooled for 1 h. Then, the bread was cut with knife vertically. The image of bread was captured by using a digital camera 50 mm Nikon D7000. Microsoft Office Picture Manager was used to determine the image with magnification up to 600 x 600 pixels. Then, the number and size of air pores were analyzed using Image J software. The image of bread was changed to greyscale contrast and converted to binary images. The length of the image pixel values are known, and then translated into units specified in the value of millimeters.

2.9 *Moisture Content*

The determination of moisture content was conducted by drying the sample for 16–24 h at 105 $\pm$ 1°C, according to the method specified [13].

3. RESULTS AND DISCUSSION

A leavening agent is a substance used in dough for bread making which produces a foaming action that lightens and softens the dough. Most leavening agents for bread making are yeast or synthetic chemical compounds, with the purpose of producing carbon dioxide. Generally, it has been known that the yeast derived from the market is the yeast imported from overseas. According to [1], the leavening agents (yeasts) currently used in Malaysian bakery industries are mostly imported from foreign countries such as Australia (Mauripan), France (Saf-instant), Canada (Fermipan), and Turkey (Gold Pakmaya). Therefore, the presence of yeasts from local fruits is yet to be exploited, especially in bakery products as a leavening agent.

Kerdas (*Archidendron bubalinum*) is categorized as the Leguminosae - Mimosaceae family [8] and contains many nutrients such as protein (9.5%), carbohydrates (31.6%), fiber (6.19%), vitamin C (25.53 mg/100 g) and minerals (100 gm) [14]. The pod of kerdas has a hard texture and green colored with a size of 6–10 cm long and 2–3 cm girth. When opened, it contains several seeds arranged between 10 and 15 mm of overlaid thin skin layer, and shows pale yellow color when it is premature, and blackish when it is ripe or over mature, as shown in Figure 1. The maturity of the fruits is closely related to some physiology and biochemistry characteristics, and the process structure that causes a change in size, color, and flavor [15].

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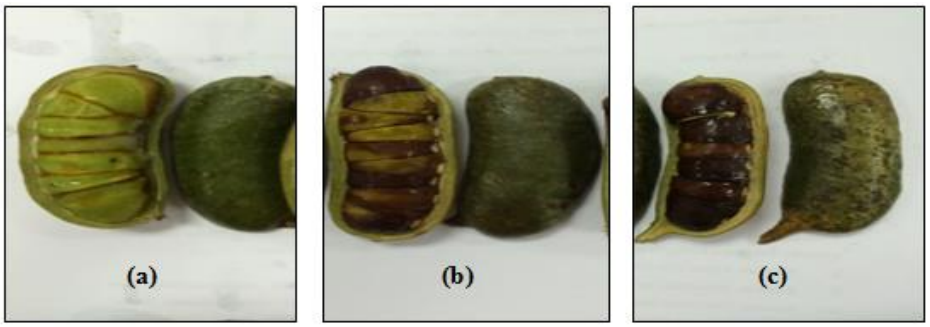

Figure 1. Kerdas fruit used in the study (a) Premature kerdas, (b) mature kerdas and (c) over-mature kerdas

Table 1 shows that there is a significant difference ($p<0.05$) between the maturity index of kerdas samples. The color observed for premature and mature kerdas was a lower value on green color compared to over-mature kerdas that approached reddish color. According to [16], the loss of green color on the fruit is the main guide of color changes when the fruit reaches maturity. The beginning of a gradual loss of color maturity index is when the color intensity changes from deep green to bright green. It has also been reported that the complete loss of green color occurs with the development of yellow, red, or purple pigment. The internal skin of kerdas shows a significant difference ($p<0.05$) of L* value according to the stage of maturity index. Over-mature kerdas has a lower value (29.99 ± 0.69) that represents low color brightness (dark). This level is defined as the final phase in the ontogeny of plant organs where cellular damage and senescence phase are approaching [16].

*Table 1: The value of mean brightness color of kerdas (L*a*b*) (n=3)*

Maturity Index	Kerdas External Skin			Kerdas Internal Skin		
	L*	a*	b*	L*	a*	b*
Premature	33.75 ± 0.95 ^{a}	1.32 ± 0.36 ^{a}	4.15 ± 1.00 ^{a}	47.89 ± 0.75 ^{c}	-2.97 ± 0.53 ^{a}	16.31 ± 0.95 ^{b}
Mature	39.26 ± 0.42 ^{b}	1.68 ± 0.25 ^{a}	5.06 ± 0.94 ^{a}	41.03 ± 0.97 ^{b}	3.32 ± 0.74 ^{b}	18.88 ± 1.39 ^{c}
Over mature	41.88 ± 1.05 ^{c}	2.39 ± 0.30 ^{b}	4.74 ± 0.78 ^{a}	29.99 ± 0.69 ^{a}	10.03 ± 0.95 ^{c}	-2.48 ± 0.51 ^{a}

*L * = highest value indicates a bright color*

*a * = redness + a = red-a = green*

*b * = yellow + b = yellow-b = blue*

*a-c * different alphabets at different coloumns show there is a significant difference ($p<0.05$)*

Based on the observation of subculture, it is found all yeasts have common yeast control. Yeast colony also has control features such as white cream, sphere form, moist surface, slimy, and floury. Some colony change color as they mature, while some become dry and wrinkled. Yeast is usually round, oval or cylinder-shaped in the form of single cells [17]. According to [18], a colony formed by yeast cells of different genera has a form of smooth, soft, rough, and thready yeast, depending on the ability of yeast to form a capsule or extracellular matrix materials. Almost the entire colony of isolated yeast has an

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equally controlled yeast aroma (sweet fruits), especially the colony that gives strong smell such as KB4, KM8, KT10, and RT11. The formation of aroma has been studied extensively in *S. cerevisiae* yeast, which generates some volatile acetate ester such as ethyl acetate (pear aroma), isoamyl acetate (banana aroma), and phenylethyl acetate (the fragrance of flowers) [19-20].

Figure 2 shows that the resulting yeasts are in the form of round and oval. According to [17], yeast is usually round, oval, or cylinder-shaped in the form of a single cell, and *S. cerevisiae* (a fungal for fermentation of sugar in cereals) is about 8 μm in diameter. Yeast has different cell sizes, where some yeast may only be 2-3 μm long, whereas other species can reach a length of 20-50 μm [17]. Cell width varies less at approximately 1-10 μm. *S. cerevisiae* cells appear as an oval or ellipsoid egg structure, surrounded by thicker cell walls [21]. Strains of *S. cerevisiae* exhibit rough colony morphology, and pseudohyphal is often associated with the disturbance in the process of fermentation, depending on the system of fermentation and other operating conditions [22].

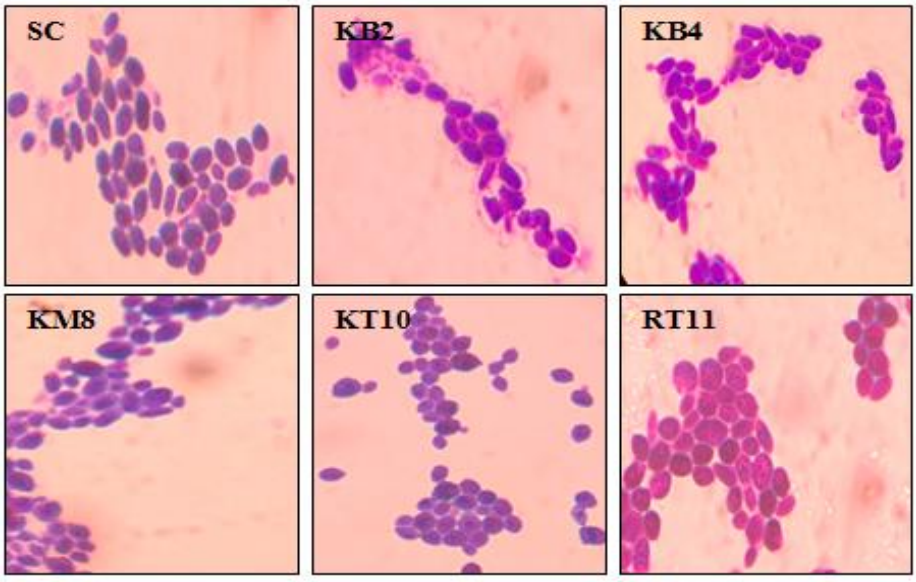

Figure 2. The morphology of yeast isolates observed under light microscope at 100× magnification. SC positive control - S. cerevisiae; KB2 - premature kerdas; KB4 - premature kerdas (without internal skin); KM8 - mature kerdas (without internal skin); KT10 – over-mature kerdas and RT11 – over-mature kerdas (without internal skin).

In order to understand yeast behavior, temperature and fermentative capacity tests were used on the series of yeast isolates. Temperature can affect the fermentation process and the metabolism of yeast. Table 2 shown all yeast strains can grow at 25, 30 and 37°C. The isolated yeast strain KB2 showed higher resistant and was able to survive at a higher temperature of 45°C. According to [1], yeast strains can survive at high temperatures, which indicate that the strains can be used in bread making. In order to speed up the process of carbon dioxide, the formation of flavors and aromas can be enhanced. The premature kerdas has the ability to grow at higher temperature range. Young maturity stage has acidic component and has high resistance against the risk of damage. During maturity process, gluconeogenesis will increase while acidity will decrease [23]. All isolates were able to grow at 37°C. The temperature range for most yeast to grow is he optimum temperature is between 25 and 30°C, and the maximum is between 35 and 47°C [24]. The growth of the yeast is more encouraged by acidic condition, i.e. between pH 4 and 4.5. *S. cerevisiae* has increased alcohol production rate at the temperature of 40°C [25]. Alcohol production is boosted by an increase in

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temperature of fermentation process [26]. At temperatures higher than the incubation temperature, cell production reduced but ethanol production rate increased [27].

Table 2. Yeast growth at different temperatures

Yeast Strain	Temperatures (°C)			
	25	**30**	**37**	**45**
SC1	+++	+++	+++	-
KB2	+++	+++	+++	++
KB4	+++	+++	+++	-
KM8	+++	+++	+++	-
KT10	+++	+++	+++	-
RT11	+++	+++	+++	-

Intensive growth (+++); moderate growth (++); low growth (+); no growth (-); positive control (SC1). SC - S. cerevisiae; KB2 - premature kerdas; KB4 - premature kerdas (without internal skin); KM8 -mature kerdas (without internal skin); KT10 – over-mature kerdas and RT11 – over-mature kerdas (without internal skin).

Table 3 showed that all strains were able to ferment all sugars provided and released carbon dioxide as observed in a Durham tube. The strains showed different time intervals in releasing carbon dioxide. KM8, KT10, and RT11 strains fermented sugars in less than 30 min (the color of fermentation broth changed from green to yellow). This indicates that those strains may initiate fermentation process immediately after inoculated in bread dough and produce more carbon dioxide, causing dough to rise and contribute to better physicochemical properties of bread [1]. According to [28], *S. cerevisiae* is capable of fermenting all sugars present in the dough, for example, glucose, fructose, sucrose, and maltose, and also 8 times faster than *P. membranificiens* that can only ferment glucose. All yeast strains including commercial yeast are not lactose-free. *S. cerevisiae* cells cannot be lactose-free due to lack of lactase or β-galactosidase system [29].

Table 3. Fermentative capacity test of isolated yeast

Yeast Strain	Carbon Sources				
	Glucose	**Maltose**	**Galactose**	**Sucrose**	**Lactose**
SC1	+	+	+	+	-
KB2	+	+	+	+	-
KB4	+	+	+	+	-
KM8	+	+	+	+	-
KT10	+	+	+	+	-
RT11	+	+	+	+	-

Carbon sources assimilation (+); no assimilation of carbon sources (-). Positive control (SC1). SC - S. cerevisiae; KB2 - premature kerdas; KB4 - premature kerdas (without internal skin); KM8 - mature kerdas (without internal skin); KT10 – over-mature kerdas and RT11 – over-mature kerdas (without internal skin).

The leavening property of bread volume with various yeast isolates from kerdas seed is showed in Figure 3. The bread with the highest volume was the bread from yeast strain KM8 (2.67 ± 0.25 mL/g). The bread was fluffy, had a larger size, and uniform. Air

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pores were formed and gave a soft texture on the bread produced. This shows that the yeast isolated from mature kerdas seeds has the potential to produce carbon dioxide and consequently a fluffy bread compared to using commercial yeast control. The control bread showed no significant difference ($p>0.05$) with the bread of yeast strains from KB2, KB4, and KM8. The results of the bread of isolated yeasts KT10 (2.06 ± 0.14 mL/g) and RT11 (2.24 ± 0.04 mL/g) showed lower volume. The breakdown of bread volume is caused by higher water soluble molecules content such as glucose, fructose, and sucrose [1].

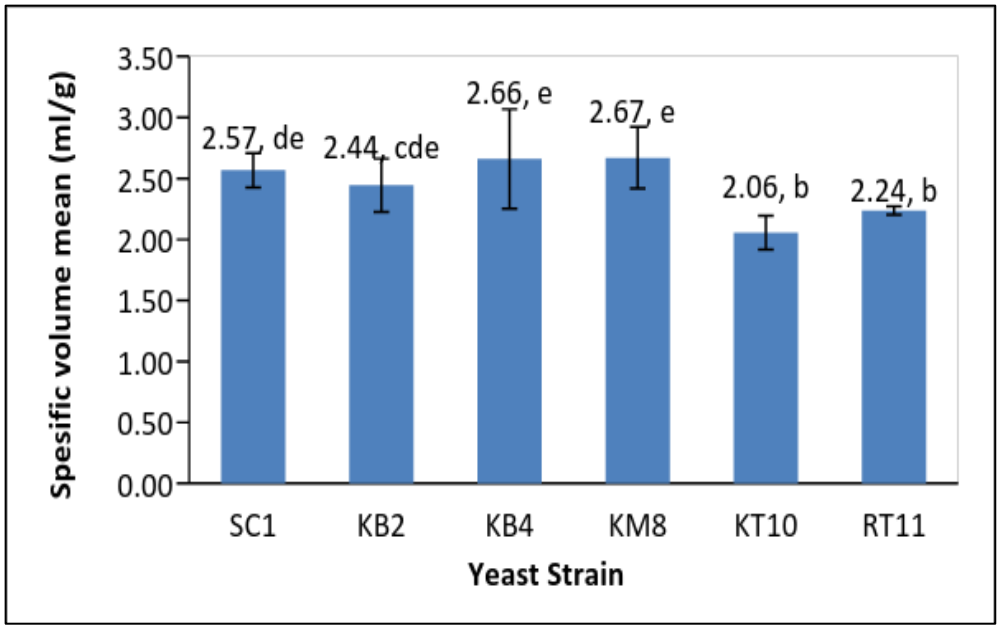

Figure 3. Specific volume of bread

Table 4 shows no significant difference ($p>0.05$) between the control bread and almost the entire bread from the isolated yeast. The highest number of air pores was recorded for the bread from yeast strain KT10 (216.00 ± 9.85 per cm^2). According to [30], during fermentation, the result of the expansion of the dough of yeast is responsible for the increase in porosity and structure stability changes. Fermentation process shows a significant move for the formation of cellular structure and the fragility of bread [31]. The higher value of the number of air pores shows that air pores are distributed evenly and gives a soft texture of the bread produced. The porosity and cell structure of breadcrumb is an important quality criterion used in evaluating the quality of bread in addition to taste, color, and texture of breadcrumb [32].

According to [33], the development and production of cell gas in dough requires controlling the cell structure and the presence of bubbles in a more opaque medium. The result shows no significant difference ($p>0.05$) for the control bread that used commercial yeast with all the bread produced by isolated yeasts. The pore size of bread under the value of 0.2 mm^2 is not counted as air pores, while the measurements exceeding 4 mm^2 are defects on the content of bread [34].

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Table 4. Number and size of air pores of breadcrumb

Yeast Strain	Analysis of Air Pores	
	Number	**Size**
	(cm^2)	(mm^2)
SC1	197.67 ± 5.13 ^{cd}	0.43 ± 0.06 ^{abc}
KB2	189.00 ± 4.58 ^{c}	0.33 ± 0.06 ^{bc}
KB4	192.67 ± 14.01 ^{c}	0.37 ± 0.06 ^{ab}
KM8	191.33 ± 14.22 ^{c}	0.47 ± 0.15 ^{bc}
KT10	216.00 ± 9.85 ^{d}	0.30 ± 0.10 ^{bc}
RT11	209.33 ± 15.14 ^{cd}	0.27 ± 0.06 ^{a}

*a-d * different alphabets at different columns show there is a significant difference ($p<0.05$)*

Based on the results in Table 5, the highest moisture content was produced by the yeast bread RT11 with the value of 36.61 ± 1.09%. There was no significant difference ($p> 0.05$) between yeast strain KB4 and KM8. The bread produced had a very soft texture and fluffy compared to other bread. The total moisture loss was in the range 1-2% of the original value of the bread. The highest moisture was recorded for the yeast bread of RT11, which was 34.50 ± 0.21%. There was no significant difference ($p>0.05$) between the yeast and bread from KB4 and KM8. The result also showed that there was no significant difference ($p>0.05$) between the bread of yeast RT11 with KB4 and KM8 for the period storage of 0 and 7 days.

Table 5. Moisture content of bread at 0 and 7 days of storage

Yeast Strain	Moisture Content	
	0 days (%)	**7 days** (%)
SC1	33.05 ± 0.75 ^{c}	32.86 ± 0.18 ^{b}
KB2	31.32 ± 0.51 ^{b}	28.59 ± 0.37 ^{a}
KB4	35.64 ± 0.12 ^{d}	34.26 ± 0.13 ^{d}
KM8	36.14 ± 1.18 ^{d}	34.03 ± 0.09 ^{cd}
KT10	33.12 ± 0.86 ^{c}	32.98 ± 0.90 ^{b}
RT11	36.61 ± 1.09 ^{d}	34.50 ± 0.21 ^{d}

*a-d * different alphabets at different columns show there is a significant difference ($p< 0.05$)*

This shows that the bread produced by isolated yeast were still in the range of 34–36% moisture within 7 days of storage. The observation of the bread found that it remained soft and fluffy compared to the control bread (32.86±0.18%). The yeast strain from isolated kerdas seed has the potential to give longer shelf life of bread. The bread produced from the isolated yeasts of *KM8*, *KT10* and *RT11* had a higher resistance to the growth of microorganisms and still maintained a soft texture within 10-14 days of

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storage. The yeast derived from mature and over-mature kerdas seed has the potential as a leavening agent. Thus, this indicates that the local plant or kerdas could be a potential source of indigenous *S. cerevisiae* strains for leavening agent in bread making.

4. CONCLUSION

From the study, it was noticed that 10 out of 12 samples tested identified the isolates were *S. cerevisiae* and it was verified through morphological tests under microscope, temperature tolerance, and fermentative capacity test. 5 yeast strains of KB2 (premature kerdas), KB4 (premature kerdas without internal skin), KM8 (mature kerdas without internal skin), KT10 (over-mature kerdas), and RT11 (over-mature kerdas without internal skin) showed better capability and potential as a leavening agent by physicochemical testing. It is evident from this finding that local fruits especially kerdas (*Archidendron bubalinum*) could be sources of new *S. cerevisiae* strains that can potentially be used as a leavening agent for bread making.

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Evaluation of Tendering Effect from Date Seed extract (*P. dactalytera*) in Knuckle Part Meat

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Abstract

Phoenic dactylifera (date) is a species of flowering plants in the family of Arecaceae. Date seeds are considered as a waste from many processing plants pitted dates produce, confectionery date syrup and date. . At this time, the seeds are used mainly for animal feed in the cattle industry, chicken and so on. With world production of dates reached 6.9 million tons in 2004, of the approximately 863,000 tons of ore date of issue. Tenderness is the major concern that affecting consumer acceptance of beef in meat industry. This study was carried out in order to investigate the effect of the bioactive compound extracted from date seed as a tenderization agent in meat. Extraction of date seed used different methods of extraction (Soxhlet and Maceration). The application of extracted on knuckle part of beef were performed and papain was used as a positive control and followed by the sensory evaluation. The analyses of cooked meat were performed in order to analyze the physicho-chemical properties of date seed extract. The result from the study revealed that the aqueous extract (Maceration Techniques) gave the best percentage of the total yield recovery (28.44%), the physicho-chemical properties of cooked meat showed that reducing power of pH after cooking. Meanwhile for the cooking yield, result showed that almost 86% of water losses during cooking for aqueous extract and positive control and 96% for negative control. According to the sensory evaluation of the cooked meat, scoring test and hedonic test were performed via One Way Anova. The result for texture (5.70±2.200), juiceness (5.47±1.756) and taste (4.70±1.950) attribute have no significance different at $p < 0.05$ between aqueos extract, and positive control. A general acceptance shows that no significance different between aqueos extract (5.60±1.976) and positive control (6.30±2.168). The result suggested that the tenderization effect of date seed improved the textural properties of knuckle part meat and can be used for tenderization in food industry.

Keyword: Date seed, Phoenic dactylifera, knuckle part, tenderization agent

1. INTRODUCTION

Phoenix dactylifera (date or date palm) is a species of flowering plants in the family oil palm trees, fruit is edible and sweet. The species is widely cultivated and is naturalized in many tropical and subtropical regions worldwide. Date seeds are considered as waste from many processing plants pitted dates produce, confectionery date syrup and date. At this time, the seeds are used mainly for animal feed in the cattle industry, chicken and so on. With world production of dates reached 6.9 million tons in 2004, of the approximately 863,000 tons of ore date of issue. There are some properties that are in the dates. it is proved by several studies and experiences of Arab countries that dates contain nutrients [1]. Date enriched with carbohydrates, proteins, fats and magnesium [1]. The use of waste it is very important for the cultivation of date and

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increase income for some sectors. According to [13], the properties of the seeds now, they reported composition is 3.1 to 7.1% moisture, 2.3 to 6.4% protein, 5.0 to 13.2 fat , 0.9 to 1.8% ash and 22.5 to 80.2% fiber.

Tenderness is the most important factor affecting consumer acceptance of beef. Beef tenderness is affected by two primary factors called background tenderness and protein (muscle fiber) tenderness [6]. Background tenderness is determined by the amount and type of connective tissue in any given cut. For example, brisket is generally very tough unless cooked properly, whereas tenderloin is almost always very tender. One major difference between brisket and tenderloin is the amount and type of connective tissue in each. Brisket has more and tougher connective tissue and tenderloin has less and more tender connective tissue. Protein or muscle fiber tenderness is affected by the strength of the actual meat fibers, which are affected primarily by aging (holding meat in an unfrozen state). Muscle fibers in meat weaken over time due to the action of enzymes, which break apart the fiber, ultimately improving tenderness. Muscle fibers becoming weaker during aging are why steaks aged 14 days are generally tenderer than steaks aged 3 days.

According [9] meat is basically made of muscle. Each cut of meat is made up of muscle fibers bound together by protein filaments called collagen. Tenderizing meat means breaking the long strands of muscle as well as softening the collagen until it turns into gelatin. This soft gelatin soaks into the meat, tenderizing it and adding moisture to make the meat juicy. This tenderizing can be accomplished through physical means like pounding, or through the chemical reactions caused when it is exposed to the acids in marinades and powdered meat tenderizers. The two main ingredients in most powdered tenderizers are papain, found in papayas, and bromelain, found in pineapples. Both enzymes attack the muscle fibers and the collagen webs that hold them together. This softens the meat and makes it tenderer. It is also why you cannot put raw papaya or pineapple in gelatin desserts. The papain and bromelain break down the gelatin, just as they do the collagen in meats [9]. The good nutritional value of date seeds is based on their dietary fiber content, which makes them suitable for the preparation of fiber-based foods. Since a large quantity of date seeds are being produced as a waste material and the seeds contain a significant amount of bioactive phenolics and dietary fiber. Palm date seeds were evaluated by Almana & Mahmoud as a source of dietary fiber. [12].

The aim of this study is to attempt producing a new natural tenderizer from the date seed via different methods and to investigate the physicho-chemical properties of the extract as well as the quality of tenderizing agent from date seed extracted.

2. MATERIALS AND METHODS

2.1 Plant Material

The dates seed (*Phoenix dactylifera)* were purchased from local market in Kuantan area,

Figure 1. Dates seed

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2.2 Reagent, Chemical and Apparatus

n-Hexane, Methanol (MeOH), dichloromethane (DCM) and Ethyl acetate were obtained from Sigma Aldrich Co. St. Louis, USA. Papain were purchased from local supplier. Mill machine (Qingdao Dahua Double Circle), oven dryer (Protech), rotary evaporator (Buchii), electronic balance (Metler Toledo) and Soxhlet apparatus.

2.3 Preparation of Crude Extracts

The dates seed were washed, cleaned, dried in a dryer at 40°C for two weeks, ground, labelled and stored in airtight container for further use

2.4 Methods of Extraction

Extraction were carried out in two different method Soxhlet Extrction with different polarity of solvent (n-hexane, Ethyl acetate, Dichloromethan and Methanol) and MacerationTechniques for aquoes part.

2.5 Meat Analysis (Tenderizing effect on meat)

The knuckle part of beef meat were marinated with the distilled water (control), date seed extracted 1ml/100g, and 2% papain (as a positive control) for 48 hour at 4 ºC. After 48 hours, the sample then were washed and drained. A 2% w/w salt were added and sample were cooked via autoclave for 15 minute at 15 psig. A different parameter have been tested for the meat sample as follow:

Table 1. Evaluated Parametre

No	Evaluated Parameter
A	Date seed (n hexane)
B	Date seed (aquoes)
C	Distilled water
D	Papain (2%)

Only the extract of the aquoues and hexane extracted were selected over others. Papain (2%) as a positif control and Distilled water as a negative control.

2.6 Sensory test

Sensory evaluation is a common and very useful tool in quality assessment of processed meat products. It makes use of the senses to evaluate the general acceptability and quality attributes of the products.Based on the scoring and hedonic test, the attributes such as juiciness, texture, taste as well as overall acceptance were examined.

2.7 Physico-Chemical Properties (pH, Cooking yield, Acidity)

For acidity the litmus paper have been used to determine the level of the pH sample pre and post cooking treatment. Cooking yield refers to the water losses during cooking process via the autoclave at 15 psi at 15 minutes. The titratable acidity were determined using AOAC methods.

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3. RESULT AND DISCUSSION

3.1 Extraction Yield

Table 2 shows the percentage of dried sample obtained after drying process. The percentage of dried sample was 61% of dry weight basis.

Table 2. Percentage of Dried Sample for P. Dactylitera seed

Sample	Wet weight(g)	Dry weight(g)	% of weight
P. Dactylitera seed	1000	610	61%

Table 3 shows the percentage of total yield extraction for different organic solvents and aqueous from both treatments. The extract of the *P. Dactylitera* seed contained different proportions of extracting. Aqueous (maceration techniques) and *n*-hexane (Soxhlet extraction) gave higher percentage of yield 28.44±0.8% and 28.8.0±0.2 % respectively compare to Dichloromethane (22.46±0.11%), Ethyl Acetate (12.79±0.2 %) and Methanol (10.59±0.5 %).

Table 3. Percentage of Total extractable Yield for P. Dactylitera seed

Sample	Extraction Yield (%)					Total Extractable Materials(%)
	n-hexane	Dichloro-methane	Ethyl Acetate	Methanol	Aqueous	
P. Dactylitera crude extract	28.8.0±0.2	22.46±0.11	12.79±0.2	10.59±0.5	28.44±0.8	**82.46±0.36**

Yield (%) = (weight of crude extract/ weight of dried sample)x 100%
Data are mean standard±standard deviation, n=3

3.2 The effect of pH , acidity on treated beef

Table 4. Quality of beef meat treated with P. Dactylitera extract at different temperature

Treatment		After Marinating (4°C)			After Cooking (100°C)		
		pH	Titrable Acidity (%)g/L as a lactic acid equivalent	Cooking Yield	pH	Titrable Acidity (%)g/L as a lactic acid equivalent	Cooking Yield
P. Dactylitera extract 6%	*n*-hexane	6	-	-	6	1.22	84%
	Dichloromethane	6	-	-	6	2.07	89%
	Ethyl Acetate	6	-	-	6	1.78	94%
	Methanol	7	-	-	5	1.66	96%
	Aqueous	6	-	-	5	1.60	86%
Positive control	Papain 2%	7	-	-	4	1.58	86%
Negative Control	Distilled water	7	-	-	7	0.594	96%

(-) = not applicable

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When beef meat was incubated at 4°C it shows that no enzymatic activity was involved during incubation. Table 4 shows the pH value for the beef meat are 6 to 7 for all treatments before cooking. When the beef meat was incubated at 100 °C (after cooking) shows that the pH value was drop significantly at 4 for papain and 5 for aqueous and methanol. Meanwhile for the other treatments it shows no changes. It suggested that the pH value has great influence on after treatment and cooking, as observed for the 2% papain and 6% *P. Dactylitera* aquoes and methanol extract. The result showed a reduction in hardness , suggesting that at pH below or above of isoelectric point of myofibrillar protein (around 5), hardness decrease It were indicated that the temperature was sufficient to weaken and break down the bonds from the long range interactions which are necessary for the presence of tertiary structure[13].

3.3 The effect of the volume of cooking yield

Cooking yield refers to water binding capacity or water holding capacity. Water-holding capacity of fresh meat (ability to retain inherent water) is an important property of fresh meat as it affects both the yield and the quality of the end product. The result in Table 4.3 indicated that the result for cooking yield vary for each treatments. For Methanol extract 96%, Ethyl Acetate extract 94%, DCM extract 89%, Hexane extract 84% and Aquoes and Papain extract 86% respectively. The cooking yield for topside beef (grilled) was 75%, slightly lower from this study due to change in proximate composition, cooking temperature or nutrients become more concentrated during cooking[12]. Water is lost during cooking, the amount depending on time, temperature, method of cooking, size of sample, heat penetration and composition leading to an increase in concentration of the fat and protein.

Meat muscle contains approximately 75% water. The other main components include protein (approximately 20%), lipids or fat (approximately 5%), carbohydrates (approximately 1%) and vitamins and minerals (often analyzed as ash, approximately 1%) [2]. Meat and meat products are considered cooked when the centre of the product is maintained at a temperature of 65-70°C for 10 minutes since the proteins will then be coagulated and the meat tenderised by partial hydrolysis of the collagen. The vegetative form of bacteria, but not spores, will have been destroyed (thermoresistant spores can survive heating above 100°C). The completion of the cooking process is generally indicated by a change of colour from red to brown (red to pink in cured products) and flavours are developed.

3.4 Sensory Evaluation

3.5.1 Scoring Test

The table 5 shows the result for scoring test that was performed via One Way Anova.

Table 5. Scoring Test

ATTRIBUTE	AQUEOS	NEGATIVE	PAPAIN
Juiceness	5.47^{b}±1.76	4.30^{a}±2.35	5.93^{b}±1.87
Texture	5.70^{b}±2.20	4.37^{a}±2.61	5.17^{b}±2.1
Taste	4.70^{ab}±1.95	5.07^{a}±2.21	3.90^{b}±2.1
Overall	5.60^{b}±1.98	4.47^{a}±2.16	6.30^{b}±2.17

From the results, texture and taste indicated that there had no significance different between the treatment using aqueous, and papain. The juiciness attribute showed that no significance differences between both treatments. The overall results shows that no significance different for both treatments.

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3.5.2 Hedonic Test

Table 6 shows the result from Hedonic test. The 9 point scale started with the lowest rate for value 1 to the highest rate, which is represented by value 9. For the juiciness, there were significance difference between aqueous and papain. For the texture, taste and overall have no significance different between negative, aqueous and papain.

Table 6. Hedonic Test

Attribute	Negative	Aqueos	Papain
Juiciness	6.23^{b}±1.382	5.87^{a}±1.737	7.37^{b}±1.712
Texture	5.97^{b}±1.712	6.10^{a}±2.057	6.80^{b}±1.955
Taste	6.40^{b}±1.163	6.80^{a}±1.349	7.07^{b}±1.507
Overall	6.17^{b}±1.020	6.57^{a}±1.906	7.17^{b}±1.984

4. CONCLUSION

In conclusion, this study was carried out to extract the crude extract from date seed using soxhlet extraction and maceration technique. Comparison between the best method from the different solvent for soxhlet extraction and aqueos extract for the maceration. This study revealed that, total extractable yield comparison from difference extraction were analyzed method for date seed are 82.46%. The best recovery are from aqueos extract (28.44%) the physico-chemical properties of crude extract were analyzed from different extraction method. From the result, pH was different between after marinating and after cooking were tested. Based on the result, shows that papain have a reducing power of pH from 7 to 4 after cooking compared to aqueos extract from 6 to 5. Based on the sensory test we have to analyse the data from scoring and hedonic test. Based on the result, the aqueous method is more stable rather than hexane. Water losses after cooking for papain and aqueos show the same result (86%) compared to distilled water (97%). Even the sensory evalution test were not good, overall result shows that there were no significant difference between aqueos extract, papain and distilled water.

Future directions of this project are proposed in line with current commercial meat tenderizer. These directions include looking into texture test for cooked meat using the extract. The rational behind this is that it is important for a consumer to have a great choise in market. Finally, the type of protease enzyme will attempt to explore in order to identify the bioactive compound with responsible to tenderizer the meat.

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Identification of Bioactive Compounds of *Thuidium tamariscellum*

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Abstract

The objective of this research to determine the bioactive compounds of the moss *Thuidium tamariscellum*. The moss was extracted with hexane, ethyl acetate, and methanol solvent by maceration method. The identification of bioactive compounds by Gass Chromatography Mass Spectra (GC-MS). The results showed that the hexane, ethyl acetate, and methanol extract each containing 9, 34, and 39 compounds. The main bioactive compounds of hexane, ethyl acetate, and methanol extract is 1,2-Benzisothiazole, hexadecanoic acid, and cytidine.

Keywords: bioactive compounds, thuidium tamariscellum

1. INTRODUCTION

Recently, people's interest in using herbal medicine increased significantly, due to the use of modern medicine can not cure the disease, and even cause unwanted side effects (Bodeker, 2000). One of the useful plants are Bryophytes which are found in various habitats, such as soil, rocks, and trees (Asakawa, 2008). Bryophyta consists of three classes of Hepaticopsida, Mosses, and Anthocerotopsida that contain many secondary metabolites and bioactive compounds (Asakawa, 2007).

Thuidium tamariscellum, family Thuidiaceae is one type of the moss, habitat is in the trunk. Based on the literature, there are no data on the content of bioactive compounds in the moss *Thuidium tamariscellum*. Therefore, the objective of this study to determine the bioactive compounds contained in hexane, ethyl acetate, and methanol extract of Thuidium tamariscellum using GC-MS method.

2. MATERIALS AND METHODS

2.1. Plant material

The plant material was collected from the forest Cangar, Batu, Malang, East Java, Indonesia in April 2015. Moss was identified in the Laboratory of Plant Physiology, Department of Biology, Faculty of Science and Technology, Airlangga University.

2.2. Extraction and Analysis of Bioactive Compounds

The moss was washed, dried and made into powder, then weighed the result was 7.2 g. The moss powder divided into three parts, each part weighs was 2.4 g. Each portion was extracted with hexane, ethyl acetate, and methanol using maceration method. The volume of each solvent is 100 ml. Maceration repeated four times. The extract was analyzed by Gass Chromatography Mass Spectra.

3. RESULTS AND DISCUSSION

Based on the results of GC-MS analysis, chromatogram profile extract n hexane, ethyl acetate and methanol each consisting of 9, 34 and 39 peak (Figure 1, 2 and 3). This suggests that

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each extract n-hexane, ethyl acetate and methanol containing 9.34, and 39 compounds (Tables 1,2, and 3).

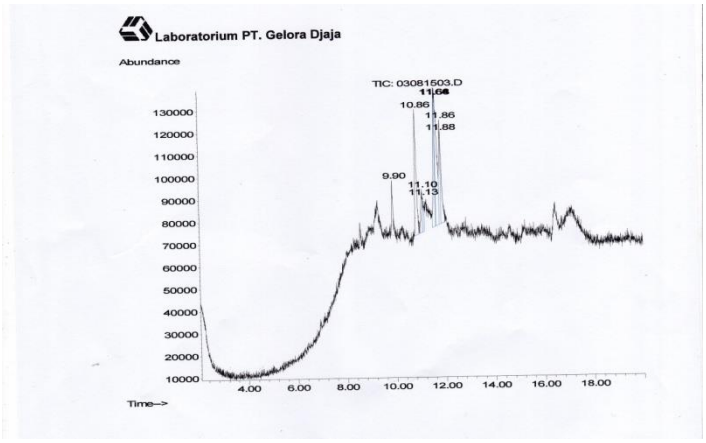

Figure 1. Chromatogram profile of n-hexane extract of Thuidium tamariscellum

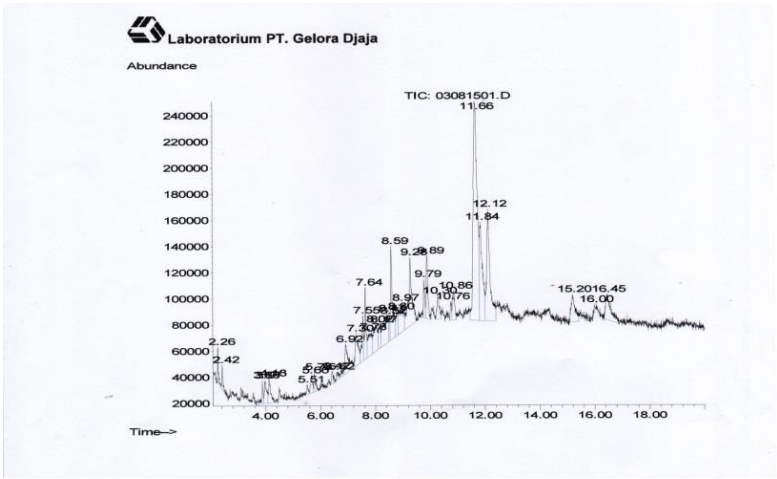

Figure 2. Chromatogram profile of ethyl acetate extract of Thuidium tamariscellum

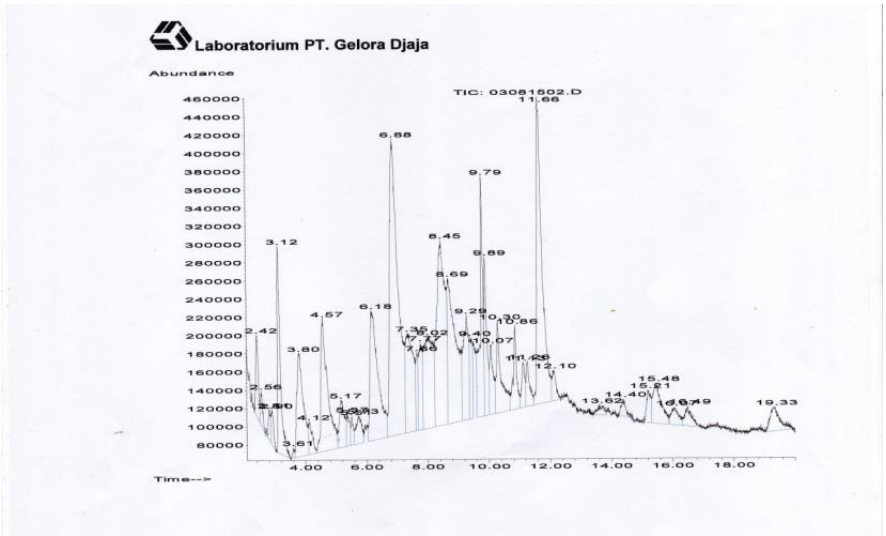

Figure 3. Chromatogram profile of methanol extract of Thuidium tamariscellum

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Table 1. Results of GC-MS analysis of the n-hexane extract Thuidium tamariscellum

Peak	Retention time	Compound Name	Area (%)
1	9.90	14-Beta-H-Pregna	6.62
2	10.86	Pentadecanoic acid	19.15
3	11.10	Thiosulfuric acid	4.85
4	11.13	4-Amino-2-(4-Amidinoamino-1-(4-	4.13
5	11.64	1,2-Benzisothiazole	15.96
6	11.66	1,2-Benzisothiazole	4.66
7	11.67	1,2-Benzisothiazole	22.99
8	11.86	1-Pentadecene	11.92
9	11.88	(cis)-2-nonadecene	9.71

Table 2. The results of GC-MS Analysis of Ethyl Acetate Extract Thuidium tamariscellum

Peak	Retention time	Compound Name	Area (%)
1	2.26	Cyclotetrasiloxane	0.56
2	2.42	Furan, 2-pentyl,-(CAS)	0.71
3	3.88	1H-4-Azacycloprop(cd)indene	0.82
4	3.97	Naphthalene	1.34
5	4.13	1,4-Dioxaspiro(4,5)decane	1.38
6	5.51	1,4-Dioxaspiro(4,5)decane	0.67
7	5.67	1,4-Dioxaspiro(4,5)decane	0.84
8	5.78	2H-Azepin-2-one,hexahydro	0.76
9	6.42	2H-Azepin-2-one,hexahydro	0.58
10	6.62	2,5-Dimethylamphetamine	0.38
11	6.92	Tetrahydroxycyclopentadienone	3.19
12	7.30	1-formyl-4-methyl-naphthalene	2.60
13	7.55	Acetic acid	1.84
14	7.64	Butyric acid	2.73
15	7.78	Tetrahydroxycyclopentadienone	2.34
16	8.01	5,9-Dimethyl-2-(1-methylethylid)	2.79
17	8.17	Thiourea	1.08
18	8.46	2-Butanol	3.84
19	8.52	4H-Pyran-4-one,2,6-dimethyl	0.65
20	8.58	Cyclohexyl 2-Methylenbutanyl K	4.56
21	8.80	5,9-Dimethyl-2-(1-methylethylid)	1.31
22	8.97	8-Acetyl-3,3,7-trimethyl-6-meth	3.06
23	9.28	E-15-Heptadecenal	6.41

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Peak	Retention time	Compound Name	Area (%)
24	9.79	Trans pinene	1.46
25	9.89	Cyclododecanone	2.71
26	10.29	2-Acetyl-4-nitrocyclooctanone	1.42
27	10.76	Hexenoic acid	0.62
28	10.86	14-Beta-H-pregna	1.66
29	11.66	Hexadecanoic acid	23.56
30	11.84	17-Pentatriacontene	7.54
31	12.12	4-Chlorobenzenesulfonamide	9.89
32	15.20	Cyclopentane	2.99
33	16.00	Octadecanoic acid	0.25
34	16.45	1-Octadecene	3.47

Table 3. The results of GC-MS Analysis of Methanol Extracts Thuidium tamariscellum

Peak	Retention time	Compound Name	Area (%)
1	2.42	Xylitol	1.14
2	2.56	1,4-dideuterio-2-methylbutan	0.38
3	2.81	N,N’-Dimethylpiperazine	0.38
4	2.90	Cycloalanylserine	0.42
5	3.13	4H-pyran-4-one,2-Hydroxy-3-met	3.38
6	3.61	2,4(1H,3H)-Pyrimidinedione	0.09
7	3.80	Formamide	3.80
8	4.12	5,6-Diamino-2,4-dihydroxypirimi	0.67
9	4.57	5-Hydroxymethylfural	5.09
10	5.17	Heptanoic acid	1.39
11	5.37	1-Heptanamine	0.52
12	5.51	Tetrahydroxycyclopentadienone	0.37
13	5.73	1-Octanamine	0.87
14	6.18	Isothiazole	6.06
15	6.88	Cytidine	13.97
16	7.35	Dodecanoic acid	4.35
17	7.66	Decahydroisoquinoline	0.90

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Peak	Retention time	Compound Name	Area (%)
18	7.78	N-(4-methoxycarbonylbutylidene)	1.82
19	8.02	1-Tridecene	4.96
20	8.45	N1N1-dimethyl-N2-n-butylformam	8.87
21	8.69	2,2'Bioxirane	6.81
22	9.29	17-Pentatriacontene	3.05
23	9.40	Palmitic acid	1.23
24	9.79	Neophytadiene	3.98
25	9.90	2-Decanone	2.34
26	10.07	Spiro(5,6)dodecane-1,7-dione	1.42
27	10.30	Butanoic acid	2.47
28	10.86	Hexadecanoic acid	1.64
29	11.13	Hexahydropyridine	0.79
30	11.27	Methyl-3-(3,5-diterbutyl-4-Hyd)	0.89
31	11.66	n-Hexadecanoic acid	9.13
32	12.10	n-Hexadecanoic acid	0.68
33	13.62	Heptadecene-(8)-carbonic acid	0.35
34	14.40	14-Beta-H-Pregna	0.40
35	15.21	1,7-dimethyl-2-oxo-7(4'-formyl)	0.79
36	15.49	Thiosulfuric acid	1.85
37	16.06	2-Piperidinone	0.71
38	16.50	1-Nonadecene	0.73
39	19.34	8-Avetyl-3,3-epoxymethano-6,6,7	1,33

Hexadecanoic acid or palmitic acid is one type of lipid components contained in microorganisms, animals, and plants. This compound contained in the leaves moss Tortula muralist (Ocuncu *et al*., 2010). Wei *et al*. (2011) indicates that hexadecanoic acid isolated from Peperomia pellucida leaf extract can inhibit the growth of bacteria Escherichia coli at a concentration of 31.25 mg / l. Arankumar and Muthuselvam (2009) indicates that hexadecanoic acid derived from acetone extracts of Aloe vera has antibacterial activity against Staphylococcus aureus and E. coli. Zani et al. (1996) reported that benzisothiazole potential as an antibacterial and antifungal primarily on Gram-positive bacteria, yeast, and dermatophytes. Cytidine is a nucleoside molecule that is formed when cytosine is attached to a ribose ring (also known as a ribofuranose) via a β-N_1-glycosidic bond. Cytidine is a component of RNA. Dietary sources of cytidine include foods with high RNA (ribonucleic acid) content (Jonas et al., 2001), such as organ meats, Brewer's yeast, as well as pyrimidine-rich foods such as beer. During digestion, RNA-rich foods are broken-down into ribosyl pyrimidines (cytidine and uridine), which are absorbed intact(Jonas et al., 2001), In humans, dietary cytidine is converted into uridine

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(Wurtman et al., 2000), which is probably the compound behind cytidine's metabolic effects. There are a variety of cytidine analogues with potentially useful pharmacology. For example, KP-1461 is an anti-HIV agent that works as a viral mutagen and zebularine exists in *E. coli* and is being examined for chemotherapy. Low doses of azacitidine and its analog decitabine have shown results against cancer through epigenetic demethylation. In addition to its role as a pyrimidine component of RNA, cytidine has been found to control neuronal-glial glutamate cycling, with supplementation decreasing midfrontal/cerebral glutamate/glutamine levels. As such, cytidine has generated interest as a potential glutamatergic antidepressant drug (Machado et al., 2010). However, further research is to test the biological activity of each extract of n-hexane, ethyl acetate and methanol *Thuidium tamariscellum*.

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Bioprospecting of Fungi Associated with *Cladiella* sp. as Antibacterial-MDR against *Acinetobacter baumannii* from Panjang Island Vicinity

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Abstract

Multi-drug Resistance (MDR) bacteria occured from the extensive use of antibiotics which affected the increase in morbidity, mortality, and expense of healthcare settings. *Acinetobacter baumannii* is a Gram-negative bacillus where it causes serious infections such as urinary tract infection, community-acquired pneumonia, meningitis, bloodstream infection, and wound infection. The ability to survive in a wide range of natural environment and form biofilm lead this organism to be more difficult controlled and treated. In order to decrease exploitation of soft coral which had pharmaceutical potential, marine fungi associated with *Cladiella* sp. was obtained as alternative source to produce antibacterial-MDR. The aim of this research was to screening and characterized fungi associated with *Cladiella* sp. which had potential against *A. baumannii*. One isolate from 8 isolates was successfully isolated and screened using by agar plug method. This potential isolate has been identified in morphology and molecular based on sequence analyses of 18S rRNA. Isolate FSP-11-A3 was identified as *Aspergillus clavatonanicus.*

Keywords: A. baumannii, antibacterial, marine fungi, MDR, soft coral, screening

1. INTRODUCTION

According to [1] World Health Organization (WHO) has identified that antimicrobial resistance is one of the three most important problems faced by public health. MDR pathogens are the most common and is often a serious problem which has the acronym "ESKAPE", abbreviation from *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. [2].

Acinetobacter baumannii (MDR-AB) is one of the bacteria that cause nosocomial infections which is difficult to be handled and controlled [3]. Nosocomial infection according to [4] defined as an infection that occurs in the hospital. This infection occurs in Intensive Care Unit (ICU) patients who relatively obtain long treatment and have a low immunity thus this bacteria become opportunistic pathogens for patients [5]. MDR-AB is a gram-negative bacilli bacteria that can cause nosocomial infections such as urinary tract infection (UTI), meningitis, bacteremia, pneumonia and wound infections [1, 3, 5, 6, 7].

One of the factors that led MDR-AB to be pathogenic bacteria that are difficult to control is the ability to form biofilms that cause these organisms can survive on less favorable conditions [8]. MDR-AB has been known to be resistant to the several classes of antimicrobial agents such as colistin, tigecyclin, AmpC enzymes, and carbapenemases in various combinations [9]. The impact of MDR-AB infections cause the mortality rate

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increased from 26% to 68%, furthermore the the longer the treatment, the higher the costs incurred by patients [10,11].

In such conditions, the discovery of antibiotics in order to overcome resistance in MDR-AB is required. Active compound from the sea as an antibacterial agent that can control MDR-AB has not been reported, moreover sea is known as a unique environments that can serve as a source for the discovery of new microbes such as bacteria, fungi, actinomycetes, cyanobacteria and diatoms that produce the potential active compound [12, 13]. Filamentous fungi derived from marine bioactive compounds have potential as an antibacterial, antiviral, and antifungal [14, 15].

Soft coral is one of marine invertebrate that can be used as a habitat for fungus to grow. This association is related in terms of nutrients, photosynthesis, nitrogen fixation and self-protection [16]. Previous research revealed that an extract from one of the soft corals *Cladiella* sp. isolated from the Andaman islands, India had antibacterial activity against *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa* at a concentration of 50µg /ml in which these studies required *Cladiella* sp. dry weight of 4.5 kg [17]. Bioactive generally produced in small amounts in each organism, utilization of the macroorganisms in this era is not relevant with the conservation issues, so that from the previous studies about bioactive of *Cladiella* sp., it is expected to have the similar bioactive compound produced by fungus associated with the organism, because microorganisms associated with macroorganisms have a symbiotic relationship that is specific to its host [18, 19], thus the fungus can be used as a sustainable source of bioactive compounds because they grow faster than macroorganisms.

Panjang Island became the objective of the sampling location because of the region's coral reefs are under threat of degradation of human activities on the north coast of Java [20, 21]. This fact became one of the factors to increase the likelihood of obtaining a secondary metabolite that has potential as an antibacterial, because that secondary metabolites are not used for growing but formed from primary metabolites under stress conditions [22]. Environmental conditions in the waters of Panjang Island is expected to be the trigger for the discovery of the potential fungi association soft coral as antibacterial agent, particularly MDR-AB.

The current research was designed to screening and characterized fungi associated with *Cladiella* sp. which had the potential against MDR-AB. The result of this research hopefully could give information about marine fungi associated with *Cladiella* sp. which produce antibacterial MDR-AB environment friendly, decrease the impact of coral reef's degradation because of the exploration of natural product from the sea. It also can give contribution to industrial pharmacy about potential bioactive compound from the sea.

2. MATERIALS AND METHODS

2.1. Collection of Sample

Sample was taken from the vicinity of Panjang Island (06^{o}34$^{'}$S and 110 ^{o}37’E) using snorkeling technique. Samples were taken at a depth of 1-3 meter. Sample was taken and put in a zip lock plastic bag to be stored temporarily in a cool box.

2.2. Identification of Soft Coral

Soft coral identification was conducted by morphological observation soft coral in the water, then compared with the morphological characteristics in accordance with the book Soft Corals and Sea Fans [23]. In addition, the microscopic morphology was also necessary to view at the shape of sclerites, so it could determine that the sample is a type of *Cladiella* sp. [24].

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2.3. Isolation and Purification

Isolation of fungi was conducted using method of [25]. Sample was initially cleaned with sterile sea water, and then cut by 1 cm^2 and slashed the inner part so that the fungi that lived in the tissue could be cultured. Sample was transferred on a petri dish that already contained MEA (Malt Extract Agar) with sea water. Sample was incubated for 3 – 4 days at room temperature. The fungus was separated by morphology of colors, shapes, and sizes.

2.4. Antibacterial PreliminaryTest

This test was performed by agar plug diffusion method [26]. MDR-AB was obtained from clinical cutures of Kariadi Hospital Semarang. Fungi associated with *Cladiella* sp. had been grown in MEA with sea water for 7-14 days so secondary metabolites have been secreted. MEA which grew the fungi were then cut cylindrical to put on the NA (Nutrient Agar) inoculated bacteria MDR-AB 1x24 hours with a density of 0.5 McFarland. Both were then incubated at room temperature for 1-2x24 hours. Inhibition zone showed that fungi produced a potential bioactive compound against MDR-AB.

2.5. Molecular Identification

DNA extraction was conducted using chelex [27]. DNA concentration was checked to minimize failure in DNA using NanoDrop. DNA Amplification was done using PCR. The process of denaturation at 95 ° C for 3 minutes, then 35 cycles (denaturation at 95 ° C for 1 minute, annealing at 56,4^o C for 1 minute, and extension at 72^o C for 1 minute), followed by post cycling in 72^o C extension for 7 minutes and Last 16^o C. The primer used for PCR 18S rRNA was ITS1 as the forward primer (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 as reverse primer (5'-TCCTCCGCTTATTGATATGC-3'). Furthermore, the gel electrophoresis to see the DNA bands formed and visualized using Geldoc. The PCR products were then sequenced and analyzed using MEGA 6.06.

3. RESULTS

3.1. Identification of Soft Coral

Purposive sampling in Panjang Island was obtained *Cladiella* sp. (Fig 1).

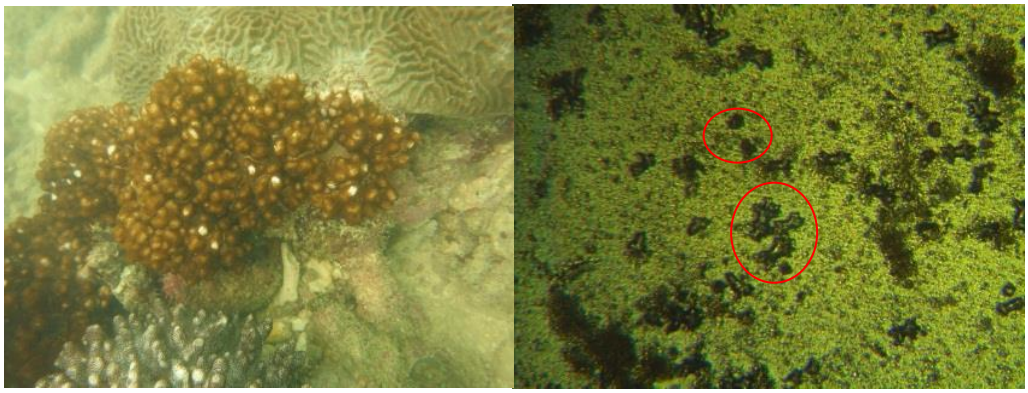

Figure 1. Colony of Cladiella sp. in the water (left); Sclerites of Cladiella sp. (right)

3.2. Antibacterial Preliminary Test

There were 8 isolates from association fungal with *Cladiella* sp and only 1 isolate was inhibited the growth of MDR-AB (Table 1).

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Table 1. Antibacterial Activity from Fungi Associated Cladiella *sp.*

Source	Isolate Code	Activity Against MDR-AB
Cladiella sp.	FSP-11-A1	-
	FSP-11-A2	-
	FSP-11-A3	+
	FSP-11-A4	-
	FSP-11-A5	-
	FSP-11-A6	-
	FSP-11-B1	-
	FSP-11-B2	-

3.3. Molecular Identification

Molecular identification of the potential isolate based on 18S rRNA, revealed that the isolate belong to the members of genus Aspergillus (Table 2 and Fig 2)

Table 2. Molecular Characterization of Fungal Associated with Cladiella *sp.*

Isolate Code	Close Relative	Similarity	Acc. Number
FSP-11-A3	*Aspergillus clavatonanicus*	99%	NR_135410.1

.

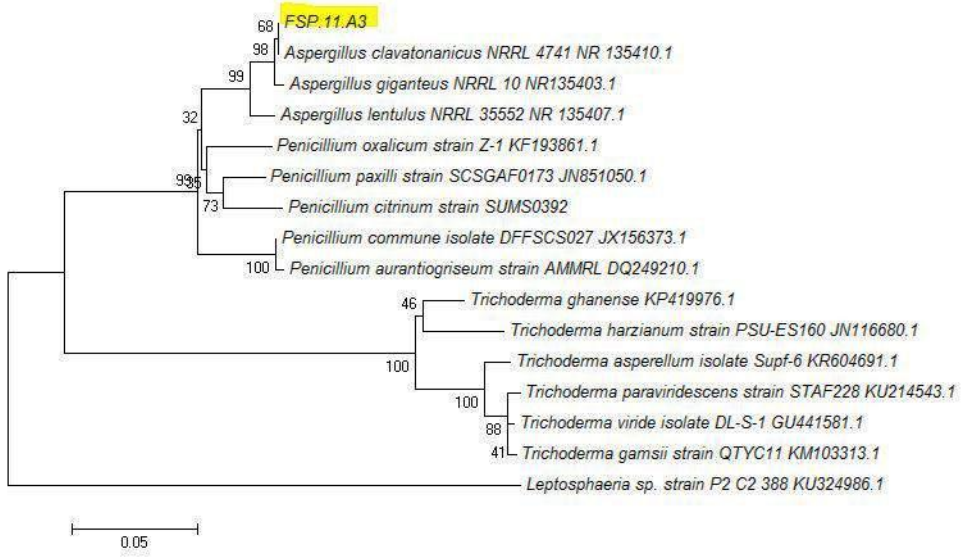

Figure 2. Phylogenetic Tree of Potential Fungal Associated with Cladiella sp.

4. DISCUSSION

Indonesian Government through Ministry of Health Policy Number 8 Year 2015 explained that the increased incident and spread of microbes that are resistant to antimicrobial were caused by the inappropriate use of antibiotics wisely and low of obedient. MDR-AB has proven to be rapidly existence toward antibiotic [9]. The

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discovery of bioactive compounds which is inhibiting the growth of MDR-AB needs to be done before there is no antibiotic that is able to control it. Many drugs that are patented derived from terrestrial, while Indonesia has become one of the countries that have the potential of marine pharmaceutical which has not been studied widely. Marine invertebrates were known to have many bioactive. One of the prospective source for searching natural product from marine environment is soft coral. Bioactive compound from this organism was identified as antimicrobial agent [28], anti-inflammatory [29], antioxidant [30], antitumor [31], anticancer [28]. Moreover many potential natural products are hampered by the scarcity of source material, for examples, 10 mg of spongiostatin is obtained from 400 kg of the sponge, 300 mg halichondrrin B from a ton sponge of *Lissodendoryx* sp. and 18 g bryostatin from 10,000 gallons of bryozoan collecte [32], this fact is harmful for equilibrium of environment. Therefore fungi associated with soft coral can be a sustainable source because of the ability to grow rapidly and the environment kindly. In addition, microorganism symbionts are responsible for producing secondary metabolites previously attributed to the host organism [18, 33, 34].

In this study *Cladiella* sp. was obtained as biota target (Fig 1) because this organism can live in a turbid waters [35]. The pharmaceutical potency of this organism also lack of information if it was compared with others soft coral like Sinularia, Lobophytum and Sarcophyton. Observation of sclerites was conducted to determine the biota target. According to [24] this organism has two kinds of sclerites, their shapes were double head and platelet (Fig 1).

8 isolates were obtained from the isolation of fungi associated with *Cladiella* sp., in which only one isolate active against MDR-AB (Table 1) encoded with FSP-11-A3. The ability of MDR-AB to produce biofilm [8] may prevent themselves from bioactive compound secreted by fungi. [36] mentioned that species growing in a biofilm can be more tolerant of an antimicrobial substances. Biofilm of MDR-AB was self-defense that protect them from unfavorable niche. This potential isolate may prevent biofilm formation by interferring with intercelluler communication. It showed chance to develope in further research.

Identification was needed to preserve this potential isolate. Identification of this fungi according to microscopic morphology and molecular based on 18S rRNA. Morphology under microscope of FSP-11-A3 involved conidiophore, vesicle, phialid and conidia. The result of sequences analysis with BLAST, this species had 99% similarity with *Aspergillus clavatonanicus NRRL 4741* (Table 2). Phylogenetic tree was constructed using Neighbor-Joining to describe relationship between this isolate and the other fungi that have antimicrobial activity (Fig 3). FSP-11-A3 was the same clade with *A. giganteus* which has the antifungal protein NN5353 that inhibits the germination and growth of filamentous fungi including *A. nidulans* and *A. niger* [37].

5. CONCLUSIONS

Marine fungi associated with *Cladiella* sp. has the ability to inhibit the growth of MDR-AB. It overcome the supply of bioactive compound which is environmental friendly. Further research still needed to discover MIC and its mechanisms to inhibit the growth of MDR-AB.

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RENEWABLE ENERGY

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Biogas Production and Biodegradability on Increasing Total Solid of Household Organic Waste

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Abstract

Anaerobic digestion is one of the potential technologies prior to assure betterment in landfill management. In fact, anaerobic digestion (AD) produce biogas which can be potential renewable energy source, energy recovery and nutrient soil replacement through digestate composting. AD is a biological method that potentially to convert organic waste into stable product for land amendment with reduced environmental impact. As to evaluate the performance of AD, it is important to interpret the relationship between solid content on AD productivity. The objective of this study is to determine methane (CH_4) production and CH_4 content of household organic waste (HOW) at different types of AD systems (wet and dry digestion). The effect of total solid (TS) content (%) on AD of household organic waste (HOW) was investigated in batch reactors over wet digestion (<10%) and dry digestion (>15%). pH level was maintained at optimum value (6.0 – 7.2) by controlling the buffer capacity of the reactors. The highest CH_4 production was 63.7 L/KgVS (TS 15%) followed by 29.8L/KgVS (TS 10%). Increasing of 5% of TS content contribute to 30 – 60% increment in CH_4 production. The result clearly demonstrate that the increasing of TS content in feedstock has certain effect on optimum CH_4 production.

Keywords: Anaerobic Digestion, Biogas, Organic Waste Treatment, Renewable Energy

1. INTRODUCTION

Household organic waste (HOW) generates from residential areas is the single-largest portion of the waste stream in Malaysia. HOW mainly constitutes from food waste and other substitutes of organic waste; is increasing and becoming a burden in landfill management due to its high biodegradability and high moisture content. Indiscriminate decomposition of this organic waste results in large-scale contamination of land, water, and air probably from HOW wastewater or so-called leachate. Organic waste disposal enhances ecological problems starting from waste handling, transportation and disposal that are prone to public health concern and environmental sustainability issues [1]. Hence, alternative methods for food waste disposal are needed to tackle the waste crisis. To date, anaerobic digestion (AD) has become an intensive field of research, since the organic matter in food waste is suited for anaerobic microbial growth [2]. Effective on 1st September, 2015, most states in Malaysia have adopted the solid waste and pubic

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cleansing management Act 2007 (Act 672) which are set to see a whole new dimension in how they will be disposing the household waste especially on food waste. Anaerobic digestion can be potential biological treatment not only concerns on organic waste treatment but also contributes to energy and mass recovery.

Anaerobic digestion is a multi-stage biochemical process in which the complex organic materials undergo hydrolysis, acidogenesis, and methanogenesis in series and each metabolic stage is functioned by different types of microorganisms [3]. To date there are two main types of AD technologies have been developed according to the total solids (TS) content of feedstock: conventional wet (≤10% TS) and modern dry (≥10% TS) technology. Dry anaerobic digestion also known as "high-solids" technology, has becoming interesting and is widely applied in waste treatment technology. There are many benefits offers from dry-AD from its simplicity such as requires smaller reactor volume, lower energy requirements for heating and less material handling [4]. According to Pavan et al. (2000), TS content of solid waste provide significant effect on anaerobic digestion performance, especially biogas and methane production efficiency [5]. Generally, with the increasing of TS contents; the total CH_4 yield increase while the efficiencies of both solid reduction and solid conversion to CH_4 decreases. The results obtained by Duan et al. (2000) showed that high-solids system could reach much higher volumetric methane production rate compared with low-solids system at the same solid retention time (SRT) in mesophilic anaerobic reactors treating sewage sludge [6]. Forster-Carneiro et al. (2008) showed that the biogas and methane production decreased with the total solids contents increasing from 20% to 30% in dry batch anaerobic digestion of food waste [7].

The putrescibility (degradability) of the fresh fed materials such as food waste was reported to be an important information for preventing failures, estimating biogas production and managing the digestion process [8]. Therefore wide differences in the waste composition and putrescibility may lead to different behaviors during the high solid anaerobic digestion. To understand the factors that determine the suitability of high solid anaerobic digestion process condition for particular substrate, a deeper analysis must be carried out. It is well known that the reasons for the organic overload inhibition of a methanogenic process are particularly related to the putrescibility of the organic matter. In order to increase the efficiency of high solid of anaerobic digestion on HOW, it is necessary to understand the effect of TS contents (%) behavior in anaerobic digestion from wet to dry technology. Hence, the aim of this study was to conduct a comprehensive comparison of mesophilic anaerobic digesters treating HOW with different TS contents ranging from 5%–15% to their respective performance on CH_4 production. It was expected that the reported work herein will reveal the role of the TS content on the behavior of the CH_4 production and hence to effective guide high solids anaerobic digestion of HOW and to optimize the operational conditions for high anaerobic digestion efficiency.

2. METHODOLOGY

2.1 Preparation of Feedstock and Inoculum

2.1.1 Preparation of Synthetic Household Organic Waste

Synthetic household organic waste used in this study is prescribed as food waste described by Chang et al., 2008. This is to provide consistency during monitoring and to reduce possible interference during the experiment. The synthetic food waste will be simulated as in Table 1. The feedstock was kept frozen (-2^{o}C) until required for feeding.

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2.1.2 Inoculum Preparation

Inoculum was collected from fabricated household bioreactor located at *Department of Agritechnology and Bioscience of Malaysian Institute Nuclear Technology (MINT).* The inoculum was stabilise and observed until reaching the stability phase; acclimatised (CH_4 = 80%).

Table 1. Composition of Synthetic Food Waste [9]

Composition	Value (%)
Moisture Content	65 – 80
Ash	3 – 5
Volatile Matters	18 – 30
Carbohydrate	40 – 60
Protein	10 – 30
Fat	15 – 40
Carbon	45 – 65
Hydrogen	6 – 7
Nitrogen	1 – 3
Oxygen	40 – 50
C/N	15 – 40

2.2 Anaerobic Reactor Design and Operation

Anaerobic digestion of household organic waste was examined using batch experiments at solid contents of 5%, 10% and 15%. The assays were conducted in 200L household bioreactor™ (NUKLEARMALAYSIA ref no:2016/L/26) with total working volume of 160L. The reactor incorporated with 2 separated ports inlets for different function of feedstock feeding, biogas measurement and biogas collection that connected to 40L×3 standard FlexFoil gas bag with single polypropylene fitting (SKC brand;USA) with single outlet for digestate sampling. Liquid and gas sampels were taken at regular time intervals. Each digester was manually mixed once a day. The biogas production volume standard temperature and pressure is measured periodically by water displacement method and calculated as volume at STP condition.

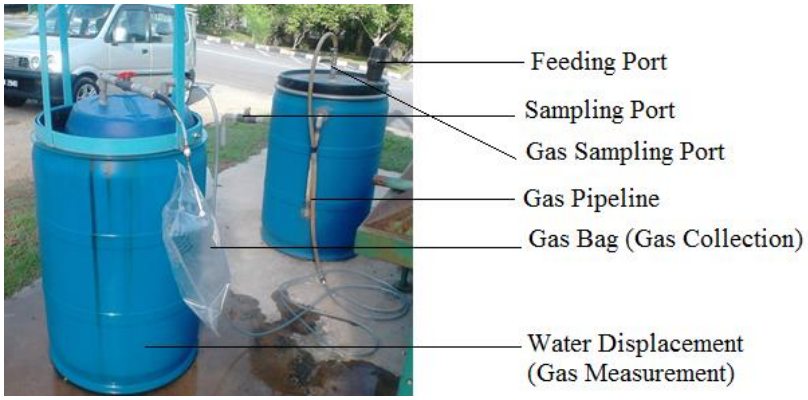

Figure 1. 200L Household Bioreactor ((NUKLEARMALAYSIA ref no:2016/L/26)

2.3 Analytical Procedures

Substrate samples were analysed twice a week to monitor total solids (TS), volatile solids (VS), alkalinity, total nitrogen (TKN), total organic carbon (TOC), chemical oxygen demand (COD) and ammonium nitrogen (N_4^+-N). All the analytical monitoring was determined according to Standard Methods (2005) [10]. Elemental

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analysis such as total carbon, total nitrogen and total sulphur were analysed using ASS based on liquid extraction method whereas nitrogen, phosphorus and potassium (N, P and K) were measured by soil extraction method using Mac CNS analyser and crude protein was determined by multiply total nitrogen values with 6.25. The biogas composition (CH_4 and CO_2) analyses was carried out by gas chromatography separation (6890N Agilent Technologies, CA, USA) with thermal conductivity detector (GC-TCD), equipped with a Hay sep N 80/100, a molecular sieve column (5A 60/100). Argon (Ar) was used as carrier gas at a flow rate of 3.0mL/min and the average velocity at 22cm/sec. The injector, oven and detector temperatures were 35^{o}C and 230^{o}C.

3. RESULTS AND DISCUSSION

3.1 Composition of Inoculum and feedstock

Table 2 shows the physical-chemical characterisation of substrate, inoculum and initial substrates operated at different TS contents. The experiments were concluded when no significant variation of CH_4 production (<1%). CH_4 production and CH_4 content (%) were observed during experiments. In addition, the reactor stability was maintained and the digestion occurred normally because a constant pH was maintained for each reactor. The average pH value was about 6.8, 7.6 and 7.2 at 5%, 10% and 15% TS, respectively. These pH values were within the permissible range for AD 6.5–8.5 but not with the optimal range 6.8–7.4. As we all know, the increase of VFA concentration contributes to the decrease of pH. It could be explained by the fact that high buffering capacity was observed in high-solids anaerobic system at TS 15%.

Table 2. Average Value Physical-Chemical Characteristics of Substrates and Inoculum

Parameter	HOW	İnoculum	Mix 1	Mix 2	Mix 3
pH	5.8	7.4	6.4	6.3	6.0
TS (%)	82	4	5%	10%	15%
Moisture Content (%)	70.1	64.8	76.5	70.5	66.3
COD (mg/L)	7380	6879	5452	6380	6550
C:N	18.5	14.2	11.2	18.3	24.5

3.2 Effect of Total Solid Content in Biogas Production

The experimental results were obtained after a period of 20 days for 10% and 15% TS contents and shorter period for 5% TS biogas reactor (13 days), when the batch assays ended with a CH_4 production less than 1%. Figure 2 shows the daily CH_4 production during the experiment operated at different TS contents (%). CH_4 production in 15%TS content is the highest (63.7L) followed by 10%TS content (27.8L). Both of CH_4 production (L/Kg VS) and CH_4 content (%) showed increasing trend with increasing TS contents. This result was in contrast with a previous work [11], in which the reactors with smaller TS contents showed higher biogas production and CH_4 percentage in the batch anaerobic digestion of food waste. It was suggested that the increasing of feeding TS contents lower than 20% has positive effect on the methane production. Increasing TS content 5 – 15% in biogas reactor induced 30 – 60% increment in CH_4 production. The decrease of CH_4 generation is detected started from day 7 until the end of the experiments due to the consumption of main degradable organic matter.

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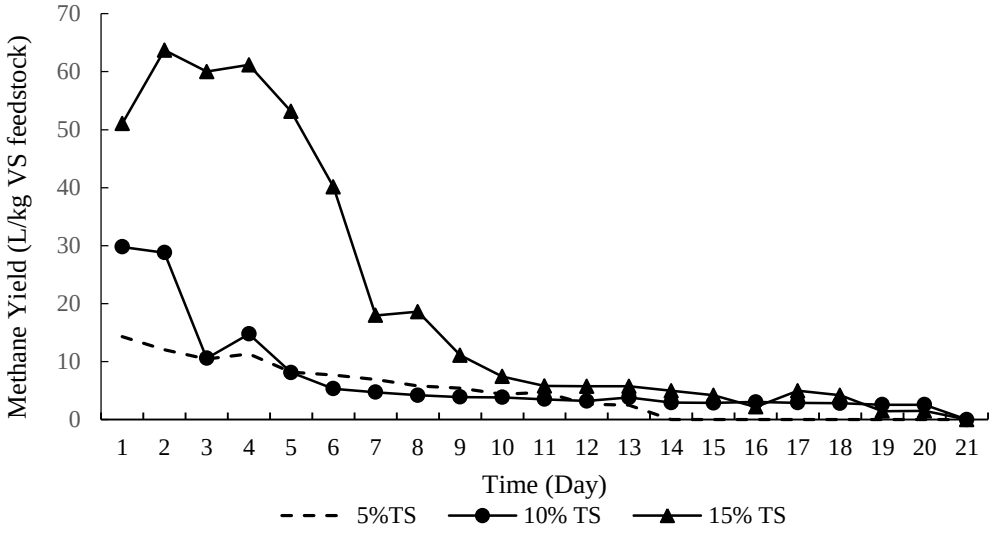

Figure 2. Daily Methane Yields of Increasing TS contents

The daily CH_4 content (%) produced from 5%, 10% and 15% TS biogas reactor are dipicted as in Figure 2. The highest CH_4 content (%) produced was 82.1% and the steady state reached between day 5 – 9 (75 – 80% CH_4). The same phenomenon was also observed in the anaerobic digestion of solid food waste where the CH_4 content imrpoved with an increasing concentration of TS content in food waste [12]. The effect and stability of CH_4 production also seen between day 10 – 14 whereas digestion process has stopped for biogas reactor with 5% TS content. The CH_4 content varied approximately 50 – 80% for 5%, 10% and 15% TS contents respectively. Increasing TS contents (%) may enhance the stability of the anaerobic digestion process due to carbon to nitrogen ratio (C:N) balance.

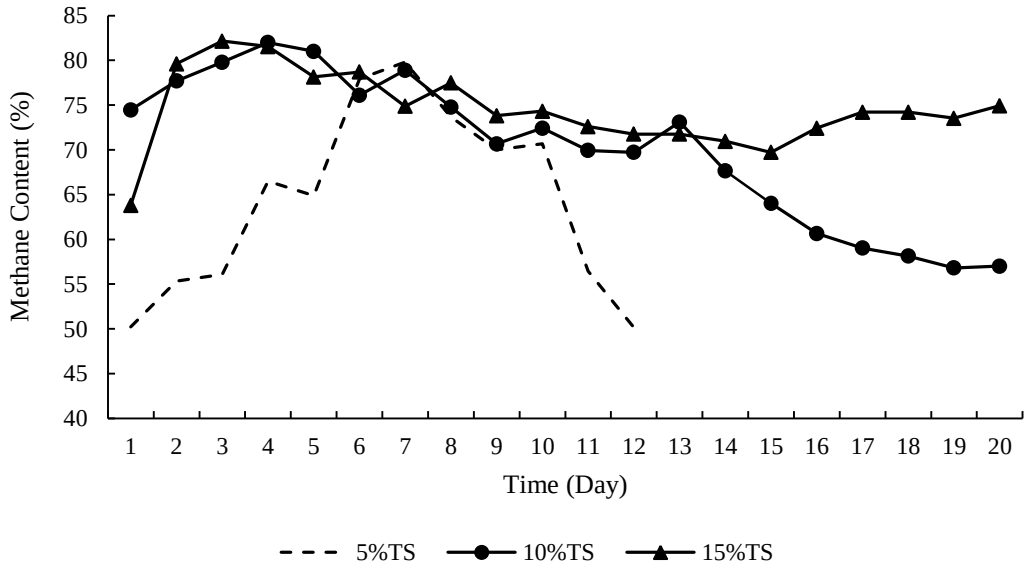

Figure 3. Daily Percentage of CH_4 Generation Increasing TS contents

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4. CONCLUSION

Adaptation of the increasing TS contents on anaerobic digestion of household organic waste was establish over a period of 13 – 20 days. The experimental methane yield was enhanced by 30 – 70% increment in CH_4 production from 5 – 15% TS content. The maximum CH_4 content percentage was observed in ranged of 50 – 80% from different operations. The CH_4 content in all reactors varied was likely due to homogeneity of the feedstock..

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Leachate Treatment using Advanced Oxidation Process with Zeolite as a Catalyst

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Abstract

Leachate of three years old active landfill was identified to have a very low biodegrability (low BOD/COD). Ozone-based on Advanced Oxidation Process (AOP) techniques have been currently developed by utilizing heterogeneous catalysts. This study treated leachate from an active landfill with the O_3/H_2O_2 with addition of natural zeolites as catalysts. It was done to show some effect of natural zeolites mass to accelerate the formation of $OH^{\cdot}$; the response of leachate matrices; and the effectiveness of the process. The study was conducted on a semi-batch reactor with volume of 500 mL. Oxygen from the ambient air was supplied using an aerator into ozone generator to produce ozone at rate of 3 L/min. At the ozone contactor ozone was contacted with 250 mL of leachate samples and 1.197 g / L H_2O_2. The addition of zeolite was done by varying its mass at 0.5 grams, 2.5 grams and 4.5 gram inside the ozone contactor. The results showed that the pH increased gradually. The best results were shown on the addition of 4.5 gram zeolites (AOP-K-4.5) which was capable of removing as much as 23% electrical conductivity , 57.14% turbidity, 28.68% organic aromatic as UV_{254} and 88.89% COD. The results showed, the more zeolites was added, the higher the removal efficiency, but in this study the addition of optimum mass of zeolites was not yet have been found.

Keywords: Leachate, Advanced Oxidation Process, Ozone, Catalyst, Semi Batch

1. INTRODUCTION

One of the main problems in solid waste management in Indonesia is the degradation of the landfill quality. In Indonesia, most landfills are designed to be sanitary landfill, but in fact become controlled landfill even open dumping in its operation (Adnan, 2008). The high operation cost of sanitary landfill and less effort in waste minimization generate high rate of cell accumulation in the landfill. Furthermore, the lack of waste sorting and segregation at collection and transportation system create a pile of cell waste with mix condition between organic, inorganic, metallic even hazardous material from household and other activities. This condition generate leachate which contain dissolve contaminants that even more difficult to be treated biologically (Tizaoui, 2007)

In Indonesia, active landfill that operate more than two years generate leachate which has characteristic of neutral pH, high concentration of COD, as well as ammonia and alkalinity with low BOD/COD. Leachate from the complete landfill has different characteristics which has lower concentrations whereas nitrite begins to form. Meanwhile, leachate from old cell has low organic concentrations and generally contain non-biodegradable organic materials (Renou et al., 2008). The technique which commonly used to treat leachate in Indonesia is the stabilization ponds (Damanhuri, 2010). It is also used at Sarimukti landfill, and based on observation of leachate treatment plant, the COD removal efficiency of only 30%.

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Wastewater treatment technology with ozone based is currently developing. The main reasons for the use of ozone are disinfection and oxidation or a combination of both (Von Gunten, 2003). In the water, ozone will be decomposed into OH, entities with the greatest oxidation power in water (Acero & Gunten, 2000; Von Gunten, 2003). Thus, the assessment of ozonation processes involves the two species ozone and $OH^{\cdot}$. The decomposition of ozone in the water can be accelerated by the Advance Oxidation Process (AOP). One of the popular of AOP is by the addition of chemicals such as H_2O_2 (Fernando, 2003; Von Gunten, 2003). The addition of 3 g / L H_2O_2 in leachate samples taken from Sarimukti landfill, have efficiency of COD up to 80%, with a contact time of 180 minutes (Sururi, et al., 2014). However, Sururi et al (2014) was diluted the leachate so that one problem of this research was to found the effeciency of the AOP process with undiluted sample.

The addition of catalysts in ozonation process is also developing and one of the catalysts used are zeolites (Ikhlaq et al., 2012). The porous media such as zeolite can be used as adsorbent media for hydrophobic properties and increasing decomposition of ozone (Chen et.al, 2008; Kwong et.al, 2008). Chen et.al (2008) using a high-silica zeolite media, where in the silica has an affinity ability to adsorb molecules of ozone and hydrophobic pollutants. Application of ozonation technique by using a catalyst appears to be a promising alternative to the leachate treatment process. Ikhlaq et al., (2012) investigated the mechanism of ozonation process with zeolite catalyst, to distinguish the ozone and OH, as well as separating the functions of zeolites as adsorbent by using artificial sample. The results showed at the ozonation process with catalyst, the $OH\cdot$ has much larger role than ozone, meanwhile the role of zeolite as pollutant adsorbent can be ignored in this process (Ikhlaq et al., 2012). Nevertheless, Ikhlaq et al., (2013), assumed that zeolites has dominant role as an ozone adsorbent. Zeolites serve as reservoirs for organic compound and ozone, so that zeolite do catalyse decomposition of pollutants by direct reaction between adsorbed ozone and pollutants on their surface where ozone decomposition reactions takes place (Ikhlaq, et al., 2012). However, the activity of zeolites depend on the silica to alumina ratios (Ikhlaq, 2013).

Appropriate environmental technology element is emphasized by the use of local zeolite. Problems that occur when local zeolite will be used is a zeolite content locally dominated by silica compared to aluminium. Aluminium is acting as a catalyst. So in this study will be conducted AOP (O3/ H_2O_2) process with the addition of zeolite and.

2. MATERIALS AND METHOD

Samples were taken as a grab sampling from the leachate collection points from Sarimukti Landfill. The measurement was taken for these parameters: pH, alkalinity, turbidity, conductivity, aromatic organic, BOD and COD. The measurement of residual ozone concentration (ROC) was done at three last point sampling due to leachate characteristic. The methodology being used refers to the SNI and the Standard Methods for the Examination Water and Wastewater 21th edition.

Natural zeolites was used, the point zero discharge of the zeolites was determined by titration methods according to Preocanin& Kallay (2006). Firstly the zeolites were washed with de-ionized water and dried in an oven at 108^0C. Subsequently, zeolite as much as 0.1 gr was added to 25 mL of 10^{-3}mol/L NaCl with continues stirring. The pH change gradually and become constant at the point of zero charge (pH_{pzc}).

The study was conducted in a semi batch with continuous ozone gas supply to the contactor filled with leachate samples. The equipment used in these studies was aerator, flow meter, ozone generator and ozone contactor which can be seen at Fig.1. The flow rate of oxygen from the aerator to the ozone generator was set at 3 L/min. Ozone generator specification were OZF - 1G , POWER 200 W , 220 V, which changed the O_2 into O_3. Ozone that generated from the ozone generator was supplied to ozone contactor (500 ml) which had previously been filled with 250 ml of leachate sample, 1.197g/L H_2O_2(AOP). Zeolites as catalyst were added with mass variation of 0.5 gr (AOP-K-0.5) ,

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2.5gr (AOP-K-2.5), and 4.5 gr (AOP-K-4.5). For a presence of a homogeneous ozone through the leachate sample, ozone gas was supplied through air diffuser and completed with magnetic stirrer.

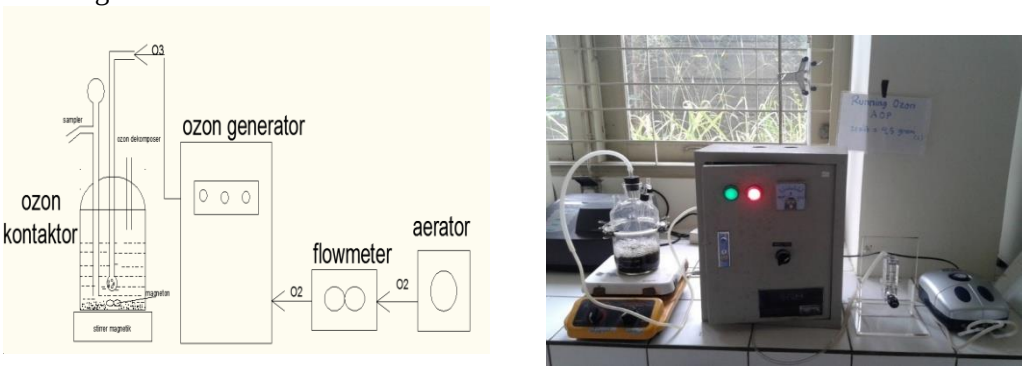

Figure 1. Reactor Configuration

Total contact time in every variation was 180 minutes, sample was taken every 30 minutes. Parameters such as ozone residual concentration dissolve oxygen, pH, turbidity, conductivity, aromatic organic, and COD were measured to see the dynamic changes of every parameter.

3. RESULT

3.1. Zeolites and Leachate Characteristics

pH PZC (Point of Zero Charge) is the pH where the zeolite is not charged or when positive and negative charges are equal (isoelectric point). pH was gradually decreasing that it would be reached a point where the positive and negative charge is equal to zero. The measurement results show the value of pH PZC zeolite used is 6.4.

Table 1. Leachate Characteristic

Parameter	Concentration
pH	7.84 – 8.11
Temperature	24– 29°C
Carbonate (CO_2^{2-})	2800 mg/L
Bicarbonate (HCO_3^-)	790 mg/L
Conductivity	24,7 – 30 mS/cm
Turbidity	80 -90 NTU
UV_{254}	33 – 37 Abs
BOD	326 – 591 mg/L
COD	4356 - 5594 mg/L

Samples were taken from influent of leachate channels which flow through the leachate treatment plant at Sarimukti. Since itis derived from young cell (active landfill), it has high concentration of COD, ammonia, and a very high alkalinity.

Table 1 shows, the pH of the leachate tend to be neutral toward alkaline with a pH range from 7.84 to 8.11. Comparison of BOD and COD only ranges from 0.07 to 0.11,

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this comparison indicates that the non biodegaradable organic matter were far more than the biodegradable. Conductivity of the leachate were at 24.7 to 30 mS / cm which shows that inorganic compounds in landfill leachate samples from Sarimukti relatively high when compared to other landfill leachate from Indonesia which is about 1000-40000 µS / cm.

At conventional ozonation process, carbonate and bicarbonate acts as a scavenger in the process of ozone decomposition. Scavenger is a term that is intended for compounds that consume OH· which can play a role either as an inhibitor or a promotor (Acero & Gunten, 2000). The high concentration of carbonates and bicarbonates in leachate samples can disrupt the process of ozone decomposition in which carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) will react with OH·to form a radical carbonate (CO3·) and inhibits the propagation process of the formation of OH ·. This is the reaction that occurs when OH · reacts with carbonate or bicarbonate ion (Von Gunten, 2003).

$$OH^{\bullet} + CO_3^{2-} \rightarrow CO_3^{\bullet-} + OH^- \quad (1)$$

$$OH^{\bullet} + HCO^{3-} \rightarrow CO_3^{\bullet-} + OH^- \quad (2)$$

However according to Acero and Von Gunten (2000), the addition of H_2O_2 can change the role of carbonate and bicarbonate to promote the chain reactions. Aromatic organic material which expressed as UV_{254} is associated with chromophoric dissolved organic carbon (CDOC). The organic material can react directly with ozone or indirectly with OH · (Von Gunten, 2003). Furthermore, the reaction that occurs when ozone and OH · react with organic predominantly were propagation reaction that produces radical superoxide although some other reactions only generate carbon radical center that will stop the chain reaction (Von Gunten, 2003).

3.2. Residual Ozone Concentration

In this study due to leachate physical characteristic, the ozone residual concentration was only measured at minutes 120, 150 and 180. The contact time was chosen at three last sampling points where there were already visible changes in physical parameters such as discoloration clearer.

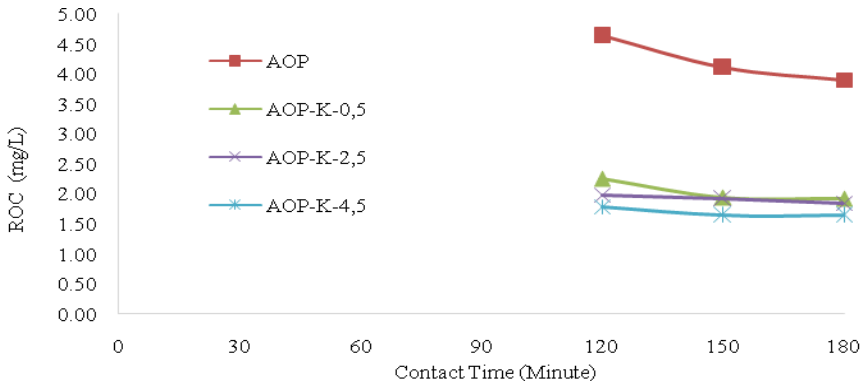

Figure 2. Residual Ozone Concentration

Figure 2 shows residual ozone concentration (ROC) on the variation of AOP (O_3 + H_2O_2), AOP plus catalyst that had mass variation of: 0.5 g (AOP-K-0.5),; 2.5 g (AOP-K-2.5); and 4.5 g (AOP-K-4.5). The graph shows, the highest of ROC was shown on the variation of AOP (AOP) without the addition of zeolite, the addition of zeolite were decreasing the ROC. The heavier zeolites, the faster ozone decomposition occurred. This phenomena was happened because the addition of zeolite as a catalyst in the process of AOP help the process of decomposition of ozone into OH·.

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3.3. Response to The Ozonization process

pH is used as an indication of the activity of hydrogen ions (Sawyer et.al, 2003). The high pH value indicates more hydroxide ions (OH-) that acts as an initiator in the decomposition of ozone (Gunten, 2003). Graph of the change in pH in any process variation during the ozonation contact time can be seen in Figure 3

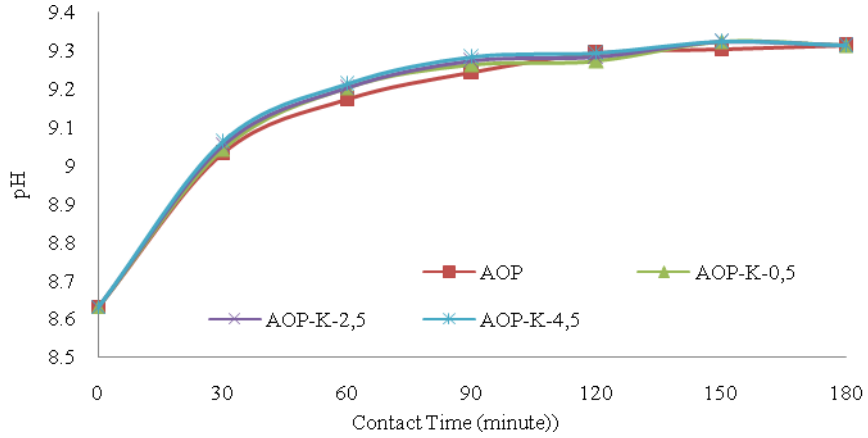

Figure 3. pH Changes on Each Variation

Figure 3 shows that all variations in ozonation process have pH increase at any time during the 180 minutes contact which indicate the formation of hydroxide ions increases. This formation can initiate ozone decomposition process and also indicate an increase of OH · during the oxidation process as shown at reaction 5.The pH of the water is important because hydroxide ions initiate ozone decomposition which involves the following reactions (Von Gunten,2003):

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \quad (3)$$

$$O_3 + HO_2^- \rightarrow OH\cdot + O_2\cdot \quad (4)$$

Reactions (3) and (4) show, the initiation of ozone decomposition can be accelerated by increasing the pH or by the addition of hydrogen peroxide.Superoxide radicals and hydroxyl radicals then act as chain carriers (Acero and Von Gunten, 2000).

3.4. Process Efficiency

3.4.1. Physical Parameter Removal

In this study, ions measured at electrical conductivity were assumed as inorganic content. All variations in the process of ozonation showed an increase in removing the electrical conductivity as shown at Figure 4 even though the difference was not significant. The difference in every variation was only about 1% due to relatively high electrical conductivity concentration or can be due to inorganic matter degradation difficulty. Although decomposition of ozone suspected more rapidly on AOP plus catalyst and OH· is more richer on that process, Acero and Von Gunten (2001) had informed that OH· only can play minor role because of its low steady state concentration, which results from high reactivity of leachate component. However, AOP K-4.5 shows the most prominent line than others which indicates the highest electrical conductivity removal efficiency.

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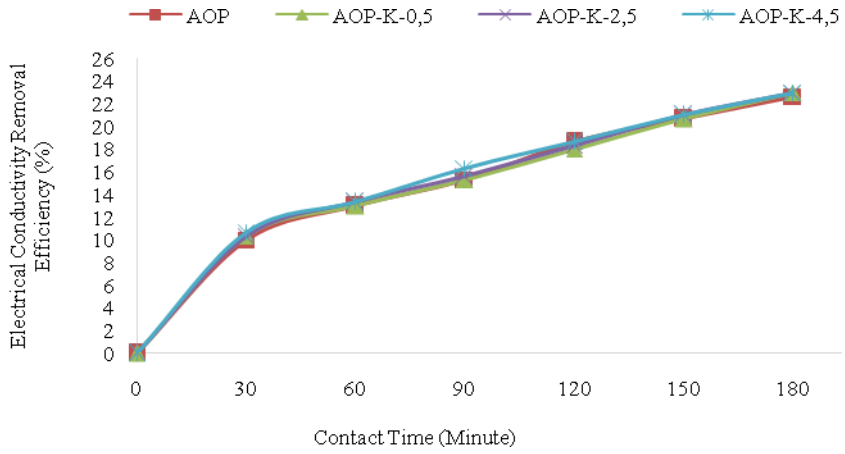

Figure 4. Electrical Conductivity Removal Efficiency

Figure 5 shows that for each variation, turbidity concentration were decreasing after the ozonation process during the contact time. The highest removal efficiency of found in AOP-K-4.5 with 57,14%, followed by AOP -K-2,5 with 44,00% and the next is variation of AOP-K-0.5 by 42,86%. The process without involving a catalyst with only AOP can only reduce turbidity up to 40,40%. Turbidity removal percentage was higher at every variation compare to electrical conductivity removel. Turbidity removalis describing capability of ozone and OH·on both organic and inorganic removal of the leachate.

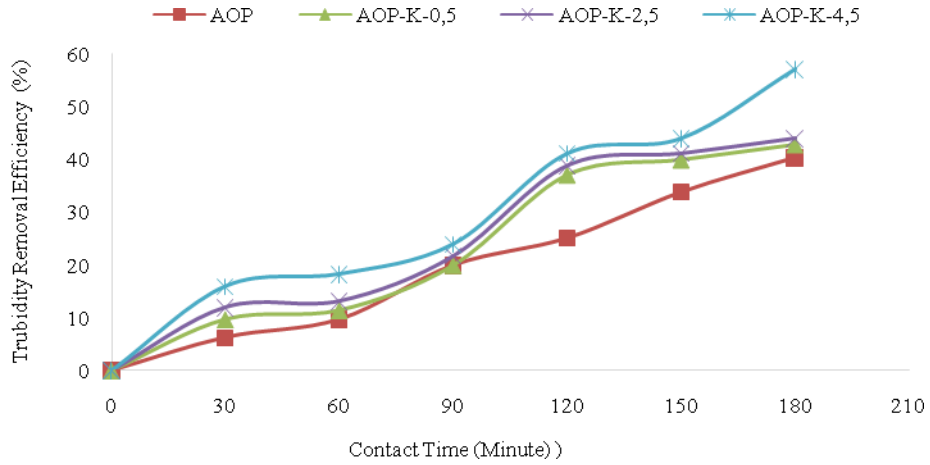

Figure 5. Turbidity Removal Efficiency

3.4.2. Organic Parameter Removal

Dissolved organic carbon is related with UV light absorption at UV spectro (Carter et al, 2012; Wang, Zhang, Shen, Chen, & Feng, 2014). UV_{254} were measured to determine the organic aromatic content, which included in NOM (Natural Organic Matter). Additionally, according to Cortez, et.al (2011), leachate has a very high content of organic matter such as humic and fulvic acid. NOM is an important parameter, where plays the role as the dominant premotor of the decomposition reaction of ozone. Reckhow et.al (1990) informed aromatic compounds having more activated site due to rich in electrons.

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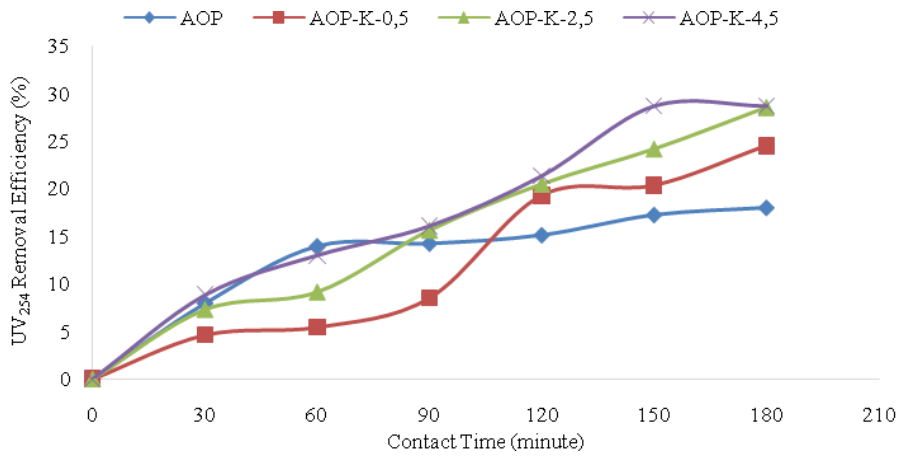

Figure 6. Organic Aromatic Removal Efficiency

The highest removal efficiency of organic aromatic was shown at variation of AOP-K-4.5 by 28.68%, followed by AOP-K-2,5 with 28.49% and AOP-K-0.5 by 24.48 %. While variations in ozone AOP process without the addition of zeolite (AOP) was the lowest removal efficiency with18%. These conditions support that more OH were produced during the variations of AOP with the addition of zeolite, it was better to degrade aromatic organic compounds.

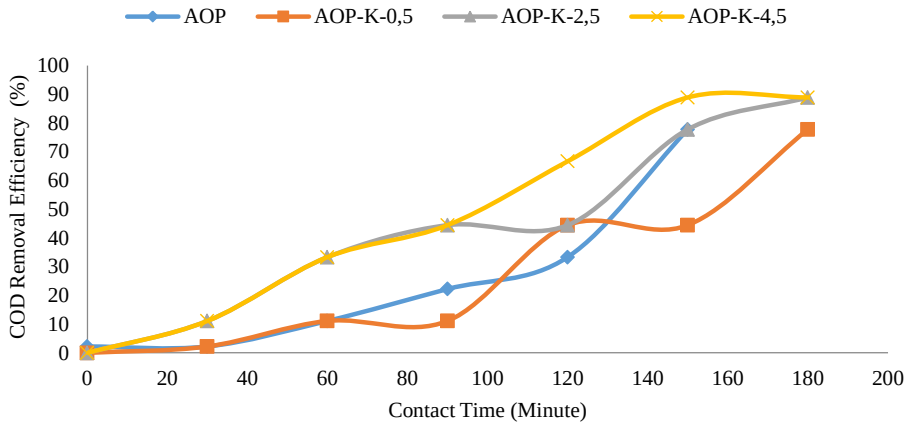

Figure 7. COD Removal Efficiency

Measurement of organic content in the leachate samples was also performed by measuring the chemical oxygen demand (COD) concentration. These parameters were measured to determine the content of biodegradable and non-biodegredable organic content. COD removal efficiency is highest in the ozone process variations with the addition of zeolite AOP-K-4.5 and AOP-K-2.5 with 88.89%, followed by process variation-K-0.5 AOP and AOP with 77.78%. COD removal caused by the process of oxidation by strong oxidizing OH · to the biodegradable organic materials and non-biodegredable which contained in the sample.Compared with organic aromatic removal, COD removal had higher percentage on every variation, this phenomena was confirmed that this process more suitable to removing diffrent stuructur of organic such as aliphatic. Direct reaction of NOM with ozone show at reaction 5-6, meanwhile reaction NOM with OH· shows at reaction 7-8:

$$O_3 + NOM1 \rightarrow NOM1_{0x} \quad (5)$$

$$O_3 + NOM2 \rightarrow NOM_2^{+\bullet} + O_3^{\bullet -} \quad (6)$$

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$$OH^{\bullet} + NOM_3 \rightarrow NOM_3^{\bullet} + H_2O \quad (7)$$

$$NOM_3^{\bullet} + O_2 \rightarrow NOM - O_2^{\bullet} \rightarrow NOM_3^{+} + O_2^{\bullet -} \quad (8)$$

A fraction of the NOM leads to carbon centered radicals (Eq. (7)). A reaction of these carbon centered radicals with oxygen subsequently leadsto the formation of superoxide radicals (eq. (8)), which react quickly with ozone to form OH · again

Zeolite had known as adsorben so that surface properties plays important rule on ozonation process. Ikhlaq et.al (2012) had informed the pH of solution plays an important role in understanding of the mechanisms of catalytic ozonation, since it affected by ozone decomposition(Fig. 3). Furthermore it determines surface properties ofcatalysts and properties of analytes being oxidised. At basic pH (pH > pH_{pzc}) the surface is negatively charged (no surface hydroxylgroups present), at acidic pH (pH < pH_{pzc}) its surface is positivelycharged, while at pH = pH_{pzc} the surface is neutral (Ikhlaq,2012). The natural zeolite was used on this process had pH pzc as much as 6.4, meanwile the pH of the solution is higher than pH pzc so that the zolite will be acted as adsorbent of O_3 and organic mater so that the reaction of ozone and organic will be at the surface of zeolite.

However, when ozone reacted with organic mater there will be transformation process, and that process will affecting decomposition of ozone. According to reaction 5-8 organic mater will play role as promotor of chain reaction so that OH · can play important role on this process regarding to RCO data on this research.

4. CONCLUSIONS

Zeolite addition at AOP process can enhance decomposition of ozone into OH·. This condition will improve the eficiency of the process to remove organic and anorganic matter on the leachate. The heavier zeolite, then the faster ozone decomposition occurred. The mechanism of AOP+Zeolite is depend on surface properties of zeolite. Need further research to determine the effect of pH, ratio of alumina and silika on zeolite.

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Performances and Emissions Characteristics of Three Main Types Composition of Gasoline–Ethanol Blended in Spark Ignition Engines

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Abstract

Ethanol as an alternative fuel for replace fossil fuels will be primary fuel of vehicles in the future. This is due to the combustion of ethanol will produces lower emissions than gasoline and its availability can be renewed (renewable energy). Some properties of ethanol is profitable when applied to gasoline engines, namely; high octane number allows to increase compression ratio that can reduce and avoid the detonation as well as increase engine power, latent heat of evaporation can lower the temperature of the exhaust so that NOx emissions can be reduced and high oxygen content can assist the combustion process to stoichiometric conditions so that CO and HC emissions can be lower. This paper will describe the characteristics of combustion of ethanol in three categories of composition, i.e. 0-20%; 25-40% and 50-100% in spark ignition engines either on the port fuel injection even in direct injection engine. The results show that at a concentration of 0-20% ethanol in gasoline, the mixture is an octane booster that can minimize detonation, increasing engine power and reduces emissions without changing the setting of operation engine. At a concentration of 25-40% addition of ethanol in gasoline is need adaptability in compression ratio depend on concentrations of ethanol in gasoline. While at the concentration of 50-100% setting operation of the machine for amount of engine parameter must be carried out simultaneously, namely the compression ratio, ignition timing and the equivalent ratio in order to produce a high performance engine and low emissions.

Keywords: Gasoline, Ethanol, Blended, Alternative Energy, Concentration

1. INTRODUCTION

The depletion of world oil reserves of fossil fuels as the main source of motor vehicles and the issue of environmental damage requires you to immediately switch to environmentally friendly renewable energy. It is based by the data that over 65% of the CO2 produced by the motor vehicle activity, the other coming from industry, households and other commercial. In addition to increasing CO2 products that have an impact on the greenhouse effect, even product emissions that result from burning fossil fuels such as CO, HC and NOx are also very dangerous for human health and survival of other creatures (1,2).

Bioethanol as a biofuel besides biodiesel and biomass developed since 1877 and was first used in Ford engines in 1908. Some of properties of ethanol are considered capable of being solution of environmental problems and limitations of fuel in the vehicle. The high latent heat of vaporization of ethanol will be able to lower the cylinder temperature and increase the density of the mixture so that will reduce NOx emissions and improve volumetric efficiency, torque and engine power (3). In addition, a third of which ethanol compounds containing oxygen will help complete combustion process to minimized CO and HC (4). Meanwhile, the octane number of ethanol higher than gasoline, allowing to apply higher compression ratio to produce more power as well (3).

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On the other hand there are some drawbacks of ethanol when compared to gasoline, thus requiring special handling to its application as a fuel for motor vehicles. The calorific value of ethanol is lower than gasoline causing specific fuel consumption of ethanol is higher than gasoline to produce same power. Ethanol solubility in water is very high causing these types of fuel is more corrosive and can damage the machine components. Ethanol as a fuel mixture in gasoline will cause demixing problems at the certain pressure and temperature so in these conditions there will be separation. Besides ethanol as a single compound, causing mixing with hydrocarbons takes azeatropica effect, which the vapour pressure of the mixture is lower than the vapour pressure of the constituent compounds.

This article is a result from review of several studies which will explain the characteristics of the combustion mixture gasoline-ethanol fuel in spark ignition engines. The fuel mixture is classified into 3 types, namely the composition of low, medium and high composition. The low composition contains percentage of ethanol to 20%, the middle type contains ethanol 25-40% and a high composition containing 50-100% ethanol.

2. COMPARISON OF GASOLINE-ETHANOL PROPERTIES AND ITS EFFECT ON COMBUSTIONS PROCESS

A property of fuel holds a very important role in the combustion process. Determination of engine operating parameters and settings based on the fuel properties as shown in table (1). By optimizing fuel properties, will obtain optimum engine performance. As a vehicle fuel, some of properties of ethanol shows disturbing differences compared to gasoline, including; calorific value, AFR, the latent heat of vaporization and others. Differences are also affected by some chemical compositions that make up the two types of fuel.

Hydrocarbons formed from nearly 90% of carbon content as a one indicator of a higher calorific value than ethanol that containing only about 50% carbons. The high calorific value of gasoline than ethanol causes potential energy generated from the combustion of gasoline 60% greater than ethanol in the same mass or volume. Thus, to produce the same energy, the necessary injection volume of ethanol is greater, so that the burning ethanol in spark ignition engines produce specific fuel consumption (SFC) which is higher than gasoline.

The percentage 35% of oxygen in ethanol helps the combustion process takes place perfectly, so as to reduce emissions of CO and HC if compression ratio and ignition settings are appropriate. Ethanol as oxygenate compounds have air-fuel ratio smaller than gasoline to reach stoichiometric conditions. In many studies the presence of oxygen in ethanol more effectively reduce CO emissions in the flue gas instead of air induced into the combustion chamber.

The density of molecules between gasoline and ethanol is equal so the mass of two fuels will be comparable in a certain unit volume. Thus the physical properties will have no effect on the combustion process. In contrast to density, viscosity of ethanol two times higher than gasoline, which is affects in fuel injection process. Therefore, to obtain the same injection volume of ethanol, the injection pressure should be increased simultaneously with increasing the percentage of ethanol.

Pressure vapour Reid of ethanol is very low that making it difficult evaporate at low temperature so the engine difficulty to start in cold situation. While the latent heat of vaporization of ethanol is two times than gasoline so get positive impact on combustion. Rising of cylinder temperature excessively and producing NOx can be suppressed with ethanol. Combustion duration and laminar flame speed of ethanol shorter than gasoline, so it will be effect to produce combustion process more stable and can enhance engine performance. Ethanol as a single compound having a boiling point in almost the entire volume of ethanol. In the combustion process it is could be effect on evaporation process, especially if ethanol dominant in the mix with gasoline.

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3. COMBUSTION CHARACTERISTICS OF GASOLINE-ETHANOL BLENDS IN LOW COMPOSITION

Differences in fuel properties require different setting operational parameters of machines for getting aim of combustion processes, namely large power, low emissions and fuel efficiency. Many studies applying the ethanol in spark ignition engines, either as a mixture with gasoline as well as a pure fuel. That's because some of the properties of ethanol are closer to gasoline so that the fuel is more properly used in spark ignition engines.

Table 1. Comparisons of Properties of Gasoline and Ethanol (5)

Properties	Unit	Gasoline/diesel	Ethanol
Chemical formula	-	$C_5 – C_{12}$	C_2H_5OH
Molecular weight	kg/kmol	114,15	46.07
C-fraction	% mass	87,4	52,2
O-fraction	% mass	0	34,7
H-fraction	% massa	12,6	13,0
H/C	Ratio atom	1,795	3
O/C	Ratio atom	0	0,5
Specific gravity	-	0,7 – 0,78	0,794
Density (at 15^{o}C)	kg/m^3	750 – 765	785 – 809,9
Stoichiometric air-fuel ratio	w/w	14,2 – 15,1	8,97
Kinematic viscousity	mm^2/s	0,5 – 0,6	1.2 – 1,5
Reid vapour pressure (at 37,8^{o}C)	kPa	53 – 60	17
Research Octane Number	-	91 – 100	108,61 – 110
Motor Octane Number	-	82 – 92	92
Cetane number	-	8	5 – 20
Enthalpyy of formation			
a) Liquid	kJ/kmol	-259,28	-224,1
b) Gas	kJ/kmol	-277	-234,6
Higher Heating Value (HHV)	MJ/kg	47,3	29,7
Lower Heating Value (LHV)	MJ/kg	44	26,9
LHV at stoichiometric mixture	MJ/kg	2,77	2,7
Heat Laten Vaporization	kJ/kg	380 – 400	900 – 920
Spesific Heat			
a) Liquid	kJ/kgK	2,4	1,7
b) Gas	kJ/kgK	2,5	1,93
Freezing point	^{o}C	-40	-114
Boiling point	^{o}C	27 – 225	78
Flash point	^{o}C	-45 s/d -13	12 – 20
Auto ignition temp	^{o}C	257	425
Vapour Flammability Limits	% vol	0,6 – 8	3,5 – 15
Laminar flame speed at 100kPa, 325K	cm/s	-33	-39
Distillation			
a) Initial boiling point	%	45	78
b) 10	%	54	78
c) 50	%	96	78
d) 90	%	168	79
e) End boiling point	%	207	79
Water solubility	%	0	100
Aromatics volume	%	27,6	0
Vapour toxicity	-	Moderate irritiant	Toxic in large doses
Smoke character	-	Black	Slight to none
Conductivity	-	None	Yes
Colour	-	Colorless to lighnt glass	Colorless

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A number of studies have shown that addition of ethanol into gasoline up to 20% will increase engine performances, such as; power, torque, cylinders pressure and lower CO, HC and NOx compared to gasoline, without major changes in operation of machine settings. This is due to the low concentration of ethanol in gasoline is an octane booster to raise RON gasoline, thereby increasing engine power, torque and reduce detonation. While the content of oxygen in ethanol helps complete combustion process that produces CO and HC emissions are lower.

3.1 CO and HC Emission

Combustion characteristics of ethanol are not only dependent on the percentage of ethanol in gasoline, but also influenced by the type and design of machines, injection and control systems as well as emissions handling system (6). The influence of concentration of ethanol to reduce CO and HC evidenced by several studies, including by Yang et al (4, 7). With addition of ethanol to 5% able to reduce CO emissions by 20% and HC by 5.2%. A bigger drop will be generated if the percentage of ethanol added, where if ethanol up to 10% will reduce emissions of CO and HC respectively up to 45% and 73% (9, 10, 11). Similarly, if ethanol is added to 20%, it will reduce emissions of CO and HC to 67% and 73% (6).

With the above results, contribution of oxygen in ethanol proved to be very effective to reduce emissions of CO and HC. This is due; the addition of ethanol 5-20% can increase the value of lambda 0.7 to 8.4% (6). The improvements make process of mixing air and fuel to the stoichiometric conditions (= 1) so that complete combustion can be achieved.

The condition is greatly assisting the process thus CO emissions continue to decrease with increasing concentrations of ethanol up to 20%. The HC emissions at a concentration of ethanol to 5% only able to decline to 5-7%, because HC is not only influenced by the availability of oxygen in the fuel but by several factors such as compression ratio and ignition timing. Nonetheless these emissions decreased significantly when ethanol added to 20% (6, 9).

It can be concluded that a mixture of gasoline and ethanol up to 20% do not require changes in operational settings on the gasoline engine. This is due to ethanol more as an octane booster that reduces detonation and increases the power and torque of the engine.

3.2 Nox and CO2 Emission

In contrast on NOx and CO2 emissions are increasing in addition to 20% ethanol. This is due to the oxygen in ethanol has trigger on increase in cylinder temperature thereby forming NOx. Similarly, CO2 emissions will increase if the concentration of ethanol in gasoline increases. As mentioned earlier, oxygen in ethanol will create the conditions to reach stoichiometric mixture so product of combustion of hydrocarbon fuels particularly CO2 and H2O will increase.

Yung-Chen Yao (12) has successfully studies on combustion of gasoline and ethanol blended up to 15% without any adjustments on a machine), both in neither carburettor system nor the injection system. In the study generated NOx emissions in the carburettor system 78% lower compared to the injection system. You can also see that the NOx emissions would be higher if greater concentrations of ethanol in the mixture (11,12)

The increase in NOx with increasing concentrations of ethanol to 20% can be minimized by performing a second injection strategy (10). But consequences of these methods will result a decrease in indicative mean effective pressure (IMEP) is significant which also affects the reduction in engine power. Increased NOx in concentrations up to 20% ethanol in gasoline is inevitable, and from all the research that has been done shows the same trend. These emissions will continue to rise if revolution and load of engine rise continuously. The high latent heat of vaporization of ethanol is not able to absorb some of the heat of combustion. This is due to the ignition delay is too short and compression

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ratios still in engine standard while duration of combustion too shorter with increasing concentrations of ethanol.

3.3 Engine Performances

Combustion of mixture gasoline and ethanol to 20% (E20) gives a very significant effect on the performance of spark engine without changing the engine settings. Engine performance usually characterized by a number of parameters, namely; power, torque and fuel consumption.

Research conducted by Gholamhassan Najafi (3) using gasoline–ethanol up to 15% showed an increase in brake power of 4.78% on each additional 2.5% ethanol in gasoline. The increase was also in line with the increase in engine rotation. Similarly, torque will be increased by 2.8% when the concentration of ethanol increased from 5% to 15%. While the specific fuel consumption decreased by 1.9% with increasing concentrations of ethanol, although the SFC increased if the engine rotation is increased (Figure 1).

For specific fuel consumption values, different results obtained by Jitendra kumar (13) where the SFC increased if the concentration of ethanol is increasing (Figure 2), although its value has decreased with the increase in engine rotation. In load variations, SFC decreases with increasing speed engine, while in speed variations show the same trend when the load increases. These results are very rational considering the calorific value of ethanol is lower than gasoline.

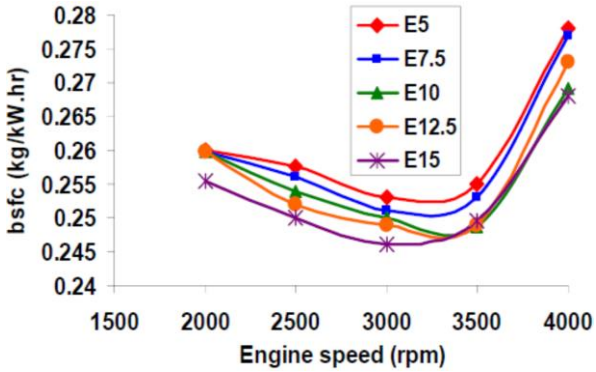

Figure 1. Values of BSFC vs RPM(41)

Differences both of SFC by research Gholamhassan and Jitendra although engine specifications are relatively similar, due to research conducted by Jitendra not make changes to the standard setting machine. In these conditions the SFC value always increases with increases of percentage of ethanol. While by Gholamhassan perform a variety of ignition timing which is intended to optimize engine performance and minimize exhaust emissions by using response surface methodology (response surface methodology).

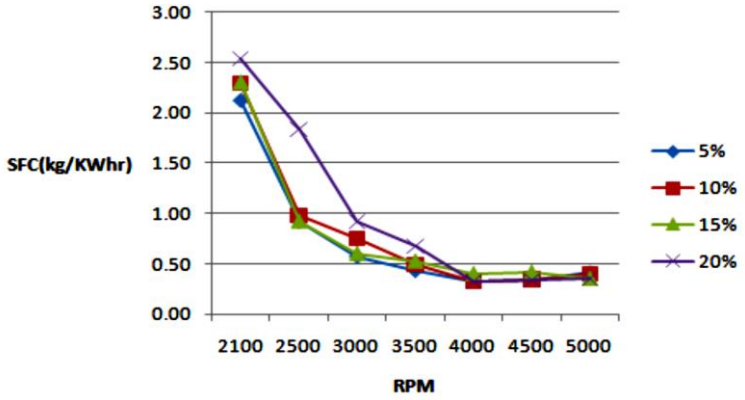

Figure 2. Values of BSFC vs RPM (111)

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Combustion duration and laminar flame speed of ethanol is short so ignition timing should be advanced. It was intended that ethanol can achieve a peak pressure shortly before complete burning. Thus will be obtained in higher combustion efficiency and lower emissions. In the spark ignition engine, fuel ignition delay by advancing the ignition timing to be implicated in the increased fuel consumption (SFC).

4. COMBUSTION CHARACTERISTICS OF GASOLINE-ETHANOL BLENDS IN MEDIUM COMPOSITION

Applying a mixture of gasoline and ethanol in medium composition (E25-E40) in spark ignition engine requires some adjustments, especially on the compression ratio. This is due to the laminar flame speed more and octane number of ethanol more dominant in the mixture. If the composition is not any adjustment to the compression ratio, it will degrade performance and increase engine emissions.

4.1 CO and HC Emission

Not too many studies that have been conducted to determine combustion characteristics gasoline-ethanol blended on medium composition, one of them carried out by the Fintas AA (14) on E25 fuel for Yamaha Vega R-110 3SO/4D7 with standard condition engine. On the research, showed the CO and HC emissions characteristic that still tends to decrease through increasing percentage of ethanol to 25%, even until 40% equal with Chen RH studies (15). Oxygen content in ethanol has primary impact on the state so CO fall down even though the engine on idle condition (14). Meanwhile, HC emissions will climb on the situation due to homogenous mixture difficult to reach on low revolution of engine, so a part of hydrocarbon compounds was not burn completely. Application of ethanol as a fuel in spark ignition engines, either as a mixture with gasoline as well as a pure fuel to give a very significant influence on the reduction CO and HC. Ethanol is a oxygenate agreed which is very helpful to reaches a stoichiometric conditions, so all of carbon compounds will burned completely. At up to 40% ethanol in gasoline do not require changing a setting machine to reduce HC and CO particulate.

Adaptability effect of compression ratio on 20–40% ethanol in gasoline would be lowered CO and HC. Re-arrangement of compression ratio based on increasing of octane number of fuels, which is known that each of increasing octane number by five required by a compression ratio. Studies of Sayin et al (16) showed that rise of CR at standard condition (9/1) to 10/1 or 11/1 have been reduce on average 18% and 17% CO and HC respectively. Similarly, if the concentration of ethanol increases, CO emissions will decline by an average of 19% if the compression ratio is suitable with octane number of fuel. HC emission reduction by increasing CR accordance with the increase of ethanol concentration due to the effects of turbulence caused by a high of CR will impact for homogeneous charge.

4.2 NOx and CO2 Emission

Consideration to arrangement some of engine parameters such as CR and ignition timing, due to decrease in emission and performance of engine by increasing of ethanol concentrations in gasoline. Besides that, increasing of ethanol percentage will lead to enhancement of cylinder temperature that caused rising of NOx emissions. Therefore, to get over the situation necessary to adjust some parameters of engine setting, mainly on compression ratio.

Adjustment compression ratio properly will reduce NOx if percentages of ethanol increase. This is due to a high of heat latent evaporation of ethanol would be absorbing some of heat cylinder (15). Similarly with the air-fuel ratio (AFR), can be set on a slightly richer condition (λ <1) to reduce the temperature rise in the cylinder. If concentration of ethanol advance continuously, so air-fuel ratio should be richer. At each increment of ethanol, a rich condition (λ = 0.9) will result exhaust temperatures lower than lean mixture (λ = 1.1). This condition causes the production of NOx will decrease due to the

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effects of oxygenate compounds (15, 16). While the CO2 emissions will be increase if concentration of ethanol in gasoline increasing too. This is proved of Sayin et al studies (16), wherein an increase in the concentration of alcohol in gasoline fuels will increase CO2 when CO decreases.

Efforts to reduce HC and CO in gasoline-ethanol combustion in spark engine will have implications for the increase in CO2 emissions. It is difficult to avoid, since ethanol is containing 35% oxygen so the stoichiometry state will produce combustion products such as H20 and CO2. Moreover, an attempt to improve engine performance by reducing emissions of CO and HC will result in increased emissions of CO2.

Improved compression ratio will also increase CO2 as a result of the increasing pressure and temperature of the mixture at the end of the compression step. The increase was caused by combustion process more properly by oxygen in ethanol which gives the effect in equivalent ratio and ignition timing better. It will have an impact on oxidation process of CO is done completely so that CO2 increases (18).

With the results mentioned above, then containing of ethanol in gasoline on 25% - 40% reduction in emissions of CO, HC and NOx requires adjustment of compression ratio to give an effect for equivalent ratio and ignition timing better.

4.3 Engine Performance

Addition ethanol more than 25% on standard engine would be decline amount of engine parameters as has been shown by Fintas research. In this studies, brake power and engine parameters other tend to increase with increase of ethanol up to 15%, both in 2000 and 3000 RPM. Meanwhile, if increased ethanol advance up to 25% almost all of engine parameters will decrease. This is affected by the compression ratio of the engine is still on standard settings (9/1) that too small for ethanol concentration is more than 20%. Discrepancy between the compression ratio and percentage of ethanol will affect in the timing of ignition which having a laminar flame speed shorter than gasoline.

As explained earlier that the spark ignition engine with standard conditions, burning ethanol to a concentration of 20% in gasoline would save fuel. This is confirmed in the research Fintas AA et al (14), in which the specific fuel consumption decreases when ethanol was added to gasoline to 20%. But if the percentage of ethanol reaches 25% the SFC value will increase, so the efficiency of the engine will follow the same trend. SFC value increases with increasing the percentage of ethanol caused by ignition timing on standards settings that tend to be too backward. In these conditions needed fuel injection longer considering by combustion duration and laminar flame speed of ethanol is shortly.

The phenomenon can be overcome by adjusting the compression ratio of the engine according to the concentration of ethanol in gasoline. A number of properties of ethanol including, oxygen, laminar flame speed, latent heat of vaporization will become more dominant in the mix. By adjusting the compression ratio, a mixture of ethanol-gasoline will be burned when it reaches its peak pressures that will produce higher power and combustion efficiency.

Research conducted by Cenk Sayin et al (18) is clearly showed the BSFC decrease with increasing compression ratio. Increased BSFC with increasing ethanol due to the calorific value of fuel alcohol is lower than gasoline, so as to reach the same value of equivalent ratio was required volume of fuels is greater. Therefore, adjustment of the compression ratio will decrease BSFC value with increasing concentrations of ethanol. In relationship with the equivalent ratio, IMEP and engine power will be increased if fuels in the rich condition together with increasing ethanol in gasoline (15).

Similarly, the value of brake thermal efficiency will be increased by raising the compression ratio and percentage of ethanol in the fuels. This is influenced by increasing the oxygen content in the mix and the delay of ignition timing. Moreover, the addition of ethanol in gasoline will increase the flame speed laminar of fuel which also is a factor in delay of ignition timing (16).

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5. COMBUSTION CHARACTERISTICS OF GASOLINE-ETHANOL BLENDS IN HIGH COMPOSITION

Unlike with the two previous compositions, which does not require setting up engine parameters, or require any adjustment compression ratio simplify, combustion of gasoline and ethanol above 50% requires the setting and adjustment of all operating parameters of the engine. In these conditions the arrangement is not done partially on one part only, but will require adjustments in all parameters simultaneously. If the orientation of our testing only get high power and torque, so it will achieved if high concentration of ethanol is applied, both at low or high load (17, 19). In this section will explain the effect of changes in operational engine settings such as, compression ratio, ignition timing and lambda on performance and engine emissions.

5.1 Ratio of Compressions

Low calorific value of ethanol can be substituted with its high octane number, to get a great power by increasing the compression ratio. Today, conventional gasoline engines are widely used, which the compression ratio be set between 9/1-11/1 with octane number 88 - 92. As has been explained in two main sections above, the percentage of ethanol in gasoline to 20% do not require changes the compression ratio of the engine. While the percentage of ethanol 25-40% involve operational setting of engine particularly in compression ratio. The changes of compression ratio depending on the increase in the octane number of fuel.

5.1.1 Engine Performance

A study by Mustafa KB et al (19) describes the effect of compression ratio on the performance and emissions of gasoline, ethanol and pure methanol. In these experiments, BTE and BMEP gasoline will decrease if the compression ratio is more than 8.5 / 1, while for ethanol and methanol both parameters will continue to increase with increasing compression ratio. Similarly, research conducted by Rodrigo CC et al (20) to change the compression ratio of 10/1 - 12/1 with E22 and E100. In the study of thermal efficiency, torque and engine power increases with increasing compression ratio on the E100. Instead of volumetric efficiency will increase if the compression ratio is increased in the use of E50-E100. The studies have also been investigated by MB Celik et al (21), and get the same results.

Meanwhile the performance characteristics of SI engine with turn ethanol concentration to 100% on the compression ratio is fixed shown by research Farha et al (22). With a compression ratio of 10/1, fuel consumption will increase with increasing percentages of ethanol. As for the other parameters will decrease with the increase of ethanol. The same result was shown by Krishna et al (18), where there was an increase of 20-30% thermal efficiency with the use of ethanol of E0 to E100.

5.1.2 CO and HC Emission

Characteristics of CO and HC of fuel blended in two earlier compositions decreased significantly with increasing the percentage of ethanol. An effect of changes in the compression ratio of the HC and CO by more than 50% ethanol is also shown by several studies mentioned earlier. Reduction of CO and HC by increasing the compression ratio of occurred significantly despite speed of engine is increasing (21, 22). Meanwhile, if blend of ethanol and gasoline applied to the same compression ratio (fixed), CO and HC emissions tend to increase with the increase in engine rotation , despite it was decline if ethanol is increasingly (22)

The reduced emission of CO and HC by increasing ethanol due to the oxygen content of ethanol is increased so as to help the process of combustion. While the increase in compression ratio in accordance with an octane number of ethanol to make the mixture more homogeneous so that the HC decreases. However, reduction of HC wills not continuously by raising the compression ratio only, but it must be followed by ignition

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timing adjustment. This is because the laminar flame velocity increasing with increasing volumes of ethanol.

5.1.3 CO2 and Nox Emission

The latent heat of vaporization of ethanol is high that have enough to absorb the increasing cylinder temperature, especially if engine at high speed or a large load. Thus the increase in NOx can be reduced by increasing the concentration of ethanol. Effects of changes in the compression ratio to the reduction of CO2 and NOx s showed on Celik MB et al studies (21), in which NOx in E50 and compression ratio by10/1 is higher than the compression ratio of 6/1, although both of them have a tendency to decrease with the increase of engine speed. Higher of NOx by E50 and CR 10/1 than the 6/1 due to they use standard setting. With these results it appears that applications for more than 50% ethanol in gasoline requires advanced on ignition timing to handle rising of combustion temperature through ignition delay of ethanol.

5.2 Ignition Timing

The setting ignition timing was more important not only to improve engine performance, but contribute to reduce combustion emissions. With increasing concentrations of ethanol in gasoline mainly to a percentage above 50%, then the ignition timing adjustment needs to be done. This is done to produce high engine performance and low engine emissions.

5.2.1 Engine Performance

Ignition timing adjustments of the concentrations of ethanol and compression ratio engine will very significant impact on performance and emissions. In research Bambang S et al (23), increased compression ratio needs advance ignition timing, so that the brake torque, brake power and BMEP will reach maximum value. On E50 with a fixed compression ratio, the engine performance will increase if the ignition timing is advanced 3o of the standard setting of 9° to 12o BTDC and the compression ratio of 9/1 (24). The fuel consumption will decrease if the ignition timing is suitable with the increase in compression ratio. Meanwhile for E85, a number of engine performance such as torque, power and engine efficiency will be increased if the ignition timing is advanced 4o of standard setting with a compression ratio of 9,2 / 1 (25).

A Study by Phuangwongtrakul et al (26) on E10–E100 shows the influence of ignition timing for the fuels with a compression ratio of 10.5/1. In these conditions it appears that the E85 has the greatest MBT than E100, by 38o BTDC ignition timing. The value of MBT to rising of ethanol percentage is fluctuated, where its value began to increase from E10-E40, but decreased from E50-E70, as well as achieving maximum value on E85 before descending to the lower E100 than E60. It is shows that to obtain maximum performance not only requires setting the ignition timing, but necessary adjustments to the compression ratio and lambda. With the increase contain ethanol in gasoline, then the compression ratio needs to be increased and the excess air in lean mixture. The low of ethanol calorific value have impact for increased fuel consumption that is proved by the length of fuel injections is longer if ethanol increases.

5.2.2 Emission

Produce NOx on ethanol fuels at a high concentration (>50%) is much lower than low concentrations, if the ignition timing is advanced. Variations ignition timing was not influenced of CO and CO2. This suggests that an increased level of ethanol in gasoline only takes advances ignition timing. Thus the gasoline-ethanol mix will be burned when it reaches the maximum torque to generate high power. Under these conditions there is a delay of ignition so that the high of heat of evaporation of ethanol is able to controls the increase of temperature cylinder, which ultimately produce NOx emissions (26).

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5.3 Lambda

The higher value of ethanol in gasoline need the equivalent ratio less than one (λ <1) to get the power and efficiency of the engine better. Lambda influence on a mixture of gasoline and ethanol are shown by Alexandru R et al (27). It's showed that to a high performance of engines is required excess air that is less than one (λ = 0.90 to 0.95) and continue to decline if lambda more than one. As for low fuel consumption obtained when lambda at the point from 1,0 to 1,05. This is due to if the concentration of ethanol rises needed greater airflow thus increasing volumetric efficiency and fuel consumption becomes less. The conditions mentioned above was also confirmed by Phuangwongtrakul studies for E10 - E100 (26).

Meanwhile, CO and HC will decrease if the lambda value is the greater. Higher of AFR is also required if concentrations of ethanol increase both on high load and engine speed (25). NOx emissions will increase in value lambda 0.85 to 1.05 and after that it will decrease. The higher the ethanol content, the lower produces of NOx when burning is set on stoichiometric and the compression ratio as well as ignition timing adjusted based of percentage of ethanol.

6. CONCLUSION

Blend of gasoline and ethanol as a fuel in spark ignition engine produces low emission compared combustion of pure hydrocarbon fuel (E0). Otherwise, use of ethanol as an additional fuel in gasoline will also increase power and engine efficiency. Consistency reduced emissions and increased engine performance on conditions of standard setting particularly in spark ignition engine is obtained if the concentration of ethanol in gasoline up to 20%. In these conditions the existence of ethanol in gasoline acts as octane booster so that the power, torque and efficiency is increased and fuel consumption decreased even detonation minimize occurrence of each cycle.

Combustion of ethanol 25% - 40% as a blended fuel in spark ignition engines will reduce engine performance and increase emissions if using a conventional engine with a standard setting, which range of compression ratio 6/1 – 10/1. To overcome it is necessary to adjust the compression ratio based on the octane number value of the mixture, which every increase of 5 octane numbers need adjustment 1 compression ratio. On the composition adjustment of the compression ratio would influence in better of excess air and ignition timing, so that in this condition is not required settings on two parameters.

Combustion gasoline and ethanol blended at high composition in the spark ignition engine requires adjustment on three main parameters simultaneously, namely compression ratio, ignition timing and lambda. It's not only depend on octane number, but type of machine and engine operating conditions are also taken into consideration in order to produce greatest engine performance

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RESTORATION ECOLOGY

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Utilization of Water Hyacinth in Decreasing Levels of *Heavy Metals in Industrial Wastewater*

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Abstract

Phytoremediation is a method to remove inorganic pollutans from contaminated environment by using plants. Many inorganic pollutans were discarded to the river by industries. Heavy metals are some contributors of waste in the river. Water hyacinth often found in the river stream, which lead to lower aesthetics of the river. However, there is one advantage to be gained from this plant. This plant can be used as an inorganic pollutants adsorbent by using of phytoremediation.

The research purpose is to reduce heavy metal levels in waste water by using phytoremediation of water hyacinth. The method of this research was done by cultivating the water hyacinth in various concentration of heavy metals and variated contact time . The concentration of heavy metals were 5 ppm of pure Cd , 3 ppm of pure Cr, and 4 ppm of Cd and Cr mixture. In growing hyacinth, addition of aeration and urea (NH_2CONH_2) were needed. The variated contact time were 0, 5, 10 and 15 days. The result reported that the ability of water hyacinth to adsorb heavy metals Cd better than Cr by 99.949 % and yield removal of 0.00831 g Cd/kg hyacinth while the removal of Cr was 91.049 %. Cr removal in a single metal solution was greater than the removal of Cd which was in a solution containing alloys (Cd and Cr).

Keywords: Cd, Cr, heavy metal, water hyacinth, phytoremediation

1. INTRODUCTION

Progress in the field of industry has resulted in an increase of waste removal, if the handling of the waste generated by the industry has not been managed well. **A**n assortment of industrial waste generated, ranging from liquid waste, solid waste and gas waste.In wastewater treatment, the way to do this is by using fitoremediation.Fitoremediation is effort to control contaminants using plant as adsorbent Plants that can be used for phytoremediation include water hyacinth, velvetleaf, water jasmine, water spinach and clover. In this experiment, it used water hyacinth because it has the ability to adsorb heavy metals well. **T**he adsorption of Cu and Zn by water hyacinth plants with initial concentration of 15 ppm and a contact time of 14 days resulted an adsorption concentration of 340.775µg/g for Cu and 158.33 uǵ/g for Zn (Syahputra,2005). Hyacinth can adsorbed Ar, Cd, Cu, Cr, Fe, Mn, Ni, Pb, V and Zn (Agunbiade, 2009). The mechanism of heavy metal adsorption by the water hyacinth include fitoextraction process, rhizofiltration, fitodegradation, fitostabilisation and fitovolatilisation.Heavy metal uptake by plant roots can occur when heavy metals are located around the roots . Plant root cells generally contain ions with a concentration higher than surrounding areas that are usually negatively charged. Plants do not have the power to choose food that is absorbed, so that the available food in the waste water directly utilized without selection. Absorbed elements rate depend on concentration of an element. The higher concentration of an an element resulted in the greater transportation rate.According to Niang(1999) , the metal-containing waste water will be positively

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charged and how to bind these metals is by fill in negatively charged object. Plant roots are negatively charged and acts as a magnet to attract positively charged elements, even the roots of dead or dry still contains negatively charged large enough to attract the positive ions of heavy metals. (Hartanti, 2006). There are two main functions involved in helping the adsorption of the metal. The first is the production of metal chelating compounds to form complex compounds that are less toxic to plants. The second is that acidify rhizospere metal solubility. When the plants are exposed to heavy metal contamination, this plant can produce fitochelate which helps in the adsorption of heavy metals. Fitochelatine is a thiol-reactive peptide that consists of glutathione, cysteine and glycine (an amino acid). Glutathione is a natural antioxidant that is used in enzyme reactions during the formation fitochelatine. Fitochelatine then store the heavy metals in the vacuole which is the nucleus of the cell and storage of plant cells. (Erni, 2011). Then metal translocated which is distribution process from the roots to other plant parts (stems and leaves) through the carrier's network (xylem and phloem). The process of adsorption of heavy metals passively occurs when heavy metal ions bind to the cell wall and the binding process can be done in two ways. The first is where the ion exchange of monovalent and divalent ions such as Na, Mg and Ca in the cell walls were replaced with heavy metal ions. The second is the formation of complex ions of heavy metals with functional groups such carboxyl, thiol, phosphate, hydroxy located in the cell wall (Moenir, 2010 There is a hole in the cell wall that serves as a conduit between one cell to another. These holes are called plasmodesmata, which can be traversed by molecules with a molecular weight of about 60 nm. Cellulose has the potential to be used as an adsorbent for as -OH group. The presence of -OH groups caused the polar nature of the adsorbent. The interaction between -OH group with a metal ion is also possible through the formation mechanism of coordination complexes because the oxygen atom in -OH groups having free electron pairs. Metal ions will interact strongly with strongly alkaline anion such as OH. The bond between the metal ions with -OH on cellulose through the formation of coordination bonds, where the lone pair than -OH binds to heavy metal ions forming complexes through covalent bonding. Cellulose binding to metal chelate Cd forming cellulose. (Erni, 2011).

The research purpose is to reduce heavy metal levels in waste water by using phytoremediation of water hyacinth.

2. MATERIALS AND METHODS

The experimental works was condusted in a 20 liter laboratory scale aerated batch reactor system containg water hyacinth. Research Variables consisted of the addition of metal containing 5 ppm Cd or 3 ppm Cr or 4 ppm mixture of Cd and Cr for 0,5 ,10 and 15 days. Temperature and pH condition of the reactor was kept at T= 30ºC and pH =7. Urea should be added to maintain the growth of bacteria. Dissolved oxygen in the mixture must be kept higher than 2 mg/l. The metal content was analized every day by *Atomic Absorbtion Spectrophotometry* (AAS). The experimental was set up such as Figure 1.

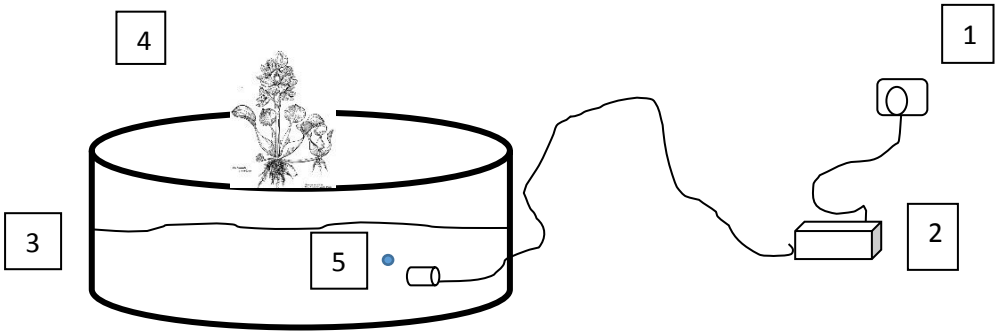

Figure 1. Experimental Set Up

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Note:

1. Electricity
2. Aerator
3. Oxygen diffuser
4. Water Hyacinth
5. Oxygen diffuser

3. DISCUSSION

From the results were known that the metal level in water hyacinth for substrate without metals was decrease after phytormediaton . The concentration of Cd was decrease as much as 3,7 ppm and for Cr was 0,3 ppm. This matter showed that the water hyacinth had the ability for degrading the metals. Water hyacinth could degradade the metals become non dangerous substance by evaporating it to air and other would be localized at root cell and plant tissues (rod and leaf).

In this reseach there were 3 variables, wastewater containing heavy metals Cd in the amount of ± 5 ppm, wastewater containing heavy metals Cr in the amount of ± 3 ppm, and wastewater containing mixed heavy metals(Cd and Cr) each in the amount of ± 2,5 ppm and ± 1,5 ppm.

The highest procentage of metals removal and yield removal by water hyacinth was achieved by wastewater containing single metal of Cd, then the mixed metals wastewater, and the last was wastewater containing single metal of Cr. Initial concentration could affected the result of degradation and influenced the yield of metal removal. Initial concentration single metal Cd was 4,989 ppm, and mixed metals contained 4,040 ppm mixed metals which contain Cd 2,455 ppm and Cr 1,585 ppm, where the initial concentration wastewater containing mixed metals less than wastewater containing single metal Cd. Meanwhile, the lowest initial concentration at wastewater containing single metal Cr of 2,6705 ppm. The comparison percentage of heavy metal removal to kind of heavy metals was given in Figure 1, and yield of metal removal was shown in Figure 2.

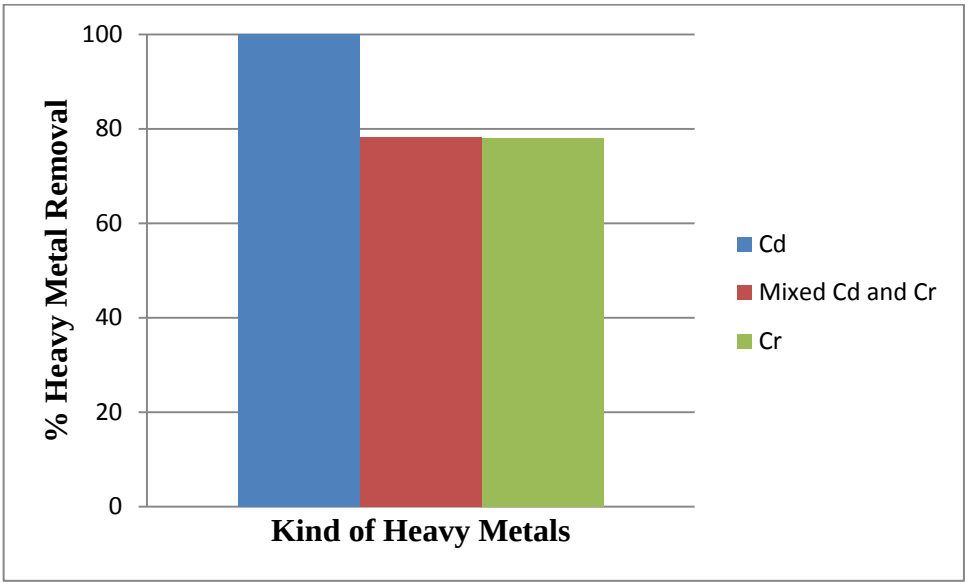

Figure 2. Percentage of Heavy Metal Removal to Kind of Heavy Metals

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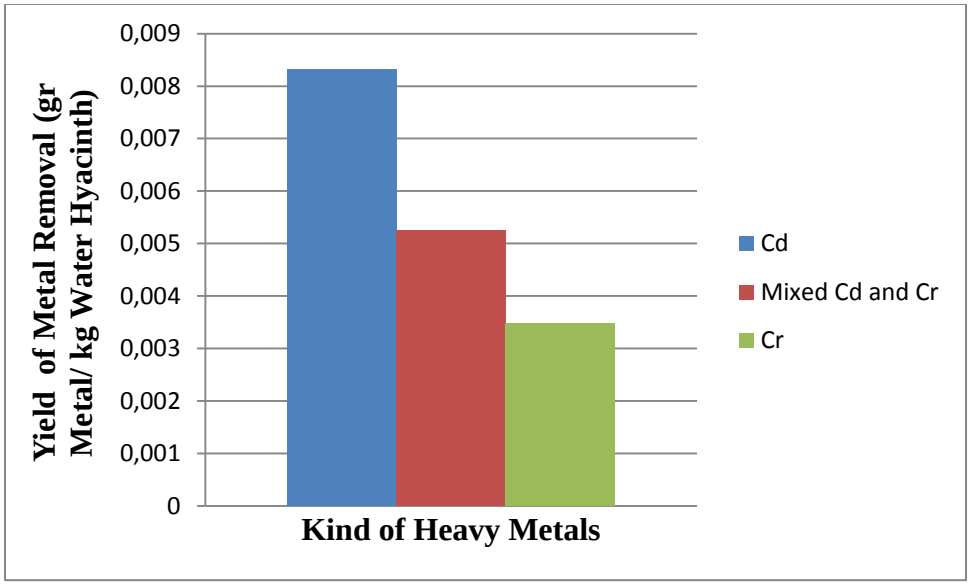

Figure 3 Yield of Metal Removal to Kind of Heavy Metals

From Figure 2 and 3 can be observed that the highest rate of metals removal by water hyacinth and yield metals removal by water hyacinth in wastewater containing single Cd metal, mixed metals and the last was a single Cr metal. The value of removal rate by water hyacinth for metals Cd, mixed Cd and Cr, and Cr respectively were 99,95%, 78,131% and 77,98%. While the value of yield removal metals Cd, mixed Cd and Cr, and Cr were 0,0083 g Cd/kg water hyacinth, 0,00526 g mixed/kg water hyacinth, and 0,0035 g Cr/kg water hyacinth. The adsorption rate using single metal concentration of Cd showed higher value than mixed metals and single metals Cr. This condition could be expressed as relation between decreasing of metal concentration to changes in time and was expressed as a linear line such illustrated in Figure 4,5 and 6. Coefficient of decreasing of metal concentration against time resulted 2.6193 ppm/day for single Cd metal, 0.1465 ppm/day for single Cr metal and 0.2067 ppm/day for mixed metals. The best coefficient of decreasing metal was Cd removal by water hyacinth.

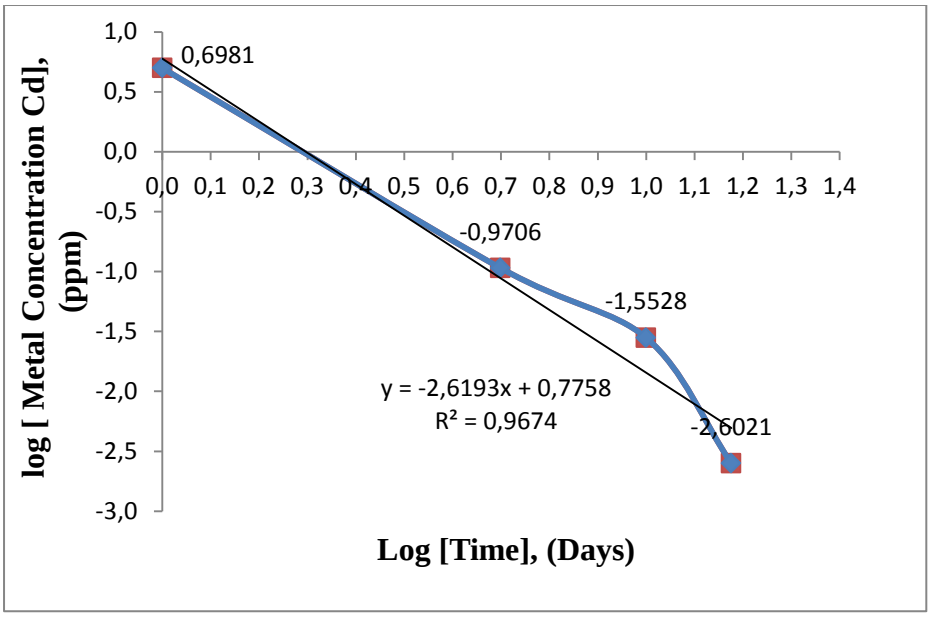

Figure 4. Relation between Metal (Cd) Concentration and time

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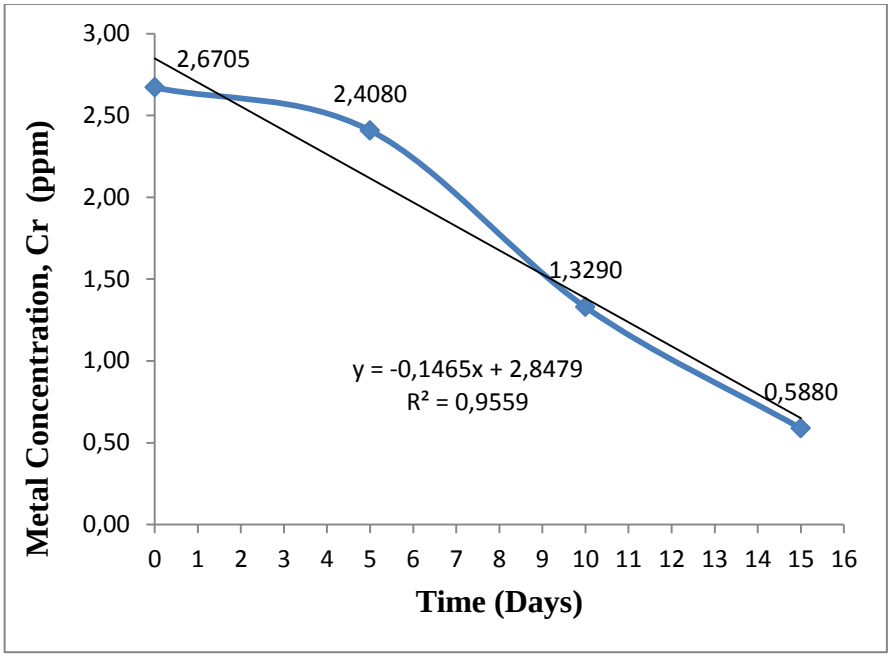

Figure 5. Relation between Metal (Cr) Concentration and time

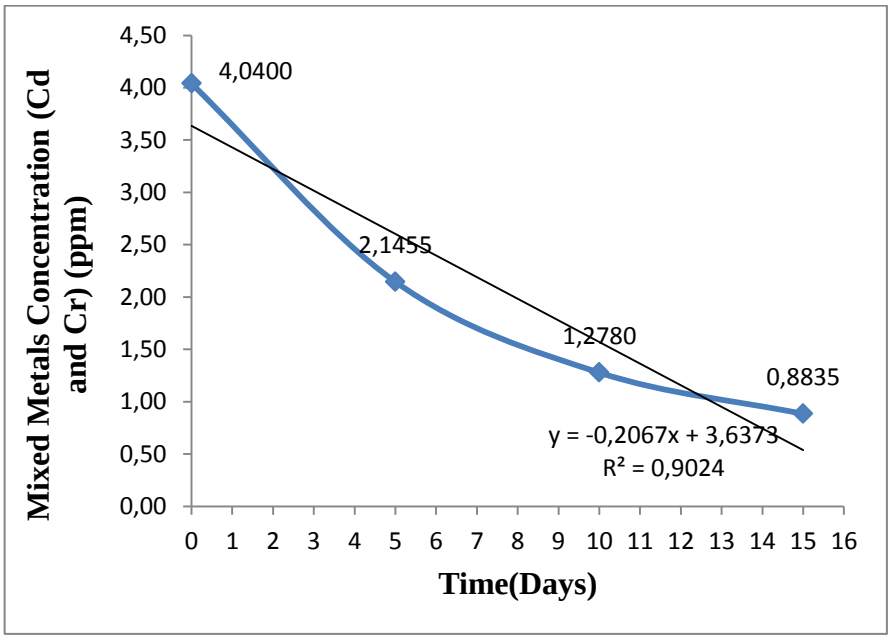

Figure 6. Relation between Metal (Cd and Cr) Concentration and time

4. CONCLUSIONS

1. Hyacinth ability to absorb heavy metals of Cd is better than Cr with 99.949% metal removal and the yield of 0.00831 g cd metal removal / kg water hyacinth.
2. Ability to absorb metal hyacinth on waste water containing one type of metal is higher than the waste water containing more than one metal.
3. Removal of Cd metal is 99.949 % (in single cd metal), greater than in mixed metals that is 91.049%.
4. Removal of Cr metal is 77.981 % (in singe cr metal), greater than in mixed metals that is 56.309%.
5. Coefficient of decreasing of metal concentration against time resulted 2.6193 ppm/day for single cd metal, 0.1465 ppm/day for single cr metal and 0.2067

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ppm/day for mixed metals. the best coefficient of decreasing metal was cd removal by water hyacinth.

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SOLID WASTE, WASTEWATER & HAZARDOUS WASTE TREATMENT

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Public Participation of Recycling Through Economic Benefit: Case Study in Federal Territory of Labuan

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Abstract

Experiencing rapid urbanization and industrialization on country development, Malaysia is facing the increasing and changes of the characteristics of municipal solid waste. Waste expected to reach 15.7 million tons in year 2020 if without any enhancement in solid waste minimization and considering the economic growth and population projection unless urgent measures are taken to reduce the disposed waste amount. Solid waste minimization crucial in reducing waste generation as it has been placed as the highest priority in solid waste management hierarchy. However, there is still lacking of solid waste minimization practices in Malaysia. Therefore, this paper will highlights solid waste minimization practices in Federal Territory of Labuan. Labuan Corporation (LC) is a local authority which administrates and responsible for public health and sanitation, waste removal and management, town planning, environmental protection and social and economic development. This paper discusses the high potential of public participation in solid waste minimization by recycling and composting through economic benefit in Labuan. Economic benefit plays a main role in encouraging recycling and composting to the consumers as it crucial for nowadays. Based from the interview, it revealed that common cause the lack of solid waste minimization practices because of low level of awareness and knowledge, negative attitude, lack of enforcement inefficiencies, as well as lack of recycling facilities. However, a good waste management system should stand aside within advanced technology and environmental awareness, education, continuous campaign of municipal solid waste minimization and more recycling facilities must be planned and should not be neglected to ensure a sustainable development for the future generation.

Keywords: benefit, economic, recycling, solid waste minimization, waste management strategies

1. INTRODUCTION

Solid waste growth has become an issue in many countries especially in developing country as it emphasized a major threat and problems to pollution, health threats of urban residents and destruction of natural resources [1-2]. Experiencing rapid urbanization and industrialization on country development, Malaysia not excluded facing the increasing and changes of the characteristics of municipal solid waste. Waste has expected to reach 15.7 million tons in year 2020. The number will keep increasing as considering the economic growth and population projection unless urgent measures are taken to reduce the disposed waste amount. [1;3;4;5]

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Therefore, in order to maintain a sustainable environment and a standard of living in the future, it necessary to apply the solid waste management strategies by implement solid waste minimization. Solid waste minimization has been placed at the highest priority of the solid waste management hierarchy [6]. Solid waste minimization consists of two basic operations: source reduction and recycling [7]. Source reduction is the most desirable way to avoid waste generation, while recycling is useful to conserve resources and to prevent materials from entering the waste stream. The success of solid waste minimization relies largely on education and increase of public awareness and their attitude to change positive habits. Besides that, enforcement efficiency and sustainable production should be taken seriously.

Separation of waste is the foundation of the recycling process which it is important to reduce amount of solid waste sent to the disposal site. Recycling is very important as waste has a huge negative impact on the natural environment if left unmanaged properly. In terms of waste recycling, industrialized countries such as Germany, Sweden, Japan and the United States have already achieved remarkable results in comprehensive utilization of resources as well as solid waste management [8]. Additionally, the recycling industry needs to be improved through increased professionalization, improved product standards, market development and better operating standards. Moreover, recycling has been identified as a significant factor towards sustainable waste management which can produce new products from old materials thus benefiting both environment and economy [9-10].

Therefore, this paper will highlight the potential of public participation in solid waste minimization by recycling and composting through economic benefit in Labuan Federal Territory. The programme targetting household in the residential area and villages. This study will revealed that economic benefit in terms of monetary reward will encourage the public to participate in recycling and composting with appropriate recommendations based on the scenario and current demands from the public.

2. BACKGROUND OF STUDY AREA

Labuan is a Federal Territory of Malaysia off the coast of Borneo in East Malaysia. Labuan's area compromised the main island 87.52km^2 with the total area 91.64km^2 as shown in Figure 1. It is made up of six smaller islands (Daat, Papan, Burong, Kuraman, Rusukan Kecil and Rusukan Besar) and located off the coast of the state of Sabah.

Labuan Corporation has been established on 1 July 2001 under Act 609, Labuan Corporation Act 2001. It is the combination between two agency which is Labuan Development Board and Labuan Municipality. Labuan has been declare as Federal Territory in year 1984 meanwhile in year 1990 Labuan Federal Territory has been claimed as IOFC (International Offshore Financial Centre). Labuan best known as its offshore financial centre as well as being an offshore support hub for deepwater oil and gas activities in the region. Virtually in Labuan focused on import and export oriented economy.

According to Malaysia's Department of Statistics population for 2010 [11] was at 86,908 and projected to increase to be at 91,300 for 2013 but reported to be at 96,800 in 2015. The majority ethnic composition is Brunei Malay which is 30,001 people, and other ethnic can be seen in Labuan such as Kadazan Dusun, Bajau, Murut, Chinese, Indian and other Bumiputeras as shown in Table 1. As of 2010 census, majority Labuan population were Muslim (76%), followed by Christian (12.4%), Buddhist (9%), Hindu (0.4%) and 2.1% others religion.

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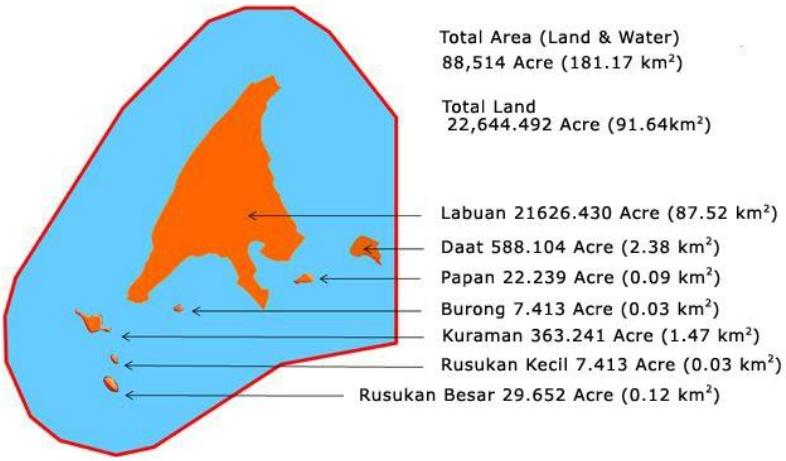

Figure 1. Map of Federal Territory of Labuan

Table 1. Labuan Ethnic Composition

Ethnic	Total
Brunei Malay	30,001
Kadazan-Dusun	7,380
Bajau	6,300
Murut	701
Other Bumiputeras	18,212
Chinese	10,014
Indian	641
Others	1,515
Non –citizen	12,144
Total Population	**86,908**

(Source: Malaysia Department of Statistic, 2010)

3. SOLID WASTE MANAGEMENT

Solid waste management in Labuan is responsibility by the Labuan Corporation (LC). Labuan Corporation is a local authority which administrates and responsible for public health and sanitation, waste removal and management, town planning, environmental protection and social and economic development.

In Labuan, Department of Municipal Services has a regular collection in commercial area for seven days a week meanwhile in villages and other residential area, the regular collection were two till seven days a week. There were divided by ten zone with one additional zone. The solid waste collection will send to the landfill site in Bukit Kalam for disposal.

4. SOLID WASTE MINIMIZATION IN LABUAN

Despite Malaysian government efforts in promoting reduce, reuse and recycling (3Rs) of materials, the amount of solid waste recycled in Malaysia remained at 10.5% of the total waste disposed which target to increase till 22% by 2020 compared to neighbouring countries Singapore recycling rate have reached 60% [12] while in Philiphines has reached 28% [13]. Although the awareness of recycling is high among Malaysian which is (82%) but only a few that has practice recycling in their daily life [14].

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Labuan municipality has put significant effort in maximizing recycling and minimizing waste under Department of Municipal Services via community approach and public awareness and participation. Labuan corporation is the municipal government for the island which responsible for the development and administration of the island. It is also responsible for the municipal solid waste management, collection and waste disposal including in Act 1990.

Thus, Labuan Corporation has taken initiative in promoting recycling for the whole Labuan residential area as shown in Figure 2. The recycling programme has been introduced since 2010. There are about 27 villages and 68 residential area in Labuan involve in the recycling programme. [15]

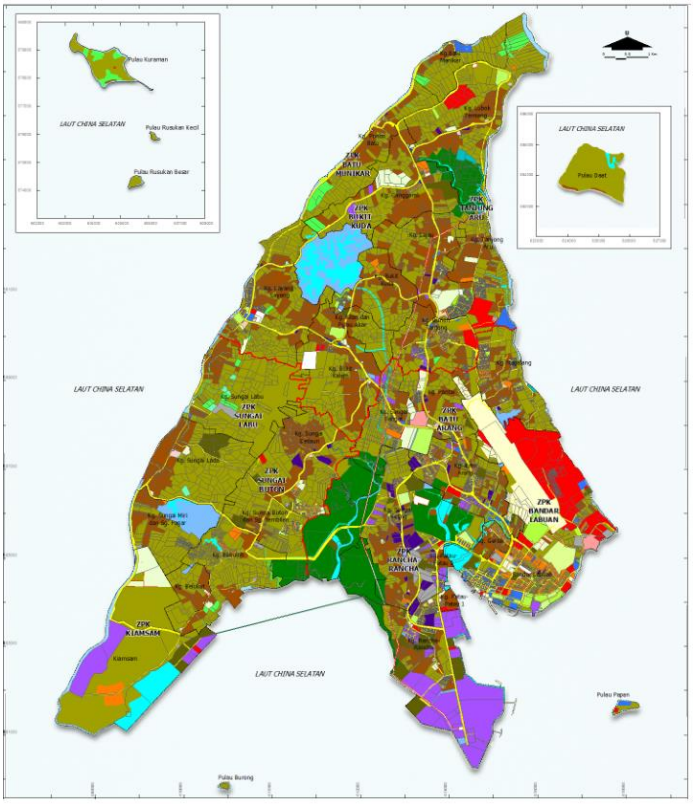

Figure 2. Map of Residential Area in Labuan

The recycling programme has become an annual event among Labuan people. It shows a positive feedback from the public and they started to practice recycling and waste separation in the house although there is no regulation on solid waste separation enforced to the state.

Figure 3 shows the recycling programme held in one of the residential area in Labuan. A banner of recycling programme will be hang to spread the programme to the villagers. Then, the staff from the municipal will open a recycling booth in front of the residential area so that the villagers can send their recycables waste. The staff then weighing the recycables waste and in return monetary reward will be given to the client according the price and type of recycables waste. Type of recycables waste that are collected such as paper, metal, plastic, box, and aluminium can. After that, the recycables waste will be send to the nearest recycling centre.

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The recycling programme has create an opportunity to the villagers to gain side income from selling the recyclables waste. Based from the interview with the client, majority of them send their recycables waste to gain money and cleanliness.

Moreover, the programme had increase villager's awareness regarding the waste separation and proper waste management. İt revealed that about 44% of solid waste recovery from the recycling programme.

Banner of Recycling Programme in front of the residential area

Client send their recycables waste

The staff weighing the client's recycables waste

Recycables waste collected

The recycables waste send to the recycling centre

Figure 3. Recycling Programme in Labuan Residential Area

5. SOLID WASTE GENERATION IN LABUAN

Solid waste generation in Labuan Federal as shown in Figure 4 are in range 31,000 tonnes in year 2010 till 2014. Meanwhile the collection of solid waste management by the municipality has increase to 20,000 in year 2014. Based on the total population in Labuan, it shows that solid waste generation per person has reached 0.37kg/person in 2014 as shown in Figure 5. Although the figure is still low but it shouldn't be neglected as it will keep increasing as population and urbanization increase. Based from the interview with the client, they mention that some causes of inactively

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solid waste minimization because of the lack of enforcement inefficiencies, lack of recycling facilities and low level of awareness and knowledge regarding the proper solid waste separation. However, majority of them agreed that the recycling programme should continuously organize by the Labuan Corporation.

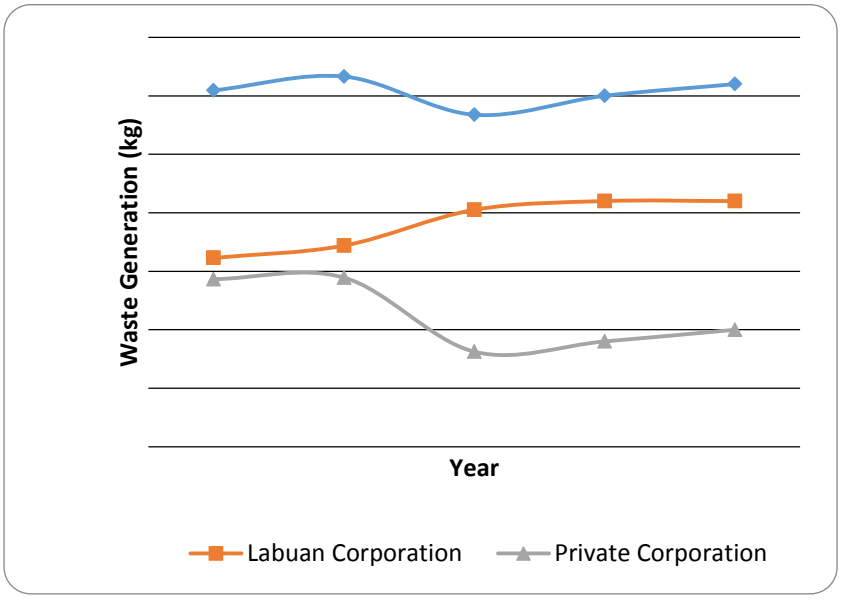

Figure 4. Solid Waste Generation in Labuan

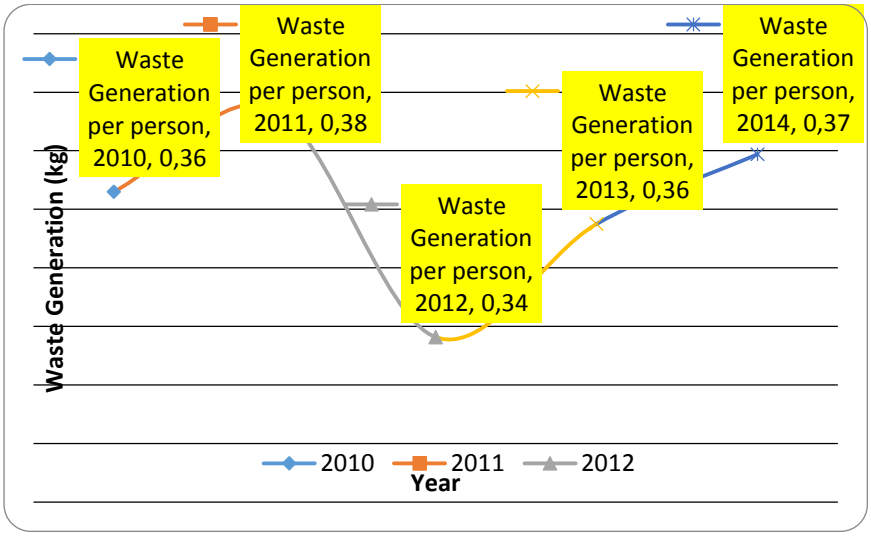

Figure 5. Solid Waste Generation per person

Labuan Corporation has actively promoting solid waste minimization practices for the whole Labuan where total of recyclables collected has reached 19, 693 tonnes in year 2010. However, the number has decreasing till year 2014 which total of recyclables waste collected decrease to 13,210 tonnes as shown in Figure 6. Paper is the highest recyclables waste that has been received which is average 15,000 tonnes per year.

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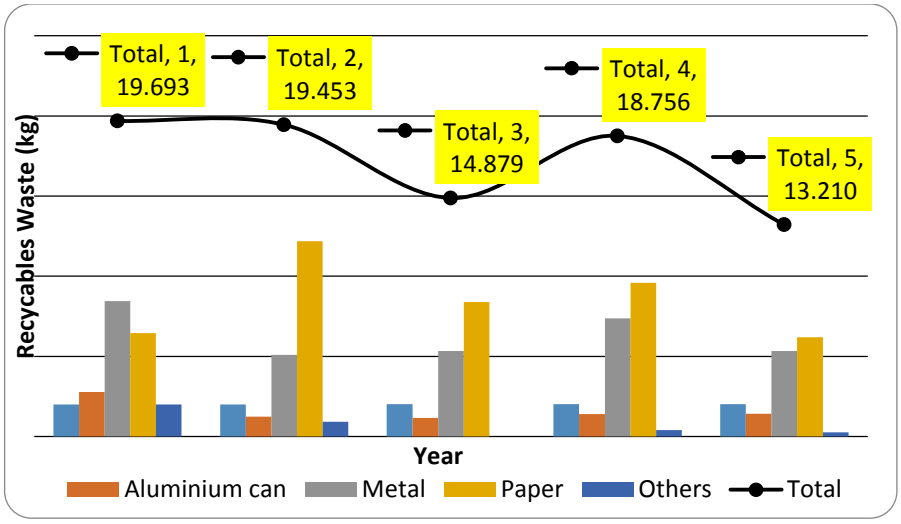

Figure 6. Recycable Waste Collection

6. COMPOSTING AS FERTILIZER

The Department of Municipal Services also actively in minimizing waste especially in fish market which using the residual of fish to make a fertilizer. The fertilizer has been sold to the public and it is economically cheap and effective. The steps of making the fertilizer were shown in Figure 7. The residual of fish collected from the market and mix it with sawdust. An inozyme has been added to the mix to speed up the reactions by providing an alternative reaction pathway. The final product then be package before sold to the public.

7. SWOT ANALYSIS

A SWOT analysis was conducted to explore the success and barriers of the recycling programme in Labuan Federal. The SWOT identified from the interviews of householders, the workers of waste collection and the staffs of Department of Municipal Services are shown in Table 2.

Table 2. SWOT analysis of recycling programme

Strength (S):	Weakness (W):	Opportunity (O):	Threats (T):
Annually Event of Recycling Programme	Not frequently collection	Frequently Collection	Limited human resource
Target household in residential area	Each residential area only once collection	Provide another recycling collection point	Limited transportation
Economic benefit	Some recycling price were low	Give other option of incentives	High cost

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Figure 7. Step in composting fish waste as fertilizer

7.1: SWOT of Recycling Programme

S1: Annual Event of Recycling Programme

The recycling programme which organized by the Labuan Corporation has been known as annually event in Department of Municipal Services. They were actively in promoting recycling and cleanliness programme. There were held many campaign and competition between villages regarding the cleanliness villages. The Municipal Services Department also had organized a competition about creativity on recyclables waste. It shows that a programme about waste has been accepted among Labuan people.

W1: Not frequently collection

The recycling programme has organized once a year however the collection is not frequently where the villagers need to collect and wait for a long time before send the recyclables. It make the villagers has limited space to store their recyclables waste in their house.

O1: Frequently collection

The municipal can plan a frequent collection of recyclables waste once a week or twice a week. It will keep the villagers more alert and aware on the schedule of the recyclables waste collection.

T1: Limited human resource

There were threats to the Municipal Services Department cause of the limited of human resource to collect frequently recyclables waste. It will become a burden to control the collection process as well.

S2: Target household in residential area

The recycling programme was targeting household in the residential area to collect the recyclables waste. It is an opportunity to the household as it will make easier for them to

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send the recyclables waste. They can save transportation cost and definitely increase their awareness on recycling. The ease to access the recycling facilities contribute to the high number of public participation [16-18].

W2: Each residential area has once collection

Unfortunately, the collection of recyclables waste in Labuan only organized once a year for each residential area. This is because of the programme cover the whole residential area in Labuan and it takes time. The period for the next collection was too long will influence the household to involve in the recycling programme.

O2: Provide another collection point

The Municipal Services Department can provide another collection point of recyclables waste collected as it will make easier for them to handle the collection. According to [19], facility accessibility affect the recycling behavior which means the accessibility of recycling facilities can affect individual decision to participate and do recycling. The more recycling facilities provided, the number of participation will increase.

T2: Limited transportation

Another collection point requires another transportation to collect the recyclables waste. The obstacles will threat to the LC which need a high cost to prepare another collection vehicle. The distance of collection point also influences the cost.

S3: Economic benefit

Economic benefit in terms of monetary reward will be given to the household that send their recyclables waste. The price were depends on the type of recyclables waste and weight. The monetary reward has encouraged them especially in the village residential area to gain side income from selling the recyclables waste. It is one of the factor of the public participate in the recycling programme. Economic benefit can attract more attention and awareness on recycling as well. It also can become an option to decide whether to recycle or not. According to [20], economic factors influence the recycle decision.

W3: Recyclables waste price were low

However, some of the recyclables waste price were low and discourage them to collect the recyclables waste. The recycling market in Labuan was limited and only a few factory or recycling centre was build in Labuan. The limited accessible of recycling facilities will decrease the public participation in the programme and the increasing of solid waste send to the disposal site.

O3: Incentives option given

Other incentives option will attract the villagers to involve in the recycling programme. It will give them more opportunity to participate and collect the recyclables waste. For example on giving a shopping discount coupon, food and beverages discount and travelling discount coupon. More incentives option will give them more choices and encourage them to collect more recyclables waste.

T3: High cost

Providing other incentives is costly as it needs to cover the expenses of the discount given. Although it might attract the public but it will be an obstacle to the LC to provide other incentives which it require a lot of money.

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7. CONCLUSION

Labuan Corporation has been actively promoting solid waste minimization to the public in order to reduce solid waste generation. This is because of the location of Labuan Federal Territory which is a small island are necessary to have a proper solid waste management. Having a limited space of disposal site encourage them to take initiative to plan a proper solid waste management and encourage the public to practice solid waste minimization through economic benefit. Monetary reward is one of the initiatives taken by Labuan Corporation to promote and encourage public in involving the recycling programme. Moreover, it also revealed that existing recycling facilities has create intention of public to practice recycling in their daily life. Thus, a proper waste management can contribute in shifting the planet towards a sustainable future.

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Solid Waste Composition at Log boom of Diversion Gombak River in Kuala Lumpur during Dry Season

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Abstract

The issue of solid waste disposal were mainly influenced by the anthropogenic activities which become devastating toward the environmental sustainability in Malaysia. The limited of historical data on solid waste composition in the river influenced the decision making process by relevant authorities and agencies. This paper aimed to identify the amount of solid waste composition at the log boom of Diversion Gombak River during dry season. Solid waste items trapped at the log boom were collected, weighed and categorized in 14 days events of sampling. Based on the 14 data of sampling, the results shows that the log boom was dominated with 52% (85.1 $\pm$ 14.8 kg) of plastic waste as it was known as non-biodegradable and buoyant items to float on the water surface for a long period of time. Baseline data from this study can generate more information in helping the relevant authorities and agencies in decision making process to improve and enhance the sustainable solid waste management and river management strategies for a better future.

Keywords: Baseline data, Dry season, Log boom, Plastic waste, Solid waste composition

1. INTRODUCTION

Klang River, Gombak River, Batu River, Bunus River, Kerayong River and Jinjang River were known as the most polluted river due to abundance of waste load in Kuala Lumpur that deriving from resettlement area and food stalls along the rivers as it has been reported to the local authorities. Solid waste that commonly found in the river known as riverine litter were mainly due to the anthropogenic activities such as illegal dumping. One of the major factors that contribute to high abundance of waste was immigrants from the other countries that dwelling in squatter settlement along Klang River with lacking of waste disposal and sanitation facilities as it has been highlighted by Chan et al. [1]. Another point of view from Daskalopoulos et al. [2] have indicated that population growth and high living standard of the people were the major factors in influencing the amount of municipal solid waste generation and its composition. Besides that, littering on the road and public areas also can be transported by winds and surface runoff into the river through drainage system. The river act as a platform to transport the various types of waste to the sea [3]. Therefore, in such situation, log boom or waste trap has been installed at the river act as a structural measure to prevent and reduce the waste load from being transported into the main river.

The systematic and comprehensive monitoring can contribute a comprehensible view on how much debris is being transported by the river [4]. However, the major issues

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faced by the relevant authorities were the financial constraint and management capacity to maintain the overall database on solid waste generation and composition for the long period of time [5]. The abundance of waste in the river requires high cost for operation cost to clean the river. Zhang et al. [6] have stated that the high cost, time consumption and commitment every year are required in order to remove the floating debris. The decision making process among relevant authorities and agencies in Malaysia on solid waste composition in the river can be complex and difficult due to the limited of historical data on the amount of waste generation and its composition [7] especially for the benefit and future planning on solid waste management and river basin management. Therefore, in such situation, this study is aimed to identify the amount of solid waste composition at the log boom of Diversion Gombak River during dry season.

2. MATERIALS AND METHODS

2.1. Description of Study Area

The Federal Territory of Kuala Lumpur is very congested with rapid development of urbanization with the increasing number of population with 1 724 500 [8]. According to Bradley [9], the urbanization rate in the basin is growing from 5% to 6% number of population annually in average over the last 15 years. Log boom of Diversion Gombak River was selected as a study area in order to identify the solid waste composition being trapped at the log boom in terms of types and weight.

2.2. Solid Waste Collection

The solid waste collection was conducted during dry season from March to the end of April 2016. In this study, 14 days of sampling for solid waste data collection for dry season have been conducted at log boom of Diversion Gombak River. However, the rate of conveyer to transport the waste to facility is normally fluctuated during operation day due to the technical issue (Example; machine breakdown) and time consumption of man power to push the waste in the river manually. Therefore, the above equation has been adjusted in range 30 minutes to weigh every nth conveyer that transported waste to facility. The total waste tonnage has been estimated by following equation below;

$$W = T_1 (w_1/t_1) + T_2 (w_2/t_2) + T_3 (w_3/t_3) + \ldots\ldots + T_n (w_n/t_n) \quad (1)$$

Where;

W= the total weight of waste transported by conveyer
T= the total number of conveyer that transported waste
w= the total weight of conveyer that were weighed
t= the number of conveyer that were weighed

After that, the amount of waste has been transported to facility, weighed and recorded.

2.3. Classification of Solid Waste Composition

For solid waste composition study, the American Society for Testing and Materials Standard test method, ASTM D5231 [10] has been applied in order to determine the composition of unprocessed municipal solid waste. The samples of waste has been manually collected randomly with the optimum sample size; 200lb-300lb (91 – 136 kg) as it has been recommended by SCS Engineers [11], Klee and Carruth [12], Britton [13]. After that, the waste composition has been classified into 11 different types of waste (plastics; organic; metal; cardboard/paper/tetrapak (CPT); glass; garden waste; bulky waste; napkins; rubber; textiles; and others), weighed and recorded.

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3. RESULTS AND DISCUSSION

The results presented here were based on a single sampling during the dry season. The descriptive results of this study has been systematically presented and discussed in the Section 3.1 and Section 3.2.

3.1. Total Weight of solid waste trapped at Log Boom

The high number of population and the areas with high number of commercial activity produced massive amount of waste in terms of quantity [14]. Although the 14 days of sampling for waste collection during operation day has been conducted under severe drought condition of El Nino from March to April 2016, the high abundance of waste still trapped at the log boom with 34534.1 kg (2466.7 $\pm$ 1609.2 kg). However, Yousuf and Rahman [15] have stated that the amount of waste generation can be affected by seasonal factors. They found that the rate of waste generation during the wet season was higher compared the rate of waste generation during the dry season. This paper was presented only for the dry season. The waste collection for wet season will be conducted on this October 2016 in order to identify any significance difference between seasonal variations condition and amount of waste generation being disposed in the river. From the other perspectives, the abundance of waste in the river was not mainly due to the effect from seasonal factor only, it probably comes from various factor such as inefficient solid waste management from authority; and low level of knowledge, attitude and practices (KAP) from residents and public. Cole et al. [16] have stated that the Local Authority has to highlight more on waste reduction from household waste management. The proper of waste management from Local Authority and relevant agencies that responsible to clean up the river required a high cooperation from the residents and public to work together in achieving the clean environment. According to Malik et al. [17], the environmental awareness program and media campaign can be a medium to share the knowledge and it required a long period of time and difficult to transform the better public mentality, attitude and their behaviors in managing the proper solid waste disposal.

Based on the Figure 1, the operation day during Event 9 contribute the highest proportions of the amount of waste trapped at the log boom with 14.9% (5142.4 kg/operation day) due to the high heterogeneous of waste condition with the mud, followed by the operation day during Event 1 with 14.3% (4932.0 kg/operation day). However, the amount of waste trapped at the log boom during Event 13 and Event 14 was low with 1.5% (501.5 kg/operation day) and 1.4% (475.9 kg/operation day) as it was a remaining waste that trapped during the last operation day in April.

3.2. Solid Waste Composition trapped at Log Boom

According to the result presented in Table 1 and Figure 2 show that plastic represent the largest portion of waste at log boom of Diversion Gombak River with 52% (85.1 $\pm$ 14.8 kg). Most of the plastic waste found in the types of food packaging, plastic bags, plastic bottles and polystyrene where it most probably derives from the littering and transported into the river by surface runoff; or the plastics were directly thrown into the river. Plastic wastes known as a major source of water pollution especially in the rivers and oceans due to its physical characteristics and floating at the water surface, such as light-weight; strengthen; resistance to corrosion; electrical insulation [18]; cheap; durable and versatile [3], and disposable items which led to the extensive use among the consumers to simply throw it in any places. The garden waste represent the second higher proportion from waste composition with 18% (29.1 $\pm$ 12.8 kg). Garden waste consists of twig, stems, tree branches and leaves were normally found during the waste collection probably derives from the nearest trees along the river that fall and carried by current rivers.

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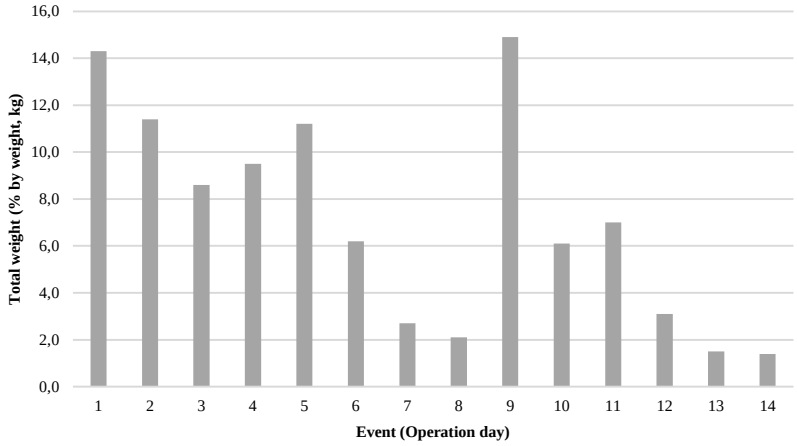

Figure 1. The total weight of solid waste trapped at the log boom during 14 days of operation day (% by weight, kg)

Bulky waste such as timber and woods represented with 8% (13.2 ± 5.4 kg) from waste composition proportion, followed by organic waste where floating fruits such as watermelon; coconuts; oranges and others were mainly found during waste collection with 7% (12.1 ± 6.5 kg). Generally, the organic waste contribute the highest proportion from household with 45.0% [19]. The organic waste become one of the most environmental issues in waste management in Malaysia because it can give an adverse effect towards the environment by emitting the high production of methane and carbon dioxide from the landfills, bad odors and leachate. However, the organic waste will undergoes degradation process once it has been thrown into the river.

Table 2. Average mean and standard deviation (SD) for each types of waste

Types of waste	Mean ± SD (kg)
Plastic	85.1 ± 14.8
CPT	2.7 ± 0.6
Organic	12.1 ± 6.5
Glass	2.2 ± 2.1
Metal	2.4 ± 0.5
Bulky	13.2 ± 5.4
Garden	29.1 ± 12.8
Napkins	5.5 ± 3.0
Textiles	1.5 ± 1.1
Rubber	1.9 ± 1.1
Others	8.2 ± 4.7

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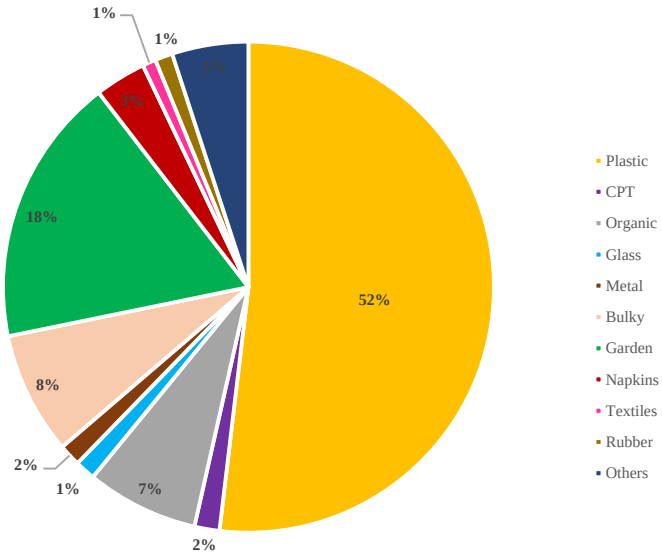

Figure 2. The proportion of solid waste composition (% by weight)

Another environmental major concerns was about the improper of solid waste disposal specifically on napkins. Napkins were contributed from waste composition with 3% (5.5 ± 3.0 kg). In such situation, it shows that the residents and public in that particular area have a low level of knowledge, attitude and practices in managing the proper of solid waste disposal.

Metal and CPT have contribute 2% of waste at the log boom with 2.4 ± 0.5 kg and 2.7 ± 0.6 kg, respectively. Glass, textiles and rubber have contribute 1% of waste at the log boom with 2.2 ± 2.1 kg, 1.5 ± 1.1 kg and 1.9 ± 1.1 kg. The metal, CPT and glass were classified as the most potential recyclable materials. However, the proportion for the metal that consisting of aluminum cans; CPT and glass were small due to their high economic value through recycling methods. Another 5% (8.2 ± 4.7 kg) of waste from others categories are consists of brooms, mops, filters, sponges and others materials.

4. CONCLUSIONS

The present study concluded that there were high abundance of waste that being trapped at the log boom of Diversion Gombak River even during the dry season. Based on the results in this study, it shows that the log boom has dominated by plastic waste with 52% (85.1 ± 14.8 kg). Results presented here were based on a single sampling during dry season. It was assumed that the high abundance of waste trapped at the log boom probably arise from the anthropogenic human activities such as improper solid waste disposal and littering which subsequently might be washed into the river through surface runoff and wind. In such situation, the waste composition information was very crucial to the relevant agencies in managing the solid waste and river because those information can help in developing better implementation of waste minimization policies and river management policies. It was suggested that the study of waste composition study will become clearer when the waste composition study conducted during wet season in order to identify the significance comparison of the abundance of waste being trapped at the log boom influenced by hydrological characteristics including river flow rate and rainfall.

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Optimization of DDT biodegradation by brownrot fungi *Daedalea dickinsii* with *Bacillus subtilis* addition

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Abstract

DDT[1,1,1-trichloro-2,2bis(p-chlorophenyl)ethane] is one of the most common persistent organochlorine pesticides (OCPs) that continues to pose serious risk to human health and the environment. Some treatment methods have been developed to reduce and minimize the adverse impacts of the use of DDT including biodegradation with brown rot fungi (BRF). However, DDT degradation using BRF has still low and consumes long incubation time. Therefore, it needs to enhance the ability in degrading DDT. Effect of *Bacillus subtilis* addition to degrade DDT by *Daedalea dickinsii* was investigated, whether *B. subtilis* would be suitable for optimization of DDT degradation by *D. dickinsii.* In this study, *B. subtilis* was added into 10 mL of *D. dickinsii* culture at 1, 3, 5, 7, and 10 ml (1 mL ≈ 6,7 x 10^8*B. subtilis* bacteria cells). The addition of 1 mL of *B. subtilis* showed the highest DDT degradation of 74,33%, compared with that by *D.dickinsii* without *B. subtilis* addition (53,61%). This study indicated that addition of *B. subtilis* can be used for optimization of DDT degradation by *D. dickinsii*.

Keywords: Bacillus subtilis, Biodegradation, DDT, Daedalea dickinsii, Optimization.

1. INTRODUCTION

Pesticides in one of important thing in agricultural sectore. In agriculture, synthetic pesticides has become an indispensable tool to control the pests. About 4 milion tons of pesticides are apllied annually for pest control. Therefore, most of pesticide reamains unused and enters into ecosystem[1,2]. The organochlorine pesticides are known to be highly persistant in the environment. One of them is dichlorodiphenyltrichloroethane (DDT). DDT is the most well known pesticide from the organochlorine group. DDT was first synthesized by Othmar Zeidler in 1874 and widely used as efficient insecticide when their ability to control insect was found by Paul Hermann Muller in 1939 [3]. The use of DDT to control malaria bearing mosquitoes earned Muller in 1948 for Nobel Prize in Medicine [4]. The used of DDT as insecticide was continued for 25 to 30 years after World war II in most countries [5].

At the end of the 1950, Rachel Carson began to gather examples of environmental damages attributed to DDT [6]. DDT became one of the most persistent environment because of its toxicity and hydrophobicity resulting in its bioaccumulation [7]. DDT has negative impact on wildlife and its toxic effects on human health via the food chain [8]. The chloric atoms in DDT and its insoluble metabolites make it highly toxic to organisms [9]. Human exposure to DDT and its metabolit products may be associated with breast cancer, diabetes, decreased semen quality, spontaneous abortion, preterm birth, early weaning and impaired neuro development in children [10, 11]. Because of its toxicity, hydrophobicity, and bioaccumulation, DDT was classified as National Priority List of the most persistent environmental pollutant by U.S. Environmental Protection Agency (EPA) and it was banned in most countries [12].

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The presence of DDT in environment is a great concern due to its persistent and toxic biological effects. Some studies have reported the presence of stable residues of DDT in air, water, soil, sediment, fish and birds more than 10 years after it was banned [13]. DDT and its metabolit products have been found in water, soil, and river sediment. Sudaryanto et al. [14] also report that DDT still found in some rivers, soils, sediment and some biotic environments in Indonesia. DDT residue was also resulting some potential risks to the human health [15]. Toxic effects on humans together with long-lasting effects on wildlife made the removal of DDT from environment has become an environmental priority.

Some DDT remediation processes have been studied including hydrodechlorination [16], reductive dechlorination using metals [17], photoremediation [18], electrolysis [19] and biodegradation [20]. Although chemical and physical treatments are more rapid than biological treatments, they are need more energy intensive, and often more expensive than bioremediation [21]. Among several methods, biodegradation is relatively low cost more secure, and efficient [20]. Purnomo et al (2010) [22] reported that *Daedalea dickinsii*, one kind of brown rot fungi (BRF) have capability to degradated DDT via Fenton reaction. *D. dickinsii* have capability to degradated DDT about 47% in potato dexrose borth (PDB) for 14 days incubation. However, its amount of degradation of DDT by *D. dickinsii* was still low and consumes long incubation time. Therefore, it needs to be developed by modifying the culture to enhance the ability of *D. dickinsii* in degrading DDT.

Some study about biodegradation using mixed cultures between fungi and bacteria had been reported that bacteria addition can increase degradation capability by fungi. Zang at al [23,24] reported that with *B. subtilis* addition increased 2-napthol degradation by *Fusaruim proliferatum* and *Aspergillus niger*. This study examined the effect of bacteria addition on DDT degradation by *D. dickinsii*. Bacteria that we used in this study was *B. subtilis*. *B. subtilis* already reported greatly degradate DDT in trypticase soy broth medium in 7 days incubation [25]. The degradation capability of *B. subtilis* depend on their capability to produce degradation enzyme. Jones et al. (2012) reported that *B. subtilis* have capability to produce xilanase as their degradation enzyme [26]. Besides, *B. subtilis* also have capability to produce biosurfactant [27]. Biosurfactant can increase the solubility of DDT to optimize DDT degradation process [28]. However, the effect of *B. subtilis* addition on DDT biodegradation by *D. dickinsii* has not been reported yet. Therefore, the ability of *B. subtilis* to optimize DDT degradation by *D. dickinsii* was investigated.

2. METHODS

2.1. Chemicals

DDT was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). *n*-hexane and acetone were purchased from Anhui Fulltime Specialized Solvent & Reagent Co., Ltd (Anhui, China). Methanol, dimethylsulfoxide (DMSO), and sodium sulphate anhydrous were purchased from Merck Millipore (Darmstadt, German).

2.2. Cultur Condition

Brown-rot fungus *Daedalea dickinsii* NBRC31163 (NITE Biological Resource Center, NBRC; Chiba, Japan) was used in this study. This fungus was maintained as cultures on potato dextrose agar (PDA; Merck, Darmstadt, German). This fungus had been incubated at 30°C . *D. dickinsii* mycelia (1 cm diameter) was inoculated into 10 ml PDB (Difco). Cultures were pre-incubated at 30°C for 7 days [21].

Bacteria *B. subtilis* was maintened on nutrient agar (NA; Merck, Darmstadt, German), which was incubated at 37°C. The colony was inoculated into 60 mL nutrient

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borth (NB; Merck, Darmstadt, German). Cultures were pre-incubated at 37°C for 21 hours with shaker condition 180 rpm.

2.3. DDT Biodegradation by D. dickinsii

After pre-incubation for 7 days, 10 mL PDB medium was added into the cultures (final volume 20 mL) and 50 µl of 5 mM DDT in DMSO was added to each inoculated flask (final concentration: 0.25 µmol/flask). Each flask was flushed with oxygen and sealed with a glass stopper and sealing tape to prevent the volatilization of DDT. The cultures were incubated statically for 7 days at 30°C. As a control, the cultures were terminated by autoclave (121°C, 15min) after pre-incubation. The experiments were performed in duplo [22].

2.4. DDT Biodegradation by B. subtilis

After pre-incubation for 20 hours, 1, 3, 5, 7, and 10 mL *B. subtilis* (1 mL ≈ 6,7 x 10^8 bacteria cells) was added separately into PDB medium (final volume 20 mL) and 50 µl of 5 mM DDT in DMSO was added to each inoculated flask (final concentration: 0.25 µmol/flask). Each flask was flushed with oxygen and sealed with a glass stopper and sealing tape to prevent the volatilization of DDT. The cultures were incubated statically for 7 days at 30°C. As a control, the cultures were terminated by autoclave (121°C, 15 min) after pre-incubation. The experiments were performed in duplo [22].

2.5. DDT Biodegradation by mixculture D. dickinsii and B. subtilis

After *D. dickinsii* pre-incubation for 7 days, 1, 3, 5, 7, and 10 mL *B. subtilis* (1 mL ≈ 6.7 x 10^8 bacteria cells) was added separately into *D. dickinsii* cultures and PDB medium was added into final volume 20 mL.DDT 50 µl of 5 mM in DMSO was added to each inoculated flask (final concentration: 0.25 µmol/flask). Each flask was flushed with oxygen and sealed with a glass stopper and sealing tape to prevent the volatilization of DDT. The cultures were incubated statically for 7 days at 30°C. As a control, the cultures were terminated by autoclave (121°C, 15min) after pre-incubation. The experiments were performed in duplo [22].

2.6. DDT Recovery

After additional incubation, 50 µL of pyrene 5 mM in DMSO (final concentration, 0.25 µmol) were added into each flask as internal standart. The cultures were homogenized with 20 mL of methanol and then washed with 5 mL acetone. The residual biomass was removed bycentrifugation at 3000 rpm for 10 min. After that, supernatant was filtrated with Whatman Filter Paper 41 (GE Healthcare Life Science, UK), the resulting filtrate was evaporated at 64°C and extracted with 200 mL n-hexane. The organic fraction was collected and dried over anhydrous sodium sulfate. The extracts were evaporated at 68°C and concentrated to dryness under reduced pressure. The concentrate was diluted with methanol then analyzed by high-performance liquid chromatography (HPLC) to quantify the amount of substrate. HPLC was conducted with a Jasco PU-1500 intelligent pump and a Jasco MD-1510 multi wavelenght detector fitted with an Inertsil ODS-3 column (150 mm) with an inner diameter of 4.6 mm (GL Science, Tokyo). The samples were eluted with 82% methanol in a 0.1% trifluoroacetic acid aqueous solution at a flow rate of 1 ml/min.

3. RESULT AND DISCUSSION

This study investigated the optimization of DDT bidegradation by *D. dickinsii* with *B. subtilis* addition. DDT degradation by *D. dickinsii* was still low and need long incubation time. *D. dickinsii* capable of degrading DDT (final concentaration 0,25 µM DDT/Flask) by 53,61% during 7 days incubation in PDB medium. In previous study, Purnomo et al (2010) reported that *D. dickinsii* have capability to degradated DDT about

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47% in potato dexrose borth (PDB) for 14 days incubation [22]. DDT degradation ability of *D. dickinsii* was depended on their ability to produced extracellular hydroxyl radical via fenton reaction [22]. The production of ·OH is a universal characteristic of wood degradation by brown rot fungi, which a part of their wood-degrading system [29].

B. subtilis was used as added bacteria because in previous study *B. subtilis* was reported have capability to enchance some organic polutant degradation by fungi. Zang et al [23,24] reported that with *B. subtilis* addition increased 2-napthol degradation by *F. proliferatum* and *A. niger*. Besides, *B. subtilis* also already reported greatly degradate DDT in trypticase soy broth medium in 7 days incubation [25]. In this study, DDT degradation by *B. subtilis* was carried out with concentration variation at 1, 3, 5, 7, and 10 mL (1 mL ≈ 6,7 x 10^8 bacteria cells). The *B. subtilis* degraded DDT by 35,38%, 66,21%, 79,51%, 84,06% and 86,44% at a concentration 1, 3, 5, 7, dan 10 mL of bacteria during 7 days incubation in PDB medium, respectively. These data showed that the hinger concentration of *B. subtilis* was directly proportional to the amount of DDT degraded. DDT degradation by *B. subtilis* at 1, 3, 5, 7, and 10 mL was showed in Figure 1. The ability of *B. subtilis* to degradate pollutants was based on their ability to produced degradation enzymes. Jones et al. (2012) reported that *B. subtilis* have capability to produce xilanase as their degradation enzyme [26].

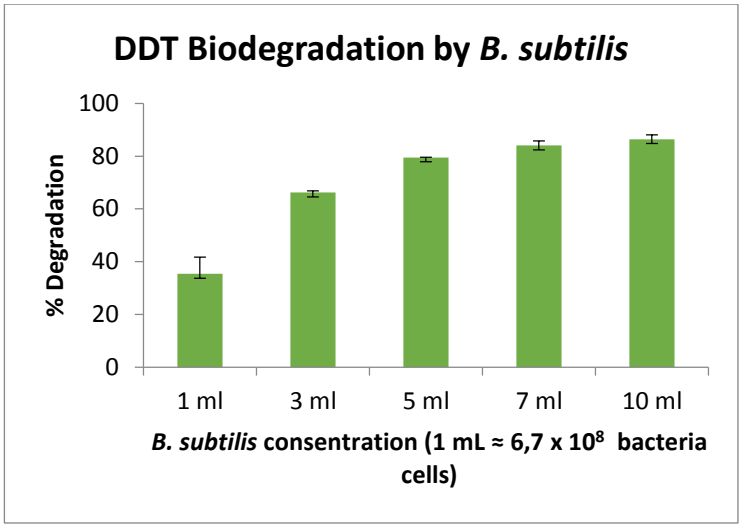

Figure 1. DDT Biodegradation by B.subtilis

For optimization process, *B. subtilis* was added into *D. dickinsii* culture at 1, 3, 5, 7, and 10 mL (1 mL ≈ 6,7 x 10^8 bacteria cells). After 7 days incubation, 74,33%, 64,20%, 57,06%, 63,24% and 67,60% of DDT was degraded by mixed cultured with the addition of concentration bacteria at 1, 3, 5, 7, dan 10 mL, respectively. Figure 2. showed the ability of *D. dickinsii* (blue bars) and Mixed cultures (green bars). Based on this figure, the addition of 1 mL *B. subtilis* to *D. dickinsii* culture enhanced the DDT degradation ability by appoximately 74,33%. Without addition of *B.subtilis*, ability of DDT degradation by *D. dickinsii* was only 53,61%. It indicated that the amount of addition of *B. subtilis* affected the synergistic relationship on DDT degradatin by *D. dickinsii*. The enhanced ability to degrade DDT can be correlated to biosurfactant produced by *B. subtilis*. *B. subtilis* is one of bacteria that can produce biosurfactant. Banat et al. (2010) reported that *B.subtilis* have capability to produce biosurfactants type surfactin [27]. In the other study Plociniczak et al. (2011) reported that biosurfactant can increase the solubility of DDT to optimize DDT degradation process [28 plociniczak, 2011]. Because of DDT was hydrophobic compound, biosurfactant, which produced from addition *B. subtilis*, can increase the solubility of DDT to optimize the DDT degradation process.

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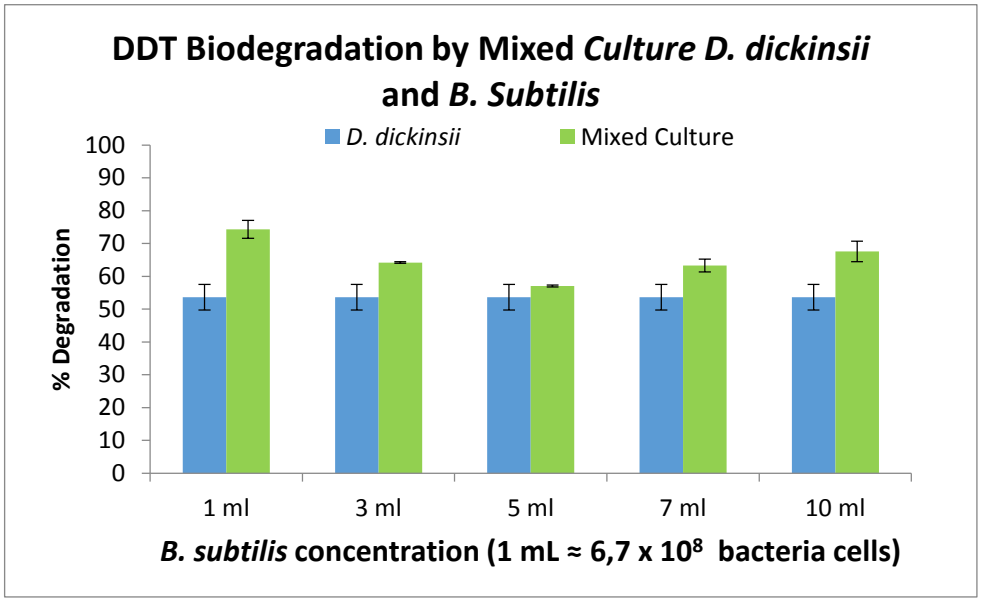

Figure 2. DDT Biodegradation by mixed culture D. dickinsii and B. subtilis

4. CONCLUSION

Effect of *Basillus subtilis* addition to degrade DDT by *Daedalea dickinsii* was investigated, which *B. subtilis* would be suitable for optimization of DDT degradation by *D. dickinsii*. In this study, *B. subtilis* was added into 10 mL of *D. dickinsii* culture at 1, 3, 5, 7, and 10 ml (1 mL ≈ 6,7 x 10^8 *B. subtilis* bacteria cells). The addition of 1 mL of *B. subtilis* showed the highest DDT degradation of 74,33%, compared with that by *D. dickinsii* without *B. subtilis* addition (53,61%). This study indicated that addition of *B. subtilis* can be used for optimization of DDT degradation by *D. dickinsii*.

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Evaluation of Waste Reduction Sustainability

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Abstract

Waste reduction at the source is currently one of the major project in Indonesia. The main challenge of this project is to maintain its sustainability. The purpose of this study was to identify the level of sustainability of waste reduction system which was carried out in Cibangkongof Bandung City and Cilengkrang of Bandung Regency. These area are using Biodigester as the main technology to treat their organic waste. The level of sustainability was measured by using Weight Ranking Teqhnique (WRT) method based on three aspects, namely social, environmental and economic. The main indicators of the environmental aspects measured by the rate of waste reduction, economic indicators measured by Cost Benefit Analysis method while social indicators measured by the level of community participation. The efficiency of waste reduction in Cibangkong was smaller which only 49.5 L/day at 0.84% compared to Cilengkrang which has 80.53 L/day. The benefits of waste reduction to society was best perceived by Cibangkong (0.535 points) than Cilengkrang (0.465 points). The results of WRT method, the level three aspects of sustainability, Cilengkrang has a score of 0.546 meanwhile Cibangkong only at score of 0.454. The result showed that the level of of waste reduction sustainability in Cilengkrang which represent the sub urban areas was better than in the Cibangkong which representing urban areas.

Keywords: Waste Reduction, TheSustainabililty Level, Environment, Social, Economy

1. INTRODUCTION

Law No. 18 of 2008 declare that waste management is consists of waste reduction and waste handling. Waste handling is a series of activities that include sorting waste handling, collection, transportation to the processing of the final disposal. While waste reduction activities is addressing the emergence of waste from the source, reuse and recycling at the source and / or at on-site processing, these activities are known as the 3R (reduce, reuse, recycle) programs.

In Indonesia, various waste reduction activities made by local governments, in particular City Waste Management Institution of the City Government and the City District Government. Under Law no.18 / 2008, every local government is obliged to establish waste reduction targets and makes every effort to achieve it. The 3R target fulfillment, have been carried out using various types of both individual and communal technologies that are considered to have an appropriateness elements, such as composting. Honnwerg, et.all, 2000, informed over the past decades composting have failed due to technical, financial, and institutional reasons in the developing countries. Some of them are due to lack of attention to the biological process necessities, poor feed stock which yields poor quality finished compost, sensible preoccupation by municipal authorities to first concentrate on providing adequate waste collection, nuisance potential, poor marketing experiences, poor integration with the agricultural community, and sometimes constrains in land requirements.

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Especially in West Java for the last few years there has been a change towards the use of technology, by using anaerobic digester which often called as bio-digester. It is considered more practical in terms of operation and utilization of the bio-digester product, which can be directly used. Edelmann, et.al, 2005, stated that anaerobic digestion is shown to be advantageous compared to composting, incineration or a combination of digestion and composting mainly because the energy it generates is quite balanced. In addition, Igoni, et al., in 2007 said that the technology could be the solution as an alternative energy in the middle of low fossil energy production and noticed the opportunities of high municipal solid waste generation.

Learning from the ineffectiveness of the composter implementation, then the use of bio-digesters should perceive about the meaning of sustainability. Based on the definition from Brundlant, 1987, and Barbier, 1987, an activity can be stated to have a level of sustainability if it takes into consideration the three main pillars: environmental, social and economy. So to be considered sustainable, 3R program should pay attention to these three pillars. Integration of sustainability toward the use of the bio-digester, is urgently needed because the implementation of waste management by communal bio-digester has developed sporadically in both the urban and sub urban of Bandung City. Based on observations, the initiation of the implementation of the 3R program with bio-digester was initiated by various parties, ranging from the local government, the private sector through public initiatives, CSR even from the community itself. Therefore, 3R program with communal bio-digester is expected to reduce waste generation.

This study aims to compare the sustainability of the 3R program with biodigester through various differences in its initiation, from the local community and local government. The study also examines: (1) The level of environmental sustainability,observed through the impact of the existence of communal biodigester related to the level of waste reduction; (2) The level of economic sustainability, observed through self-reliance funding on the implementation of the 3R with manual biodigester and the level of its benefit for the society; (3) The level of social sustainability, observed through the level of community participation in the implementation of the program of communal biodigester; and (4) The level of waste redcution sustainability by using biodigester in both urban and sub urban area.

2. MATERIALS AND METHOD

This research will discuss each pillars of sustainability and its integration which consist of social, environment and economic pillars that can be seen in the scheme in Figure 1.

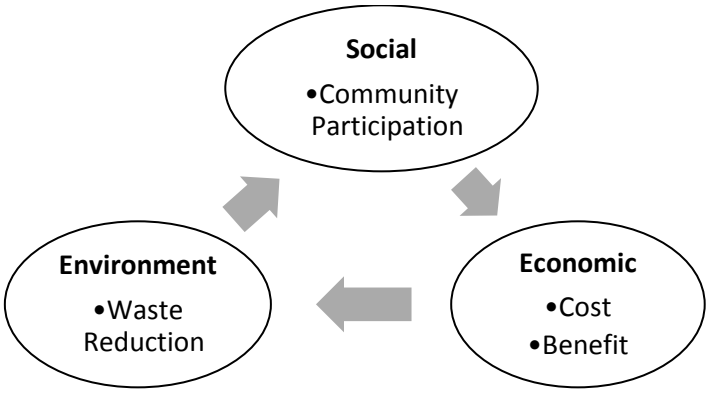

Figure 1. Research Scheme

The study were dominantly conducted as a field study which covered two study areas. The indicator in selecting the study area were (1) the use of bio-digester as the communal technology in 3R program, (2) different initiation, and (3) represents the differences between urban and sub urban area. The selected area was Cibangkong (RW 11) in Bandung City which represent the urban area and has initiative from stakeholders

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such as government, academic institution (UNPAD) and private sector (Biomethagreen). Whereas Cilengkrang (RW 17) in Bandung sub-districts represents the sub-urban area with initiative solely comes from the community and it has other 3R activities which being in active implementation, such as composting actions and bio-pori actions.

The biodigester specification in both study area are made of fiber and resin, the process use water and waste with ration of 1:1, meanwhile the contrast are as follows (1) Cibangkong was using trapezoidal in shape with total volume is about 550 L, continues feeding, waste segregated, (2) Cilengkrang : trapezoidal in shape with total volume is about 150 L, semi continues feeding, waste segregated and chopped.

The measurement on environment sustainability will focus on the waste reduction. Waste reduction will calculated based on waste generation, waste input to bio-digester and the ration of bio-digester capacity with waste input. The waste input was directly measured and conducted using the Indonesian National Standard (SNI)19-3964-1994, while the waste generation of each area study were calculated based on the previous study which was conducted by Dwihapsari, 2013. The key parameter of waste characterization in bio-digester process was measured in accordance to the standard method for the examination of water and wastewater which can be seen at Table 1. The purpose of waste characteristic measurement was to determine the substrate feasibility of bio-digester in order to produce gas and fertilizer. Parameters that being measured for waste characterization are Moisture, Organic Carbon, Nitrogen Total Kjedahl (NTK), and Phosphate.

Table 1. Key Parameter Method Measurement

No.	Parameter	Method
1.	Moisture	Gravimetry
2.	Organic Carbon	*Wakley and Black*
3.	Nitrogen Total Kjedahl	Kjedahl analyzer
4.	*Phosphate*	Spektrofotometry

The social sustainability will focus on measurement of the level of community participation meanwhile the economic sustainability measurement will focus on determination of financial analysis to gain the cost data, the pay-back period data and the level of beneficiaries of bio-digester that the community experienced. Both pillars were measured by using questionnaires with research design questionnaire scheme that can be seen in Figure 2. The questionnaire which was designed using Likert Scale and also using related secondary data. Slovin formula was used to determine the number of sample in each study area. The secondary data were used for analyzing the cash flow in calculating the pay-back period. The data needed are the investment data for biodigester manufacturing, revenues and expenses which are incurred due to the 3R activities, such as income of gas and liquid fertilizer sales and bio-digesters operation and maintenance cost. The entire unit calculations on the financial aspects were using Rp.per year.

The analysis will be conducted on every aspect of sustainability separately in order to get the comprehensive review which used qualitative and quantitative analysis methods. Meanwhile the comparison of the degree of the sustainability of 3R program for both study area will used the Weighted Ranking Method.

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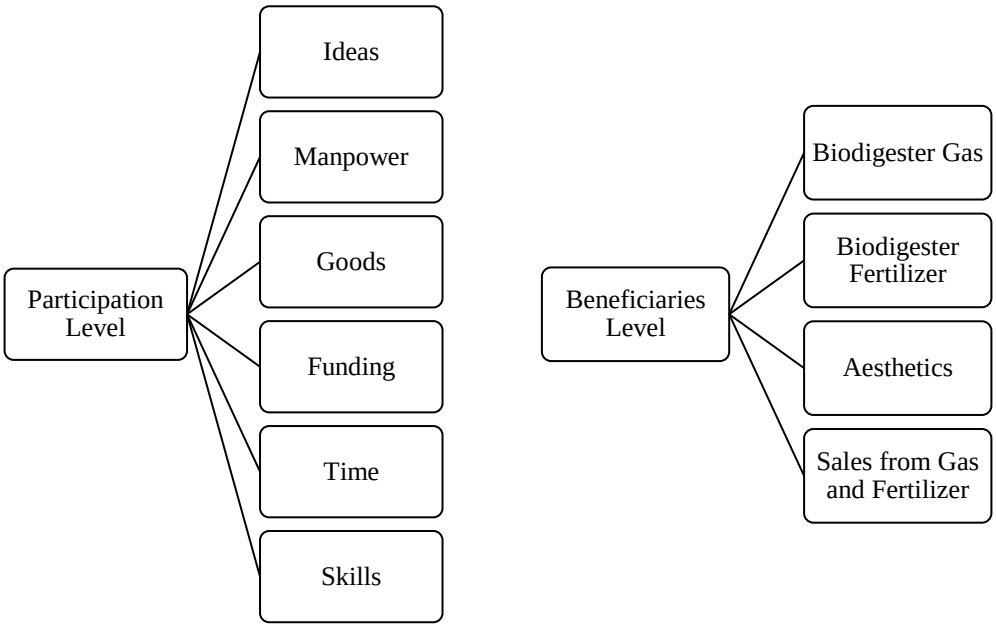

Figure 2. Research Questionnaire Design Scheme

3. RESULTS AND DISSCUSSION

3.1. The Level Of Environment Sustainability

Ratio of waste characterization on key parameter can be seen in Table 2. However, moisture content of the waste in Cibangkong are 90.9% and Cilengkrang was only 78.8%.The waste moisture in Cibangkong were larger than in Cilengkrang, the condition was caused by the differences of waste composition. Cibangkong waste composition was dominantly consists of food scraps or processed foods that are mixed with water, while Cilengkrang was dominated by agricultural products. Moisture content play a role to serves as a medium to transport critical nutrients (Honnwerg, et.all, 2000). Furthermore, both of the study area has waste moisture content above 60% which will restrict air movement and can resulted in anaerobic conditions (Lardinois and van der Klundert, 1994).

Table 2. Waste Characterization Comparison

Study Area	C:N	C:P	N:P
Cibangkong	31.84	1592.11	50
Cilengkrang	30.53	8141.67	266.67
Standard Theory	50	250	5

Table 2 also informs ratio C:N between two study area were not much different. However, low content of phosphate in both of study area (Cibangkong 0.076% and Cilengkrang 0.0012%) create the ratio of C: P and N: P were significantly different than the standard. Higher phosphate content (6 times) than in Cibangkong also due to the waste composistion differences. The content of phosphate in food scraps or processed foods is higher than in agriculture product for which phosphate is part of a nucleic acid that is present in all organisms and is an essential component of RNA, DNA, ATP, and phospholipids but in nature it is not available in large quantities (Waluyo, 2007). Compared with the ideal conditions in the biodigester process, the C: N ratio was below standard which mean that the waste has low nitrogen content and the low content of

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phosphate causing the ratio C: P and N: P above standard. Based on the waste characteristic the biodigester in both study area needs a phosphate supplied as a starter in order to enhance the process of their bio-digester.

The most important part from environmental aspect is waste reduction. The waste generation from RW 11 Cibangkong and Cilengkrang up to 2.9 m^3/day and 0,565 m^3/day in sequence. The percentage of reduction due to Biodigester facility in Cibangkong was only 1.69%/day (0,49 m3/day), meanwhile the percantage in Cilengkrang reach 14.25% (0,8 m3/day). Actually, those percentage can be upgraded by maximizing the capacity of biodigesters. According to our calculation, biodigester in Cilengkrang was only used only 9% of their capacity and in Cilengkrang was up to 53%. This condition described that we need increasing public participation to increasing waste reduction by bio-digester.

3.2. The Level Of Social Sustainability

The level of Social Sustainability can be seen by the community participation. Sudradjat, 2000, stated that community participation consists of participation in forms of of ideas, man power, goods, funding, time and skills. The comparison of participation level in both of study area that divided into three categories (high level, medium level and low level) can be seen at Figure 3.

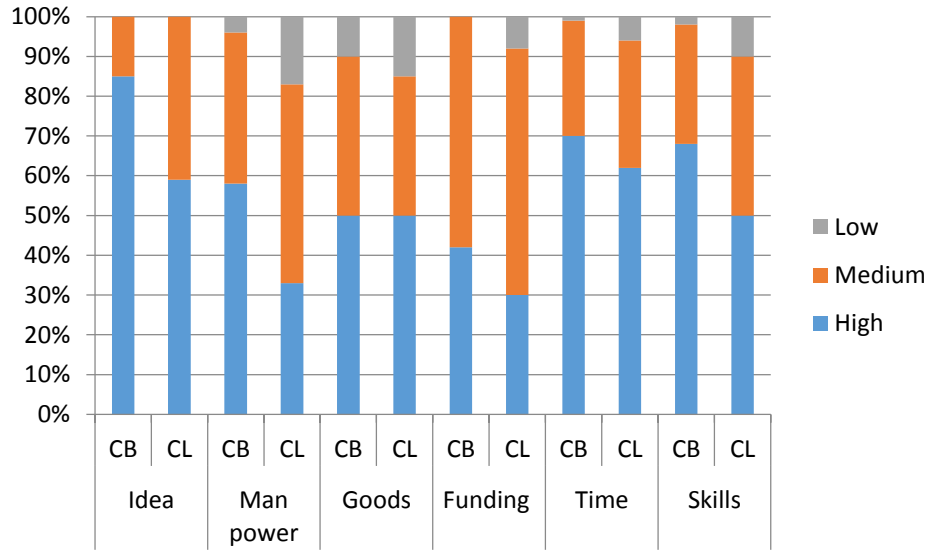

Figure 3. Proportion of Community Participation

The high level in all forms of participation can be seen more dominant in Cibangkong meanwhile the medium level were dominantly shown in Cilengkrang except participation in forms of goods. The community of Cibangkong prefer to participate more in forms of idea, time and skills meanwhile community of Cilengkrang prefer more in giving of their time and idea. Those phenomena were influenced by the difference of social economic level. The social economic characteristic in urban area shows that the education level were relatively higher, the types of profession force them mostly occupied more in the work places and the community contribution were relatively diverse.

The score of participation level in Cibangkong reach up to 3.6 points meanwhile Cilengkrang reach up to 3.3 points, even though their level was average (the highest participation reach up to 6 points) but Cibangkong community participation level were higher than Cilengkrang. This data shows that the community acceptance of Bio-digester in their area were quite high.

3.3. The Level Of Economic Sustainability

The level of Economic Sustainability can be seen by two aspects, which are the pay-back periods and the level of beneficiaries.

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A. The Pay Back Periode

The pay back periods were calculated by make use of the investment costs and the cash flow analysis. Although the bio-digester in Cibangkong were granted from Unpad (Padjadjaran University), in this study the investment cost were taken into account so that the comparison will be equal. Based on direct interview and secondary data the investment cost for developing the bio-digester in Cibangkong and Cilengkrang in sequences were up to Rp. 28.000.000,- and Rp. 18.000.000,- where the contrast due to the capacity differences and in the process of developing the bio-digester. The cash flow analysis was calculated by taking into account the revenue and the expenses in the operation of 3R program implementation in each study area. The analysis were included not only the operation and maintenance of bio-digester but also other 3R activities that reduce waste (such as waste bank, communal composting and bio-pore action) along with other supported activities (such as the transportation of waste to landfill).

The revenue were mainly come from the retribution of waste management from the community (dues in Cibangkong Rp.5000,- per house/month meanwhile Cilengkrang Rp. 10.000,- per house/month). The other revenue comes from both sales of the bio-digester products which were gas and fertilizers and other 3R activities products. The sales gained in both study area for Cibangkong were respectively reached up to Rp.960.000/year and Rp. 12.000.000/year meanwhile Cilengkrang reached up to Rp. 120.000/year and Rp. 20.400.000/year. Based on this data, it shows that in the point of economic view the bio-digester alone would not support the cash flow of the area, meanwhile other 3R activities were more economic reliable for the community. In general, the revenue in Cibangkong (Rp. 42.960.000,-/year) were considerably higher than in Cilengkrang (Rp. 40.320.000,-/year) due to several differences such as number of households, the dues per households and especially the active level of 3R programs in the area.

The expenses were calculated by taken into account the cost for operating and maintaining the 3R program in the area which can be divided into fixed costs and variable costs. The differences in element of the fixed costs other than waste employee wages in the study area were the waste transportation costs in Cibangkong as it was included in City waste management agency service area, meanwhile Cilengkrang were not. These facts causing the cost of fixed costs in Cibangkong were relatively higher than in Cilengkrang. The operation and maintenance cost with similar elements for 3R activities in Cibangkong (Rp. 5.321.000,- per year) were considerably lower than Cilengkrang that reached up to Rp. 9.057.000,- per year due to the differences in variability in 3R activities. The pay back estimation for each study area can be seen at Table 3.

Table 3. Estimation of The Payback Period

No	Items	Units	Cibangkong	Cilengkrang
1	Investment Cost	per year	Rp 28,000,000	Rp 18,000,000
2	Cash Flow	per year	Rp 2,359,000	Rp 18,663,000
3	Payback Periods	year	11.87	0.96

Table 3 informed that the payback period of Cilengkrang were shorter than Cibangkong. These phenomena showed that the variability of 3R activities and the active part of the community will give positive contribution in economic reliability and sustainability.

B. The Level of Beneficiaries

The level of beneficiaries of bio-digester can be measured by the perception of the community. Direct benefits of the existence of the bio-digester were gas and fertilizer that can be used, meanwhile indirect benefit were in the form of aesthetics and the sales of

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bio-digester products. The comparison of beneficiaries' level in both of study area can be seen at Figure 4.

The communities in both of study area were classified to have a high level perception of the beneficiary to the bio-digester existence in their area. The Cibangkong give a better perception than Cilengkrang. This phenomenon happened because in Cilengkrang, based on direct observation, there were more other 3R activities with better implementation such as bio-pore actions, waste bank and composter actions, meanwhile in Cibangkong the main activities were the bio-digester.

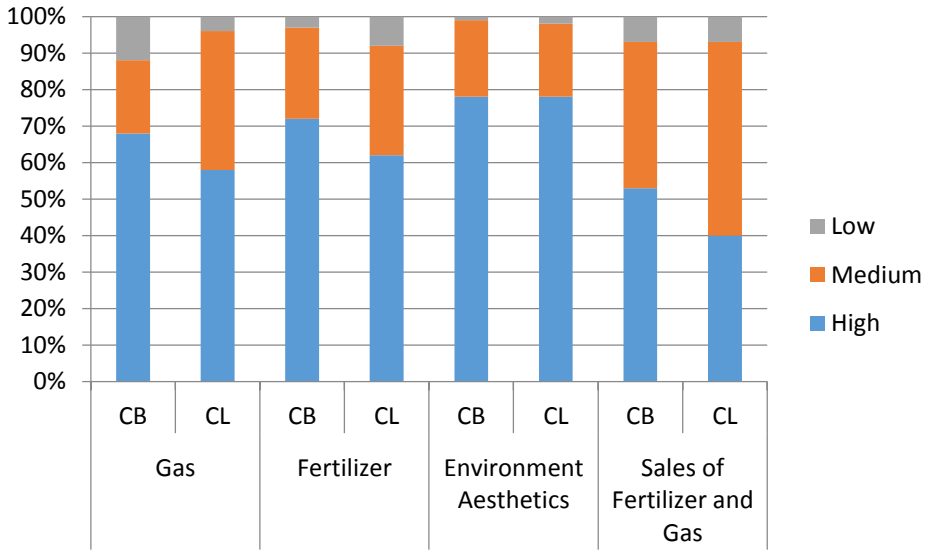

Figure 4. Proportion of Beneficiaries Level

The perception of beneficiaries' level in Cibangkong reach up to 3.7 point, meanwhile Cilengkrang reach up to 3.5 point which means that the community in both study area have a good perception to the bio-digester, they see the bio-digester gave many benefit to their environment, especially to the aesthetic and their product which can be directly used, such as gas and fertilizer.

3.4. The Comparison Of Sustainability Level

The level of waste reduction sustainability in both of study area will comprise of every aspect taken into consideration in environment, social and economic sustainability as an indicator. The method used was the Weighted Ranking Method. The first steps were indicator scoring process (IS) and determine the importance factor coefficient (IFC). The list of indicators and its value based on the previous explanation can be seen at Table 4. The estimation of indicators score for each aspect can be seen at Table 5. The calculation of IFC can be seen at Table 6. The final calculation of the weighted ranking technique of the waste reduction sustainability can be seen at Table 7.

Table 4 shows the list of indicators that were going to be used in measuring the degree of importance from each sustainability aspects (environment, social and economic). All indicators at Table 4 with each values that has been stated previously were used to determine each score of sustainability aspect.

Table 4. Lists Indicators of Each Aspect of Sustainability

Aspect Of Sustainability / Indicator	**Value**		**Units**
	CB	**CL**	
Environment			
Waste input to Biodigester	49.5	80.53	L/day
Ratio of waste input to biodigester capacity	0.9	5.4	%

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Aspect Of Sustainability / Indicator	Value		Units
	CB	CL	
Social			
High perception of contribution on Idea	85.57	59.04	%
High perception of contribution on Man Power	42.27	20.48	%
High perception of contribution on Goods	49.48	50.6	%
High perception of contribution on Funding	44.33	31.33	%
High perception of contribution on Time	69.07	61.45	%
High perception of contribution on Skills	65.98	49.4	%
Economic			
The Investment Cost	Rp 28,000,000	Rp 18,000,000	Rp/Year
The Cash Flow	Rp 2,359,000	Rp 18,663,000	Rp/Year
The Pay Back Periods	11.87	0.96	year
High perception of Gas benefit	67.01	56.63	%
High perception of Fertilizer benefit	73.2	62.65	%
High perception of Aesthetics benefit	76.29	75.9	%
High perception of Sales benefit	53.61	38.55	%

Table 5 shows the results of the weighting process to determine the score for every aspect of sustainability. In this study, the economic aspect was divided into two sub aspects (cost sub aspects and benefit sub aspect) because each sub aspect had significant part to determine the sustainability. It shows that in Environment and Economic Aspect (especially in Cost sub-aspect), Cilengkrang had significant higher value than Cibangkong. Meanwhile in term of Social and Benefit sub aspect, Cibangkong were relatively higher thatn Cilengkrang. However the differences between both place on these two particular aspect is not too significant.

Table 6 shows the continuation process of the weighted ranking process in determining the degree of importance between each aspect of sustainability which using the Delphi method. The result shows that environment and benefit had the same degree of importance in 3R program sustainability followed by respectively social aspect and cost aspect.

The comparison of sustainability level for both study area were calculated based on the IS and IFC that has determined before. The result on Table 7 shows that Cilengkrang had better sustainability level than Cibangkong. Sustainability level in Cilengkrang had reach to 0.575 point, meanwhile Cibangkong had reach 0.425 point. The gap of the waste reduction sustainability level was not significantly high.

From the estimation for every sustainability aspects in Table 6, it showed that environmental and benefit aspect have the same degree of importance. Eventhough Cibangkong had slightly higher support from its social and benefit aspect than Cilengkrang, but there were significant gap on the environment and cost aspect.

Table 5. The Estimation of IS for each Aspects of Sustainability

Aspect Of Sustainability / Indicator	Score of IS	
	CB	CL
Environmental		
Waste input to Biodigester	0.190	0.310
Ratio of waste input to biodigester capacity	0.072	0.428

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Aspect Of Sustainability / Indicator	Score of IS	
	CB	CL
Total Score of Environmental Aspect	**0.262**	**0.738**
Social		
High perception of contribution on Idea	0.094	0.065
High perception of contribution on Man Power	0.107	0.052
High perception of contribution on Goods	0.103	0.106
High perception of contribution on Funding	0.093	0.065
High perception of contribution on Time	0.084	0.074
High perception of contribution on Skills	0.090	0.068
Total Score of Social Aspect	**0.570**	**0.430**
Economic		
The Investment Cost	0.130	0.203
The Cash Flow	0.037	0.296
The Pay Back Periods	0.025	0.308
Total Score of Cost Aspect	**0.193**	**0.807**
High perception of Gas benefit	0.117	0.098
High perception of Fertilizer benefit	0.116	0.099
High perception of Aesthetics benefit	0.178	0.177
High perception of Sales benefit	0.125	0.090
Total Score of Benefit Aspect	**0.535**	**0.465**

Table 6. The Estimation of IFC

No.	Aspects	1	2.1	2.2	3	Sum	IFC
1	Environment Aspect		0.5	0.5	1	2	0.333
2	Economic Aspect						
2.1	Cost Aspect	0.5		0	0	0.5	0.083
2.2	Benefit Aspect	0.5	1		0.5	2	0.333
3	Social Aspect	0	1	0.5		1.5	0.250
							1.000

In this study the point of sustainability level differences emerge due to the environment aspect especially the waste reduction. Cilengkrang had been more able to reduce their waste up to 80.53 L /day compare to Cibangkong which only almost half of it, which was 49.5 L/day. The other aspect that influence the gap between Cibangkong and Cilengkrang was the cost sub aspect.

Table 7. The Estimation of Sustainability Level

No.	Aspects	IFC	IAC		IFC x IAC	
			CB	CL	CB	CL
1	Environment Aspect	0.333	0.262	0.738	0.087	0.246
2.1	Cost Aspect	0.083	0.193	0.807	0.016	0.067
2.2	Benefit Aspect	0.333	0.535	0.465	0.178	0.155
3	Social Aspect	0.250	0.570	0.430	0.143	0.107
					0.425	**0.575**

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4. CONCLUSIONS

Waste reduction sustainability level in sub urban area was better than urban area. The sustainability level in sub urban area emerge due to the environmental and economic aspect. The dominant factor in environmental aspect was the waste reduction level and the beneficiaries level for the economic aspect. This study finds that the bio-digester have a good acceptance from the community but it has to be integrated with other 3R activities to enhance its economic sustainability.

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Impact of Compotition Membran Chitosan and Zeolite Composite for Batik Colouring Wastewater Treatment

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Abstract

Wastewater from colouring process of batik use only 5 % of dye and the rest became waste. Dye in wastewater related to high COD (*Chemical Oxygen Demand).* Membrane technology was one of wastewater treatment technology that was expanding nowadays. Membrane could be produced from anorganic material such as zeolite and organic material such as chitosan. The optimum composition had been analyzed from zeolite and chitosan as a membrane to remove color and COD in wastewater. It's also analysis about rejection and fluks from the membrane.

The variables of this study were variation of composition from zeolite-chitosan and variation of pressure in the reactor. Variation of composite composition were chitosan-zeolite 1:1, 1:2, and 2:1. The membrane system used cross flow current for running. Quality of COD and color substance had been analyzed in the permeate. Structure of membrane also had been analyzed with SEM EDX microscope.

The highest rejection of COD and colour were resulted by composition membrane chitosan and zeolite in 2:1 at 1,5 bar pressure. COD rejection is 94,5% and color rejection is 92,55%. The highest flux was resulted by composition membrane in 1:2 at 1,5 bar pressure that is 80,3 L/m^2.h. The best composition of chitosan and zeolite membrane for removing COD and color in batik wastewater was 2:1 composition.

Keywords: COD, color substance, chitosan zeolite, batik wastewater, membrane

1. INTRODUCTION

Textile industries were important sector in the global industry. Their contribution could not be denied as the satisfaction of basic human and world economies. The textile industry was one of the consumers of water for dye and chemical processes at the production process. After used in the production process, the trace materials could not be used again and discharged in the effluent. This effluent would cause problems if not treated before.

The content of the textile wastewater was usually rich from textile dye, chemical oxygen demand (COD), a complex chemical compound, inorganic salts, total dissolved solids (TDS), pH, temperature, turbidity and salinity (Verma et al., 2012). The contribution of textile wastewater was formed from the stages of the production process. Stages of the production process consisted of dyeing, printing and washing (Li et al., 2012). Most of the textile industry used synthetic dyes because of the cheap price, the color was durable, easy to obtain and use. However, the wastewater was still colored and has difficult degradation (Chatterjeeet al., 2007). One type of small-scale textile industry that spreading everywhere in Indonesia was the batik industry.

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One of the technology for wastewater treatment that was growing rapidly was membrane technology. Mechanical wastewater treatment using membrane had several advantages. The advantages were simple in its operational processes, could take place at room temperature, non-destructive nature, so it did not result in a change (degradation) of substances that could be separated either physically or chemically, as well as most of the membrane could be reused. Therefore, the membrane could be categorized as clean technology.

Zeolite was a natural polymer that was often used as a raw material for making inorganic membranes. Natural zeolites had the ability in a variety of chemical processes such as adsorbents, catalysts, and ion exchange. Application of zeolite in membrane technology as a medium of absorption and separation simultaneously (McLearyet al., 2006). The structure of zeolite were microporous membrane of uniform size, thermal stability, mechanical, and chemical good.

The other membrane types, namely organic membranes which could be made from chitosan. Chitosan was a product made from chitin, a natural biopolymer in the world. Most potent source of chitin was the outer shell of crustaceans such as crabs, lobsters, shrimp and insects (Hale, 1986). Permeability of chitosan was high enough and its hydrophilic (Nawawi and Hassan, 2003). Chitosan membrane still had some flaws to be applied to textile wastewater processing unit because the membrane was easily shrink when dry, mechanical strength was not very good (Chen et al., 2004).

In this research would be conducted synthesis between zeolite and chitosan to obtain a zeolite-chitosan membrane structure was compact and united. So it could be used as a filter media in the process of separating the waste water from the contaminant.

2. MATERIALS AND METHODS

2.1 Study of Literature

Study of literature was conducted from early stage research to draw conclusions to obtain adequate theories that support the implementation of the research. Literature that were used included textile wastewater characteristics, definitions and types of membranes, characteristics of chitosan and zeolite, preparation of chitosan and zeolite membranes, membrane permeability, permselektivitas membrane, membrane performance testing with a cross flow reactor. The study of literature came from supporting materials such as text books, national and international journals, article. The study of literature was expected to be the basis which enables this final project.

2.2 Activation of Zeolite

Based on earlier research (Sari, 2014), natural zeolite was destroyed by means of ball miling and sieved. Then the zeolite was washed with a solution of HCl for 24 hours. After soaking 24 hours, zeolite was rinsed with distilled water until the content of HCL missing. Zeolites which had been washed and then dried in the oven.

2.3 Preparation of Chitosan

Preparation of chitosan based on weighing the mass variation of chitosan. Then the chitosan powder with a mass of 5 grams dissolved in 1% acetic acid with a volume of 100 mL. Then the solution was stirred for 1 hour with a stirrer magnetic stirrer to form a clear viscous solution fawn.

2.4 Making of Membrane

Phase manufacture of membranes used phase inversion methods. Phase inversion method was a process of changing the form of a liquid phase into the solid phase. The first step was weighing the zeolite powder with a variation of the mass of 2.5 gram of zeolite, 5 grams, and 10 grams of washing had been done before. The mass weighing based on variation of chitosan and zeolite in the ratio 1: 1 (5 grams of chitosan: 5 grams of zeolite), 1: 2 (5 grams of chitosan: 10 gram zeolite), and 2: 1 (5 grams of chitosan: 2.5 gram of zeolite). Zeolite had been stirrer beome powder in 100 ml NH4Cl then taken

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sediment. The precipitate zeolite and chitosan solution which had previously been made put in a glass beaker and stirred with a magnetic stirrer at a speed of 600 rpm. The printing process was done by mixing a solution of the membrane PEG (Poly Ethylene glycol). The first step was to weigh as much as 40 grams of PVA and diluted with distilled water to 200 ml in a state of boiling. Then added a solution of PVA membrane solution cooled as much as 50 mL and 25 mL of PEG. The materials are mixed with zeolite-chitosan which is then heated with stirring by using a hot plate stirrer. After the solution had been thickened printed using a small petri dish and allowed to stand for 24 hours.

Figure 1. Membrane Chitosan-Zeolite

2.5 Membrane Aplication on Cross Flow Reactor

Membranes that have formed were then tested in a cross flow reactor. Diameter membrane that had been printed size of ± 5 cm. Wastewater that had varied in capacity in the collecting basin with a capacity of 30 L which would then be pumped to the membrane reactor. The first thing was the water drain taps on the membrane for ± 1 hour so that the pore - pore membrane could work effectively. In addition, as a start-up early to set the reactor pressure. Then the waste water flowed into the reactor with corresponding pressure variations.

Part of the cross flow reactor was a manometer, valve, by pass, hoses, and nuts. The function of the manometer was to show that the pressure inside the reactor running. Valve and by pass was used for unscrew the flow so that the pressure in the reactor could be arranged. Diverter hose served as a medium of initial collector tub to the sump.

In the early running, and by pass valve was opened and closed slowly to start up the early pressure. After the pressure in the reactor was varied. Wastewater used previously deposited first so the rest of the night or candles can float and reduced so that the running time does not inhibit membrane.

The waste water passed the membrane filtered or called permeate was accommodated in a container for subsequent analysis of COD and color. The water coming out of the by-pass would be accommodated in the tank as retentate. At the beginning of running time spent was 80 minutes by taking the permeate hose 5 minutes. At each variation of pressure and composition, before running the waste was analyzed early in advance to determine the change after filtration.

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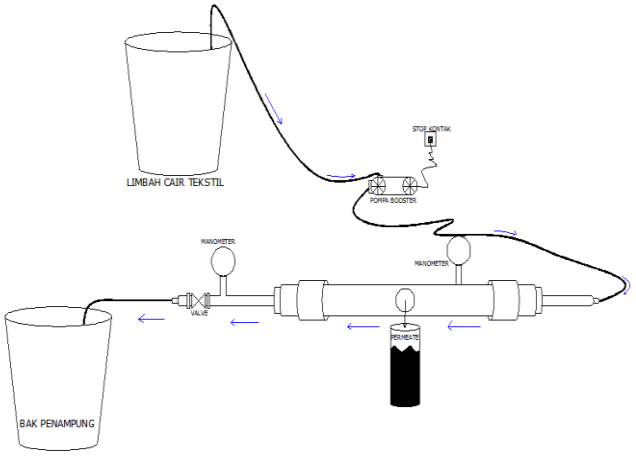

Figure 2. Crossflow Reactor

3. RESULTS AND DISCUSSION

3.1 Rejection Value

The composition of the chitosan - zeolite 2: 1 discount rejection was the greatest because of the influence of chitosan more of zeolite, making the chitosan membrane pores tighter. The relationship between rejection and composition that the higher the chitosan composition of the zeolite would produce higher rejection. It could be explained that the membrane was thin porous layer. Membrane pores formed by the polymer matrix making up the membrane, so more polymers (cellulose acetate), the polymer matrix resulting in tighter pores smaller (Suseno et al, 2003) . Addition of inorganic materials such as zeolites which served as porogen and also provided mechanical strength that chitosan was able to last longer when tested in the reactor.

Table 1. Characteristic Wasterwater

Membrane Variation	First Colour Value (Pt-co)	First COD Value (mg/L)
Composition 1:1 Pressure 1,5 bar	7950	16893
Composition 1:2 Pressure 1,5 bar	7450	16657
Composition 2:1 Pressure 1,5 bar	8050	18052

The composition of the chitosan - zeolite 2: 1 discount rejection was the greatest because of the influence of chitosan more of zeolite, making the chitosan membrane pores tighter. The relationship between rejection and composition that the higher the chitosan composition of the zeolite would produce higher rejection. It can be explained that the membrane was thin porous layer. Membrane pores formed by the polymer matrix making up the membrane, so more polymers (cellulose acetate), the polymer matrix resulting in tighter pores smaller (Suseno et al, 2003) .Addition of inorganic materials such as zeolites which served as porogen and also provide mechanical strength that chitosan was able to last longer when tested in the reactor.

COD contained COD analysis carried out because of its high levels as well as a coloring agent in the coloring process used synthetic dyes. Staining and rinsing produced wastewater that was colored with a COD (Chemical Oxygen Demand) high (Hadiwidodo et al,. 2009).

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Table 2. Rejection of Colour

Time (minutes)	1:1 Composition	1:2 Composition	2:1 Composition
5	66.67%	67.11%	81.99%
10	68.55%	69.80%	83.23%
15	67.92%	70.47%	82.61%
20	68.55%	67.79%	84.47%
25	71.07%	69.13%	83.23%
30	72.33%	71.14%	84.47%
35	73.58%	73.15%	85.09%
40	72.96%	74.50%	86.96%
45	74.21%	71.81%	87.58%
50	74.84%	73.15%	86.96%
55	74.21%	75.17%	86.34%
60	74.84%	74.50%	88.20%
65	75.47%	77.18%	88.20%
70	79.25%	76.51%	91.30%
75	78.62%	79.19%	93.17%
80	79.87%	79.19%	92.55%

Table 3. Rejection of COD

Time (minutes)	1:1 Composition	1:2 Composition	2:1 Composition
5	2.70%	19.50%	29.20%
10	3.80%	27.10%	33.80%
15	24.80%	33.70%	35.60%
20	29.20%	15.70%	43.90%
25	27.00%	18.50%	38.40%
30	28.10%	33.70%	26.40%
35	31.40%	31.80%	39.30%
40	39.20%	32.70%	31.90%
45	42.50%	32.70%	38.40%
50	44.70%	33.70%	39.30%
55	41.40%	30.90%	49.40%
60	42.50%	32.70%	54.00%
65	43.60%	37.50%	71.50%
70	44.70%	40.30%	80.70%
75	48.00%	42.20%	88.10%
80	53.60%	43.20%	94.50%

In the graphics it could be seen that the decrease in the concentration of COD generated by the composition of the chitosan membranes with zeolite 2: 1.

The flux and rejection were inversely. The less flux, the greater the resulting rejection because the number of particles filtered. The rejection on the composition of 2: 1 occurs because the density of pores formed by the large number of zeolite and chitosan mixture. The more tightly the more particles were capable restrained. The smaller the pore size the higher the selectivity (Mulder, 1996).

Despite % rejection by the membrane against the concentration of COD in waste of batik dyeing was high, but the end result of COD in the permeate still not meet the quality standards appropriate Java Governor Regulation Number. 72 Year 2013. There was due to high levels of organic dye stuff and the substances dissolved in the waste coloring still escape while filtering. Thus the need for initial pretreatment thus supporting membrane selectivity performance was already high in rejection

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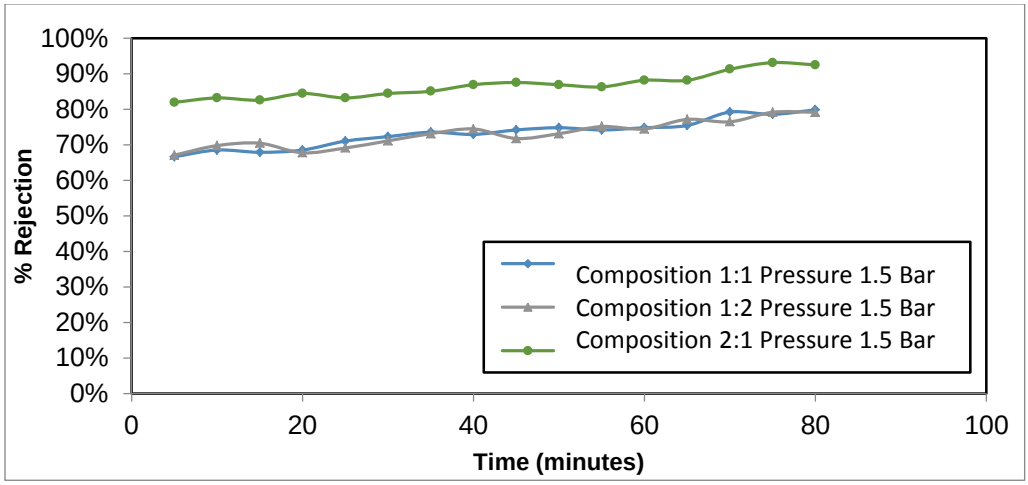

Figure 3. Rejection of Colour

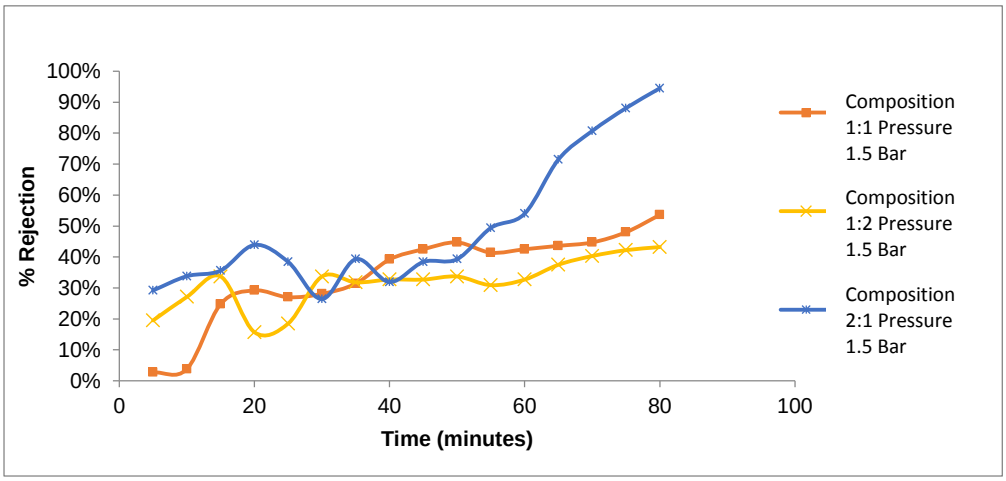

Figure 4. Rejection of COD

3.2 Flux

Membrane flux value was also a function of pore size, porosity, thickness and structure of the membrane (Sari, 2014).

Table 4. Membrane Flux

Time (Minutes)	Flux at 1:1 Composition (L/m^2.hour)	Flux at 1:2 Composition (L/m^2.hour)	Flux at 2:1 Composition (L/m^2.hour)
5	78.4	68.8	80.3
10	30.1	43	38.3
15	28.4	32.2	24.6
20	21.1	23.5	18.7
25	15.3	22.6	14.2
30	9.3	12.6	11.4
35	7.7	9.3	9.3
40	4.2	6.6	5.5
45	2.9	4.9	3.6
50	2.6	4.3	4.3
55	1.8	4.1	5.3
60	2.4	3.2	3.2
65	1.9	2.4	2.8
70	1.6	1.6	2.6
75	1.4	1.1	2.3
80	1.1	0.8	1.8

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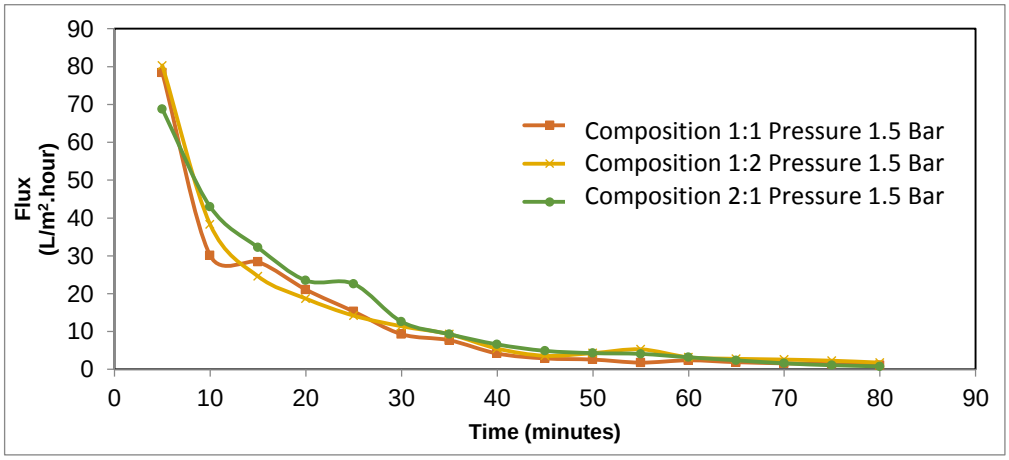

Figure 5. Flux Membrane

It could be seen that the composition of the chitosan - zeolite 1: 2 in every pressure produced the greatest flux in the 5th minute is 80.3 L / m2.hour at a pressure of 1.5 bar and 79.3 L / m^2.hour at a pressure of 1 bar. The difference was very much on the composition of the chitosan membrane zeolite 2: 1 where the flux generated in the 5th minute only 22 L / m^2.hour at a pressure of 1 bar and 68.8 at a pressure of 1.5 bar. The flux produced by each membrane from the 5th minute to the 80th minute downhill. The longer the running time, the flux reactor that produced and decrease in line with the filtered particulate buildup that caused the formation of fouling. Traits - traits fouling formation itself was to begin decreasing the resulting permeate to permeate not come out at all.

The composition of the membrane also affected flux generated. In forming the membrane, the pore structure of composite membrane was influenced by several factors, including the concentration of the polymer was chitosan, the wall-forming material intentionally added to improve the mechanical properties of the membrane is a zeolite. Flux at chitosan-zeolite 1: 2 composition resulted in the greatest flux due to the addition of zeolite more.

Zeolites were capable of forming a pore surface area on the sidelines of chitosan bigger so that they could pass more fluid and water permeability of the membrane was formed then be tinggi.Celah resulting permeate can pass through the membrane quickly. Speed is what makes values permeate the membrane permeability increased. The addition of inorganic material in chitosan membrane can increase the permeability of the membrane (Bokau, 2013).

3.3 Characteristics of Membrane

Membranes had been used for filtration will experience a build up of pollutants on the surface, caused the surface of the pores are closed and happened fouling

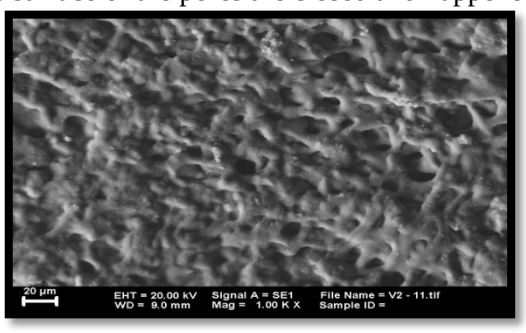

Figure 4. Membrane Porous

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EDX analysis results showed that the particle content filtered by a membrane. Figure 4.21 showed that the content was filtered out is the amount of organic substances where there were elements of C, N, and O. There were some other elements such as Cl, Al, S, and P. This showed that the organic content in wastewater more filtered.

Table 2. Element Contents

Atom	Nomor Atom	Kandungan (%)
C	6	51,44
O	8	46,06
Na	11	0,52
Al	13	0,11
Si	14	0,45
P	15	0,37
S	16	0,49
Cl	17	0,46
Ca	20	0,09

4. CONCLUSION

The highest rejection value for the colour parameter is 2: 1 composition of the chitosan-zeolite with a pressure of 1.5 bar which is 92.55%. The highest value for the parameter COD rejection was also generated on-zeolite chitosan composition variation 2: 1 with a pressure of 1.5 bar which is 94.5%. While the value of the highest flux generated by the composition of the chitosan-zeolite 1: 2 in the amount of 80.3 L / m2.hour at a pressure of 1.5 bar. Composition of chitosan and zeolite capable to removal most excellent color and COD was on the composition of 2: 1 with a reactor pressure of 1.5 bar.

ACKNOWLEDGEMENT

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Laundry Wastewater Treatment Using Membrane Fılter Synthesized from Zeolıte and Chıtosan

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Abstract

The increasing number of laundry industries, has caused the increase of wastewater generation. One method to reduced the pollutant in wastewater was using membrane filtration. Zeolite and chitosan were materials that have been often used as membrane material because it have a good ability in the film-forming, easily processing and availability of abundant. The aim of this research analyze the effect of using membranes synthesized from zeolite and chitosan for flux and removing pollutants mainly for the parameter of TSS and surfactant in laundry wastewater. The filtration process used cross-flow method because it required lower operating costs. Variations in this research were the mass composition ratio of zeolite and chitosan 1:1; 2:1 and 1:2 (membrane Z1C1; Z2C1 and Z1C2) with a total mass used of 15 g, and pressure of 2 bar. Where as the concentration of wastewater used in the process was 100% without the addition of distilled water or dilution. The research data showed composition ratio of optimum zeolite and chitosan, the membrane flux and rejection or removal efficiencıy. The optimum compotition ratio of zeolite and chitosan was 2:1 (membrane Z2C1). The highest flux in membrane filtration was membrane Z1C1 at 10th minute that was 39,99 L/m^2.jam. Where as the highest TSS and surfactant removal efficiencies at membrane Z2C1 at 20th minute and membrane Z1C2 at 60th minute were 90,71% and 97,04% respectively.

Keywords: laundry wastewater, membrane, zeolite and chitosan

1. INTRODUCTION

The increasing number of population in Surabaya, in both native and immigrant resulted in the increase of laundry business. The increasing number of laundry business was causing more waste produced. This increasing number of laundry industry caused pollution in the water body on the downstream of Surabaya River (Kusumo, 2011). Almost all wastewater from laundry business was disposed through a sewer or septic tank without any advanced processing, which would potentially caused contamination of the groundwater and water bodies in the vicinity. Laundry wastewater contained chemicals originating from detergents with high concentrations, include phosphates, surfactants, ammonia and nitrogen, as well as a high level of dissolved solids (TSS), turbidity, BOD and COD (Ahmad and El-Dessouky, 2008). Thus, the increasing amount of laundry business will lead to an increased of surfactant concentration in the water body if the waste was continuously discharged into water body without any treatment processes.

Meanwhile based on the characteristics test of initial laundry effluent, the obtained TSS value was 560 mg/L and the surfactant concentration was 186,4 mg/L. The level of both parameters either TSS or the surfactant exceeded the applied quality standards in the province of East Java, where the quality standard for TSS was100 mg/L and surfactant was10 mg/L (East Java Governor Regulation No. 72 Year 2013).

One method to reduced or lowering the level of contaminants in the wastewater was by using membrane filtration. Membrane filtration was an alternative wastewater treatment technology which had an advantage of its high efficiency for separation. In

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addition, processing by membrane technology also had advantages for its relatively cheap operating costs, environmentally friendly, and space efficiency (Muliawati, 2012). Some studies have been done to get the right methods of laundry wastewater treatment in order to complied the effluent standards for the laundry business. Nasir, et al (2013) has been conducting a research on laundry wastewater treatment process using ceramic filters made by natural clay and zeolite to lower DS, TSS, pH, COD, BOD, DHL and surfactants.

Membrane was a layer (barrier) or selective barrier, placed between two semipermeable phases which could pass some certain components, and hold the other ones (Mulder, 1996). Membrane materials were significantly affecting the separation process. Zeolite and chitosan were materials that can be used as membrane material, because they had good capability in film-forming, processing, and they are also abundant. Chitosan could be obtained from shrimp shells that are abundant in Surabaya.

This study was conducted to analyze the effects of using membranes made from zeolite synthesis and chitosan in pollutants reduction, mainly to the TSS and surfactant parameters in wastewater from laundry business. The use of quality standard for the laundry business in this study was as the effluent control or permeate. The filtration process was using cross-flow stream because it required lower operating cost. After obtaining the quality of effluent produced, it was expected to be the alternative laundry wastewater treatment so that it can help to overcome the environmental pollution problems.

2. MATERIALS AND METHODS

2.1. Zeolite Activation

Zeolites that will be used for the membrane forming should be activated. Activation was done chemically by acidification. The goal is to remove inorganic polluter. This acidification will cause the cation exchange with H^+ (Ertan, 2005). The zeolite used in this membrane forming was in the form of powder. Before the chemically activation was done, zeolites were sieved using a sieve (mesh), and taken in a uniform size that were 100 mesh. Afterward, dissolved 250 g zeolite into 350 mL 1 N HCl for 24 hours. Then, after being soaked in HCl, zeolite was rinsed with tap water and distilled until the solution became neutral (pH closer to 7). This washing was done for 6-7 times to make the zeolite neutral. Then zeolites were dried in an oven at 100^o C for 24 hours to removed the liquid on it.

2.2. Solution Preparation and Membrane Molding

The zeolite preparation was begun by weighing the mass of zeolite suitable with the ratio for making membranes that were 7,5 g, 10 g and 5 g. Then put them in a beaker glass and added Iso Propanol Alcohol (IPA) as much as 35 mL and stirred it using a magnetic stirrer for 10 minutes at a speed of 600 rpm to homogenized the solution. The addition of IPA in the membrane forming was to dissolve the zeolite. Next, the zeolite solution was added by a 3,5 ml solution of NH_4Cl. The addition of NH_4Cl solution functioned as a washing solution to removed contaminants that may be presented in the zeolite solution, and prevented the growth of microorganisms on the membrane. Afterward, the zeolite solution was stirred again by a magnetic stirrer and settled.

The preparation of chitosan solution was started by weighing the chitosan powder in accordance with the mass ratio for the membrane forming, that were 7,5 g, 5 g and 10 g. Then, the chitosan powder was poured in to a beaker glass and added with a 5% solution of acetic acid (CH_3COOH) as much as 100 mL, to dissolved the chitosan. After the acetic acid was added, then it was heated on an electric heater or using a water bath (temperature 95° C) while stirring manually with a stirrer glass for 10-15 minutes until the chitosan was completely dissolved and turned into a homogeneous brownish yellow solution.

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In creating membrane, the first step to be done was pouring the zeolite precipitate into chitosan solution, and stirring it manually with a stirrer glass, continued with stirring the solution using a magnetic stirrer with a speed of 600 rpm for 10 minutes to homogenized these two solutions. After being mixed, then heated it on an electric heater. The mixture heating could be done in a saucepan or a larger glass beaker to prevent the solution from being charred or too dry. In addition to the above, heating could also be done in a water bath at a maximum temperature of 95° C. The heating was done for 1 hour while manually stirring the solution by using a glass stirrer.

At the beginning of heating, the mixed solution was added by additive i.e. Poly Vinyl Alcohol (PVA) and Poly Ethylene Glycol (PEG) solution, 30 mL respectively. PVA addition aims to reinforced the membrane or as an adhesive substance (Nisa, 2005). While the PEG addition was functioned as additive which could form more uniform membrane pores. According to Farha and Kusumawati (2012), the addition of a certain amount of PVA could improve the membrane structure it self, increased the strength of membrane and also stabilized the formed membrane, while the use of PEG was for the formation of membrane pores that known as porogen.

After the heating was completed, membrane solution was not given any treatment for a minute in order to make it cooler, and then poured in petri dish with small diameter, 5. Then heat it in oven at a temperature of 100^{o} C for 24 hours to removed or reduced the water content contained in the solution.

2.3. Membrane Reactor Application

The steps in membrane reactor application is as follows (Sari and Damayanti, 2015) :

a. Membrane resulted of zeolite synthesis and the membrane are molded in ± 5 cm diameter by using a dish.
b. Membranes that had formed were tested on crossflow reactor.
c. The membrane was placed in the hole where the permeate discharges. The laying of membrane must be ensured to cover the hole tightly to prevent leakage. The membrane reactor laying was done with an array of rubber-gauze coarse-fine gauze-membrane-fine-mesh gauze rough-cover.
d. The liquid waste in a storage tank (feed) with capacity of 10 L which will be pumped by a booster pump to the membrane reactor. To made the membrane pores work effectively, distilled water was flowed to the membrane for ± 1 hour. Then the wastewater flowed into the reactor at a pressure of 2 bar (Gustian, 2006).
e. In the initial running, the valve and by pass were opened and closed gradually to increased or reduced the pressure up to 2 bar. After reaching a pressure of 2 bar, the permeate was accommodated in a tube or tub for TSS and level of surfactant analysis next. The water coming out of the by-pass will be accommodated in a tank as retentate or concentrate. The membrane test was done 60 minutes (1 hour) with each variant and the permeate were taken every 10 minutes.
f. The resulting permeate volume was measured, and calculated membrane flux.
g. The resulting permeate was analyzed for TSS and surfactant parameters, and then calculated TSS removal efficiency.

2.4. Permeate Analysis

The analyzed pollutant parameters were TSS and surfactant parameters. TSS analysis was done by using gravimetric analysis method in reference to the SNI 06-6989.3-2004. For the analysis of surfactants or detergents using MBAS method (APHA, 2005).

2.5. Membrane Morphology Analysis

Analysis of membrane morphology was using a Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX). SEM analysis was conducted to determined the membrane pores while EDX was determining the composition of

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elements contained in the membrane. SEM analysis was performed before and after the membrane used for laundry wastewater filtration process. Membranes used for the SEM-EDX analysis was the membrane that produced the highest flux value.

The first step that had to be done in SEM EDX analysis was to dry the sample, the membrane must in dry condition. Then the membrane was immersed in nitrogen liquid for a few seconds until the shape was hardened (Muliawati, 2012). Then the pieces of membrane which will be used was cut by tweezers and covered with pure gold (coating). Pure gold serves as a conductor. The next step was to take a picture of membrane surface with a certain magnification. Magnification used in this study was 1000 times magnification.

3. RESULTS AND DISCUSSIONS

3.1. Effects of Membrane Filtration on Permeate Flux

Diametre of the membrane which was contacted with the foul water when operating membrane reactor was 2,9 cm, so the area (A) that used for the flux value calculation was 0,00066 m^2. The membrane was operated for 60 minutes or an hour with permeate sample taken at 10 minutes interval, so the time value (t) used for the calculation was 0,167 hour. The permeate volume (V) which obtained from the operation was measured in mL unit, then in the volume value converted to Liter unit. In conclusion, the flux value will used L/m^2.hour unit as measurement.

Table 1. Membrane flux value

Time (minute)	Z1C1		Z2C1		Z1C2	
	Volume (mL)	Flux (L/m^2.h)	Volume (mL)	Flux (L/m^2.h)	Volume (mL)	Flux (L/m^2.h)
10	4,4	39,99	4,2	38,17	3,3	29,99
20	3,2	29,08	2,9	26,36	2,2	19,99
30	2,3	20,90	2,2	19,99	1,8	16,36
40	1,1	10,00	1,9	17,27	1,5	13,63
50	0,9	8,18	1,6	14,54	1,5	13,63
60	0,6	5,45	1,3	11,81	1,2	10,91

From the value shown in Table 1 it can be concluded that the membrane flux value which obtained from the operation were mostly categorized in the ultrafiltation membrane type, in which the value were around 10 – 50 L/m^2.hour (Mulder, 1996). The membrane tended to produced smaller value of permeate volume and flux value as the operation time gone on. From the obtained result, the highest value of permeate volume and flux value was obtained using Z2C1 membrane. The average volume and rejection value produced by Z2C1 membrane were 2,35 mL and 21,36 L/m^2.hour respectively.

Based on Ciabatti, et al., (2009), and Sari and Damayanti (2014) the flux value tend to decreased as the time gone on. This phenomon was caused by the occurence of fouling at the membrane which triggered by adsorbtion of pollutant into the membrane, accumulating the particles and making a layer on the surface and pores inside the membrane which will causing the decrease of membrane's flux (Nasir, *et al*., 2013).

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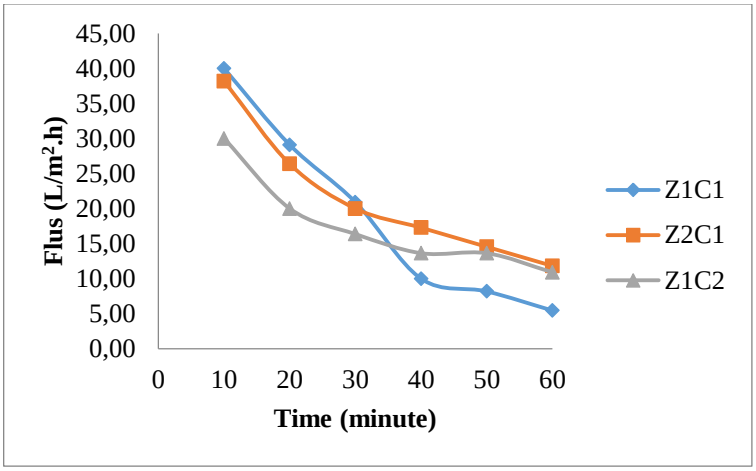

Figure 1. Membrane flux value

3.2. Effects of Membrane Filtration on TSS Rejection

Determining TSS value was useful for measuring the level of foul water pollution and determining the efficiency of water processor unit. Too much TSS can hindering sun light to passed through the water layer, interfering with the photosynthesis process, urging the necessities to determined the TSS limit value for processing foul water before dispatched to the water body (Rahmawati and Azizah, 2005). The measurement of the TSS level was conducted with the use of gravimetric method (APHA, 2005). The initial TSS concentration value of laundry foul water before filtrated was 560 mg/L.

Table 2. Permeate concentration and TSS rejection

Time (minute)	Z1C1		Z2C1		Z1C2	
	Concentration (mg/L)	Rejection (%)	Concentration (mg/L)	Rejection (%)	Concentration (mg/L)	Rejection (%)
10	120,0	78,57	72,0	87,14	140,0	75,00
20	128,0	77,14	52,0	90,71	124,0	77,86
30	144,0	74,29	156,0	72,14	164,0	70,71
40	80,0	85,71	140,0	75,00	148,0	73,57
50	92,0	83,57	128,0	77,14	108,0	80,71
60	76,0	86,43	140,0	75,00	100,0	82,14

Based on Table 2, the highest value of TSS rejection value obtained from the variant composition ratio of zeolith and kitosan 2:1 (Z2C1) at the 20th minute with 90,71%. While the lowest value of TSS rejection value obtained from the variant composition ratio of zeolith and kitosan 1:2 (Z1C2) at the 30th minute with 70,71%.

The TSS rejection value tended to rised as the operation time gone on. This phenomon was caused by the occurence of fouling at the membrane which made the selectifity capability risened because of the diminution of the membrane pores, the longer time the operation taken the more pollutant taken place and trapped inside the membrane's pore causing the pore to keep diminuting, making the pollutant harder to passed through the membrane pores (Wahyuni and Damayanti, 2015).

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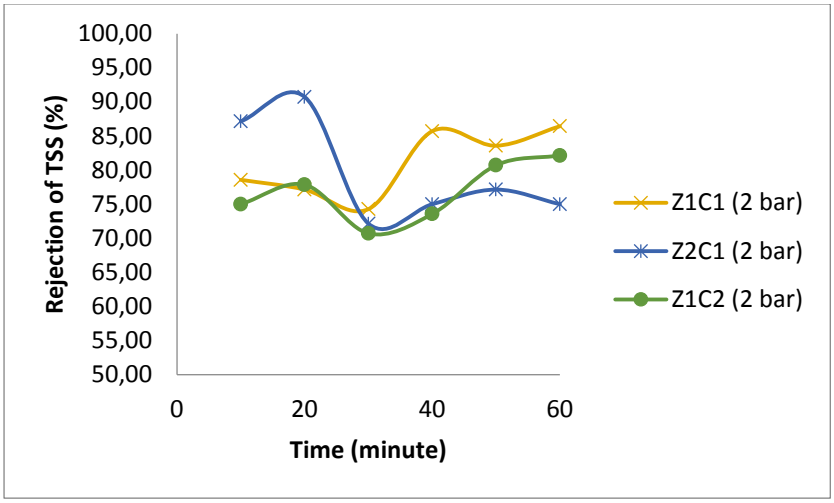

Figure 2. TSS rejection of membrane

3.3. Effects of Membrane Filtration on Surfactant Rejection

Surfactant analysis was performed using MBAs (Methylen Blue Active Surfactant) by adding methylene blue substance that binded to surfactant and was analyzed by UV-Vis spectrophotometer (APHA, 2005). The legible concentration was the level of anionic surfactant in the waste sample that binded to the methylene blue. The surfactant concentration of the analysis before the wastewater treatment using membrane filtration was 186,4 mg/L.

Table 3. Permeate concentration and surfactant rejection

Time (minute)	Z1C1		Z2C1		Z1C2	
	Concentration (mg/L)	Rejection (%)	Concentration (mg/L)	Rejection (%)	Concentration (mg/L)	Rejection (%)
10	31,9	82,87	18,7	89,95	29,3	84,26
20	27,5	85,27	14,7	92,10	16,1	91,35
30	24,2	87,04	14,2	92,36	15,4	91,73
40	23,2	87,55	9,5	94,89	14,0	92,48
50	21,8	88,31	9,1	95,14	11,9	93,62
60	18,7	89,95	5,5	97,04	9,3	95,01

Based on Table 3, it was known that the highest surfactant rejection value was obtained on the 2:1 variant composition ratio of zeolite and chitosan (Z2C1) in the 60th minute with an amount of 97,04%. While the lowest possible surfactant rejection value obtained on the 1:1 variant ratio composition of zeolite and chitosan (Z1C1) in the 10th minute with an amount of 82,87%.

Surfactant rejection was increased due to the increasing of operating time. But there was a difference in the increasing rejection of each variant. When the surface of membrane has been contaminated, the membrane pore was getting narrower and in longer time it will form a cake, when it was formed, then the solute will also restrained which caused the rising of rejection coefficient (Espendiller *et al.*, 2010).

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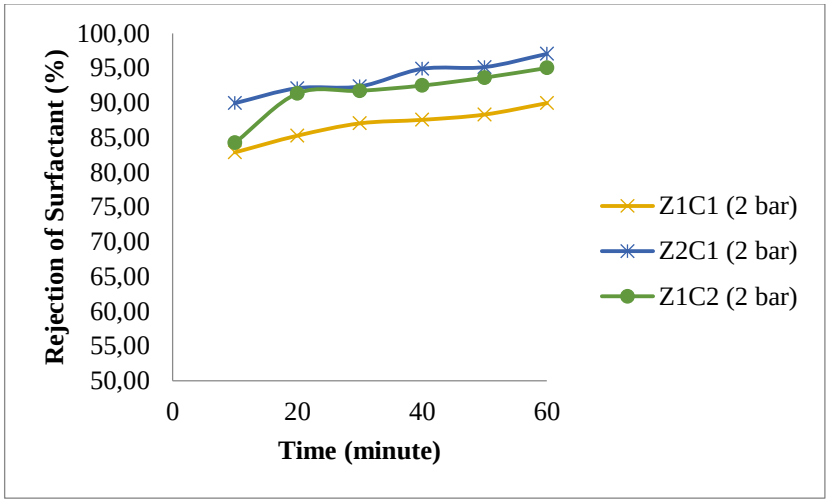

Figure 3. Surfactant rejection

3.4. Membrane Morphology Analysis

Analysis of membrane morphology was using a Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX). SEM analysis was conducted to determined the membrane pores, while for EDX to determined the composition of elements contained in the membrane. SEM analysis was performed before and after the membrane was used for laundry wastewater filtration process. Membranes used for SEM-EDX analysis was the membrane that produced the highest flux value that was Z2C1 membrane which produced total flux in the amount of 128,15 L/m^2.hour.

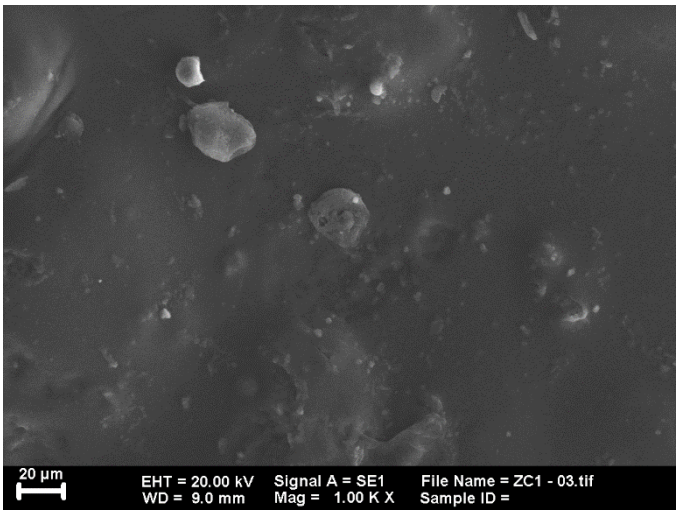

Figure 4. SEM analysis of membrane before operation

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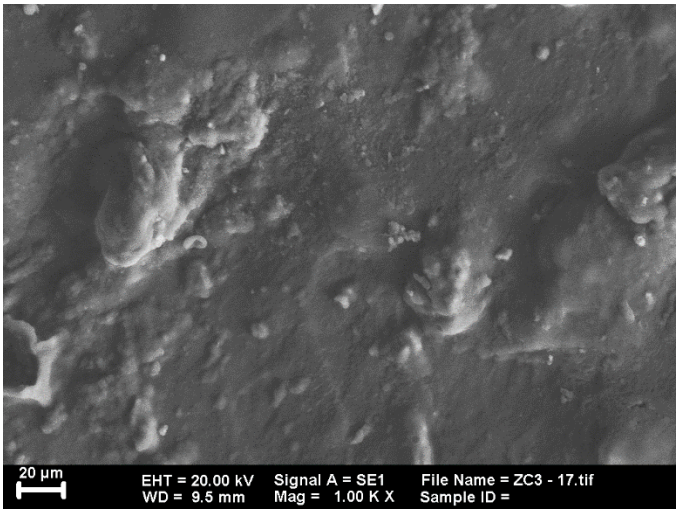

Figure 5. SEM analysis of membrane after operation

The first step done for SEM EDX analysis was drying the sample, so the membrane must be dried. Afterwards, the membrane was immersed in nitrogen liquid for a few seconds until it was hardened (Muliawati, 2012). Then the pieces of to-be-used membrane were cut with tweezers and covered with pure gold (coating). Pure gold was functioned as a conductor. The next step was to taken e a picture of the surface of membrane with a certain magnification. The magnification used in this study is 1000 times magnification.

Based on Sari and Damayanti (2014) research, the membrane looked increasingly congested due to the fouling. Fouling caused blockage on the pores of membrane due to a material build up on the surface of membrane. Membrane fouling occured not only at the top surface or outer layer of membrane which made up a cake but it was also possible to be happened in the membrane lining. Pollutants entering into the inner layer occured due to the pressure exerted during the membrane operation. The pressure will push the deposition of particles on the surface of membrane.

4. CONCLUSION

From this research, we can take some conclusions, as follows:

1. The best membrane was membrane with 2:1 zeolite and chitosan ratio (Z2C1) because it produced permeate volume and total flux as much as 14,1 mL and 128,15 L/m^2.hour.
2. The best flux value was 39,99 L/m^2.hour obtained from the variant of membrane with 1:1 zeolite and chitosan ratio (Z1C1) in the 10th minute.
3. The best TSS rejection coefficient with a percentage of 90,71% found on the membrane with 2:1 zeolite and chitosan ratio (Z2C1) in the 20th minute. While the best surfactant rejection coefficient with a value of 97,04% found in the membrane with 1:2 zeolite and chitosan ratio (Z1C2) in the 60th minute.

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Effect of Salt Concentration and C/N/P Ratio on the Performance of Membrane Bioreactors for Treating Synthetic Produced Water

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Abstract

Fundamental studies of membrane application in wastewater treatment are essential for a detailed understanding of the organic and inorganic pollutants removal. In spite of the membrane bioreactor (MBR) has been widely used to remove oil and grease (O&G) and chemical oxygen demand (COD) from produced water of containing high salt content, the use of MBR to remove organic and inorganic pollutants from secondary oil recovery operation needs to be verified. Two submerged MBRs of plate and frame module were used in this study to remove O&G, COD, NH_3, and PO_4^{3-} from synthetic produced water of moderately low salt content 17.5 g L^{-1} or less with two variables of salt content and C/N/P ratio. The results showed that the change in salinity does not affect the performances of the MBR to remove O&G, COD, NH_3, and PO_4^{3-}. Despite of the change of C/N/P ratio from 100/10/1 to 100/2/1 as shown by lowering the sludge volume index from 105.55 to 40.88 mL g^{-1} and that from 107.57 to 41.49 mL g^{-1} for salt contents of 10 and 17.5 g L^{-1}, respectively, can increase the efficiency of MBR, the membrane fouling occur due to the growth of filamentous bacteria. The application of submerged MBR of using different salt contents and C/N/P ratios provides valuable insight into the treatment of produced waters for getting optimal performance of the existing bioreactor.

Keywords: activated sludge, C/N/P ratio, membrane bioreactor, salt content, synthetic produced water

1. INTRODUCTION

Produced water is a term commonly used in the oil and gas industries to describe water that is produced as a byproduct along with the oil and gas. Oil and gas reservoirs often have water as well as hydrocarbons, located sometimes in a zone that lies under the hydrocarbons, and sometimes in the same zone with the oil and gas. According to its caharacteristics, produced water is considered an industrial wastewater because of high content of organic and inorganic pollutants and is required to be treated before releasing into the environment or employing beneficial re-uses for produced water. The levels of mineral, organic and oil in produced water have been estimated to be as high as 300000, 1500 and 565 mg L^{-1}, respectively, with the ratio of produced water to oil production of approximately 3:1 [1]. However, the rate of oilfield produced water production is expected to increase as oilfield ages. The volume of produced water from oil and gas wells does not remain constant over time and has been reported that an annual rate of production to be aproximately 10 – 20 time higher than oil production [2]. The produced water discharged into the environment can result into degradation of receiving water and its environment and constitutes health hazard for both animals and plants [3]. Currently, oil and gas operators treat produced water via one or more of technological approaches because it can lead to economic value through incremental oil recovery from waterflood projects [4], or the treated water can be released into the environment.

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A number of physical and chemical methods, such as gravity separation, air flotation, coagulation, membrane filtration, and adsorption, have been proposed for the treatment of produced water and are able of removing dissolved organic, inorganic, and toxic substances [4-5]. However, the application of these technologies still produces sludge as a pollutant byproduct that requires further treatment. Goverment regulation specifies water quality requirements and standards relating to produced water before releasing it into the environment [6]. Biological treatment is one of alternative technologies and is an important and integral part of any wastewater treatment plant that can treat produced water having high content of soluble organic and inorganic impurities. Biological treatment using aerobic activated sludge process can remove dissolved hydrocarbons to yield environmentally friendly byproducts and has been in practice for well over a century. Biological treatment is basically a biochemical phenomenon, dependent on the natural process that requires large land areas for the installation of treatment facilities and additional time for complete biodegradation of organic pollutants. Excessive mineral and hydrocarbon concentrations can reduce the efficiency of biodegradation process. The development of activated sludge process is still needed for enhanced biodegradation of hydrocarbons presented in produced water.

Membrane bioreactor (MBR) is the combination of a membrane process like microfiltration or ultrafiltration with a suspended growth bioreactor and has become well established worldwide over the last decade as an activated sludge process option for advanced treatment of municipal and industrial wastewater [7]. The membrane filtration system of MBR in effect can replace the secondary clarifier in a typical activated sludge treatment system because it has the ability of separating biomass from treated oilfield-produced water. Even though the application of MBR has been sucessfully treating high strength domestic and industrial wastewater [8], fouling membrane needs to be taken seriously because it is the major factor affecting the performance of MBR and quality of the effluent released into the environment. High strength produced water can be successfully treated by using the MBR under conditions of high mineral content, depending on the types and characteristics of wastewater and setting of the MBR [9]. The performances of the MBR to remove O&G and COD from produced water with a salt concentration of 50 g L^{-1} have been reported in the ranges of 95 - 99% and 83 - 93%, respectively, with increasing of organic loading rate (OLR) from 0.3 to 2.6 kgCOD m^{-3} d^{-1} [10]. The use of the MBR feeding with an OLR of 2.6 kgCOD m^{-3} d^{-1} has been sucessful to remove O&G and COD in the ranges of 85-94% and 82-95%, respectively, with salt content in the produced water increased from 100 to 250 g L^{-1} [11]. The fact that many reservoirs in Indonesia have been entering secondary oil recovery phase where water flooding operation has been done as an effort to increase production from both oil and natural gas wells [12]. Produced water from oil and gas operations of these types of reservoir can contain low concentration of salt. In many cases, fouling could be due to chemical binding, C/N/P ratio, hydrophobicity of the membrane, electrostatic attractions, etc [13]; however, the influence of C/N/P ratio on the MBR performance is still not fully understood.

The objectives of this study are as follows: (1) to assess the performance of MBR using acclimatized non-halophilic bacteria to remove O&G, COD, NH_3 and PO_4^{3-} from produced water of moderately low salt content and (2) to evaluate the influence of C/N/P ratio on the performance of MBR caused by the fouling membrane and the change of mixed liquor suspended solids in bioreactor.

2. MATERIALS AND METHODS

2.1 Synthetic Produced Water

This study used two synthetic produced waters [14] with different concentrations of sodium chloride, as shown in Table 1. The produced water was always prepared just before using it to avoid any contamination caused by microbial fermentation. Characterization of the produced water was carried out at the Physical Chemistry

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Laboratory of the Department of Chemistry, Bandung Institute of Technology. Table 1 shows the characteristics of produced water. The addion of crude oil into a synthetic produced water as the sources of carbon and energy for bacterial growth was obtained from PT. Saka Indonesia Pangkah Limited. The content of salt in produced water was adjusted at 10 and 17.5 g L^{-1} by adding NaCl. The ratio of C/N/P was set with the addition of NH_4Cl, i.e., at the ratios of 100/10/1 and 100/2/1. The addition of surfactant (Tween 80) with a ratio of Tween 80/crude oil equals 1/8 was intended to maintain the stability of oil-water interface. The addition of acclimated activated sludge as inoculum in the form of non-halophilic culture of not commonly used for oily wastewater treatment, obtained from the Microbiology Laboratory of Chemical Engineering, Bandung Institute of Technology.

Table 1. Characteristics of synthetic produced water

Component	Molecule	Concentration (mg L^{-1})	
		Using by Pendashteh et al., 2012	Using during this study
Minerals			
Calcium chloride	$CaCl_2.2H_2O$	60	60
Potassium chloride	KCl	2000	2000
Magnesium chloride	$MgCl_2.6H_2O$	50	50
Sodium bicarbonate	$NaHCO_3$	800	n/a
Sodium chloride	NaCl	35000 – 250000	10000; 17500
Nutrients and other supplements			
Crude oil (in mL L^{-1})	-	0.25 – 3	2
Ammonium chloride	NH_4Cl	n/a	n/a
Potassium phosphate	KH_2PO_4	n/a	n/a
Ratio of Tween 80 to oil	-	1/8	1/8

Remark: n/a means that the concentration used was dependent on the experimental run

2.2 Membrane Bioreactor

This study used two MBRs consisting of: (1) storage tank with a volume of 10 L, (2) bioreactor of 14-L total digester effective volume with the dimensions of 30-cm length, 12.5-cm width, 40-cm high, (3) ultrafiltration membrane filter (Toray) of 22x0.5x30-cm^3 plate-and-frame module made of polyvinylidene diflouride with 0.11-m^2 surface area and 0.08-μm pore, and (4) effluent tank of volume of 10 L, as shown in Fig. 1. For the experiments run, the MBR was equipped with timer, homogenizer, two dosing pumps, compressor, rotameter, diffuser, pressure gauge, and water level sensor. A water level sensor was connected to the dosing pump to having a fixed rate of water flow entering the reactor. The pressure gauge was attached at permeate pipe to measure the transmembrane pressure during the operation of MBR. A rotameter was mounted at the air intake pipe to control the flow of air into the reactor. A homogenizer was connected with timer to maintain a homogenous produced water that entering the reactor. At the bottom of the bioreactor was equipped with a diffuser to supply air from the compressor into reactor to maintain the reactor always under aerobic conditions with DO concentraion of approximately 3 mg L^{-1} and serves as provider effect benefit of scouring the membrane filter to reduce membrane fouling.

2.3 Acclimatization of Activated Sludge

Acclimatization of activated sludge was carried out to having a bacterial culture that has been adapted to different conditions of produced water. Aerobic bacteria used in this work could survive at different concentrations of salt and oil. The acclimatization of bacteria to the change in salt concentration from 0 to 0.5 to 1 to 2,5 to 5 to 10 and to 17.5 gNaCl L^{-1} was performed in a batch reactor of 5 L at C/N/P ratio of 100/10/1 for every three days by which the addition of 1-L subculture into a new bacterial culture was carried out every level of increased salt concentration. Bacterial cultures with salt contents of 10 and 17.5 gNaCl L^{-1} were maintained as stock culture for future use. The

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acclimatization of bacteria to the change in oil concentration from 0 to 5 to 10 to 25 to 50 to 100%(v/v) was performed in similar MBR with a MLSS concentration of ± 6000 mg L^{-1} for every stock culture. The MBR was operated at a volumetric flow rate of 4.67 L d^{-1} with a COD concentration of 2600 mg L^{-1} until reaching a steady state condition.

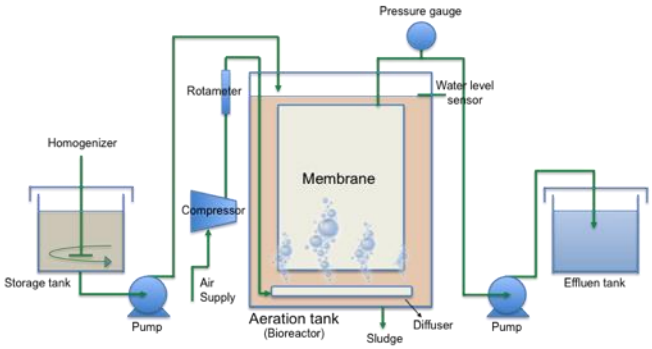

Figure 1. Schematic of membrane bioreactor

2.4 Experimental Procedure

This study used two submerged MBRs (see Fig. 1) with the same mixed liquor conditions of 25°C and pH 7 to remove O&G, COD, NH_3 and PO_4^{3-} from a synthetic produced water. The flow rate of air was regulated using a rotameter at 8 L mn^{-1} to having a DO concentration of approximately 3 mg L^{-1}. The experiments were carried out in the submerged MBR with a membrane flux of 1.93 L m^{-2} h^{-1}, setting up with fixed hydraulic retention time of 2.75 d, sludge retention time of 80 d, initial COD concentration of 2600 mg L^{-1}, initial O&G concentration of 1640 mg L^{-1}, OLR of 0.945 gCOD L^{-1} d^{-1} for 135 d. The operating procedures of MBR-A with a salt content of 10 g L^{-1} and MBR-B with a salt content of 17.5 g L^{-1} were performed under three different conditions of: (1) acclimatization phase with a C/N/P ratio of 100/10/1 for 21 days, (2) first stage of digestion with a C/N/P ratio of 100/10/1 for 80 days, and (3) second stage of digestion with a C/N/P ratio of 100/2/1 for 65 days, as can be seen in Table 2. During the acclimatization phase, the MLSS concentrations were 5834 ± 87,7 mg L^{-1} for MBR-A and 6655 ± 643 mg L^{-1} for MBR-B. The performances of the MBR to remove O&G, COD, NH_3, and PO_4^{3-} from produced water were evaluated based on the data monitored at inlet and outlet of the MBR treatment system.

Table 2. Stages of the experiment

Stage	Time (d)	MBR-A		MBR-B	
		gNaCl L^{-1}	C/N/P	gNaCl L^{-1}	C/N/P
Acclimatization	0 – 21	10	100/10/1	17.5	100/10/1
1	22 – 97	10	100/10/1	17.5	100/10/1
2	98 – 135	10	100/2/1	17.5	100/2/1

2.5 Analytical Methods

The parameters of COD, O&G, NH_3, and PO_4^{3-} were analyzed according to Standard Method [15]. For the analysis of COD, a sample dilution to having a $HgSO_4/Cl^-$ ratio of 10/1 was required due to the Cl^- concentration of higher than 2000 mg L^{-1} was found in produced water. The protein fraction of EPS (EPSp) were measured using folin phenol reagent method [16], whereas the corresponding polysaccharide fraction (EPSc) was determined by phenol-sulfuric acid method [17]. The EPS total was estimated as the sum of these two components. The levels of COD-mixed liquor, MLSS and SVI were monitored once for every 2 or 3 days to having an insight on the stability of MBR and its steady state condition. All analyses were carried out in duplo. A complete control of the MBR conditions should be carried out every day whereas the main physical and chemical

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parameters of pH, DO, temperature, floc formation and ΔP should be monitored during the day.

3. RESULT AND DISCUSSION

3.1 Microbial Growth

The growth of bacteria in biorector was quantified using the values of MLSS to represent the dry weight biomass and COD-mixed liquor to represent the organic matter. Fig. 2 shows the variations of MLSS and COD-mixed liquor for 135-day experiment. One-way ANOVA analysis of the MLSS values verifies that the steady state conditions of both MBR-A and MBR-B were reached at a significant *p* value of 0.05 after 21 days of the experiment. The average values of MLSS and COD-mixed liquor measured under steady state conditions are presented in Table 3. The average MLSS values of MBR-B with a salt concentration of 17.5 gNaCl L^{-1} higher than those of MBR-A with a salt concentration of 10 gNaCl L^{-1} were verified due to the minerals adsorbed on the surface of bacterial cells can lead to increased MLSS value. The average COD values of MBR-B higher than those of MBR-A could be due to the microbial population dynamics in response to increasing loading of minerals in a biorector suggested that: (1) many microorganisms produce the exopolysaccharides (EPS) as a strategy for growing, adhering to solid surfaces, and surviving adverse conditions [18-19], (2) accumulation of unmetabolized substrate or falling out of intermediate product of metabolism are possible in fermental activity changing [20-21], and (3) autolysis of bacterial cells could be a major cause of sheath hollowing [22]. The results (Fig. 3) of the EPS test verified that the protein content in the MBR-B was higher than that in the MBR-A due to the MBR-B contained higher mineral levels can lead to the imbalance of osmotic pressure between the bacterial cell and its environmental condition. This phenomenon can be verified with increasing of the COD value in bioreactor.

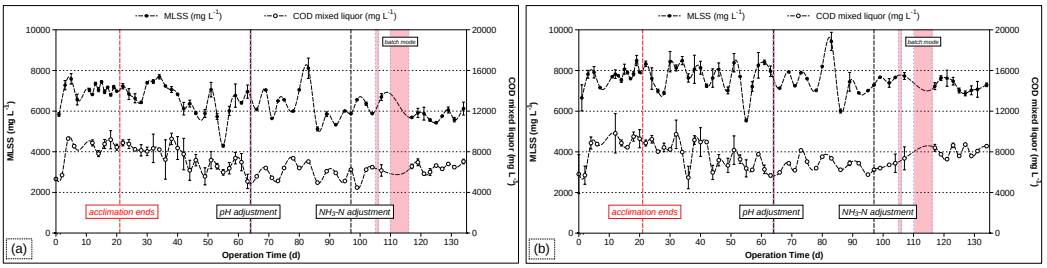

Figure 2. Variations of MLSS and COD-mixed liquor in: (a) MBR-A with a salt concentration of 10 gNaCl L^{-1} and (b) MBR-B with a salt concentration of 17.5 gNaCl L^{-1}

Table 3. Average concentrations of MLSS and COD-mixed liquor in MBR; HA means C/N/P ratio of 100/10/1 and LA means C/N/P ratio of 100/2/1.

Parameter	MBR-A		MBR-B	
	HA	LA	HA	LA
MLSS (mg L^{-1})	6455 ± 788	5960 ± 388	7634 ± 759	7352 ± 279
COD-*mixed liquor* (mg L^{-1})	6875 ± 1213	6308 ± 653	7300 ± 1184	7692 ± 760

The results (Table 3) show that the MLSS values decreased from 6455 to 5960 mg L^{-1} for MBR-A and from 7634 to 7352 mg L^{-1} for MBR-B with changing of C/N/P ratio from 100/10/1 to 100/2/1 could be due to the lack of N element is one of the main limitations on nutrients needed for synthesis of DNA, RNA and enzyme co-factors for bacterial growth and metabolism. A high metabolic activity leading to the increased biomass yield has been confirmed as the EPS values for C/N/P ratio of 100/10/1 as shown in Fig. 3 are high.

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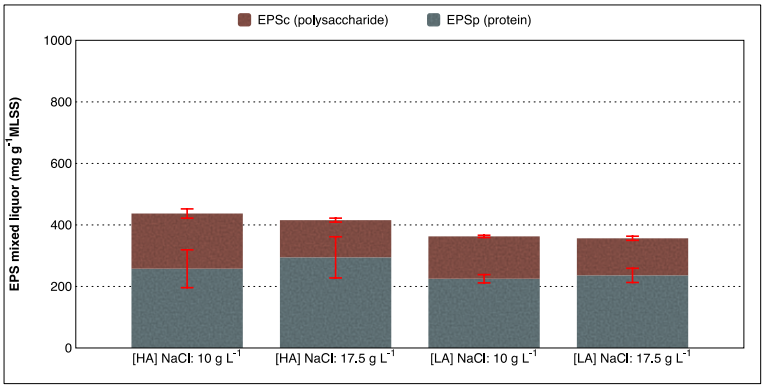

Figure 3. Average EPS concentrations in MBR;
HA means C/N/P of 100/10/1 and LA means C/N/P of 100/2/1.

Figure 4 shows a similar pattern of the variations of pH for MBR-A with a salt concentration of 10 gNaCl L^{-1} and MBR-B with a salt concentration of 17.5 gNaCl L^{-1} that: (1) the pH values tend to gradually decrease in the range of 7 to 5.5 for C/N/P ratio of 100/10/1 due to the oxidation of NH_4^+ to NO_2^- and then to NO_3^- can release the H^+ ions in solution during nitrification hence the pH value decreases (see the variations of pH from the 0-day to 64th day of the experiment), (2) the pH fluctuations are high due to the adjustment of pH of added $NaHCO_3$ to increase its concentration from 0.8 to 2.4 g L^{-1} in solution at the days of 65, 72, 77, 84, 90 and 98 during the experiment can result in a higher pH and consequentially increased water-holding and emulsifying capacity [23] (see the variations of pH from the 62nd day to 97th day of the experiment), (3) the change in C/N/P ratio of culture media from 100/10/1 to 100/2/1 with added NH_4Cl can lead to a slow decrease in pH even if the addition of 0.4 g L^{-1} of $NaHCO_3$ affecting the increased pH is close to neutral pH of 7 (see the variations of pH from the 98th day to 135th day of the experiment). The low pH reduced protein degradation and increased NO_3^- due to nitrification compared with high pH. The pH treatments had no effect on efficiency of microbial protein synthesis [24]. The pH of a solution is one of several important factors that determine the survival and growth of bacteria for achieving a better efficiency of biological wastewater treatment process.

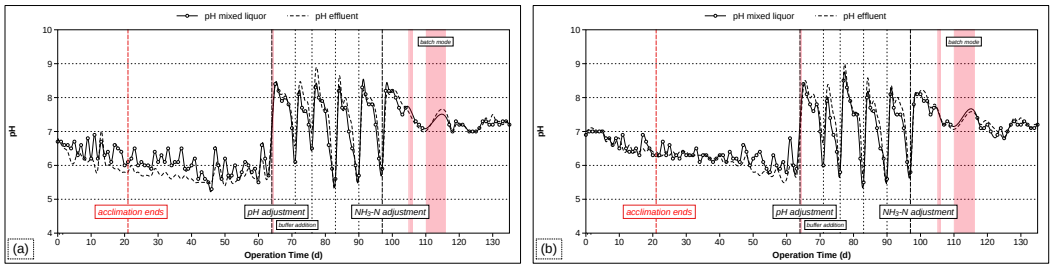

Figure 4. Variations of pH in: (a) MBR-A with a salt concentration of 10 gNaCl L^{-1} and (b) MBR-B with a salt concentration of 17.5 gNaCl L^{-1}

3.2 Performance of the MBR

The results (Fig. 5) for the experiments of either using MBR-A or MBR-B show that: (1) the variations of effluent COD concentration and COD removal efficiency fluctuate slightly until the steady state conditions are achieved after 21 days of the experiment and (2) the COD removal efficiencies are quite stable even if the effluent COD concentrations vary slightly due to the variations of EPS production in the reactor. The effluent COD will increase due to the increased bio-resistant products of recalcitrant organic compounds and EPS do pass through the membrane filter as a consequence of the N content in C/N/P ratio of 100/10/1 is high. An analysis of the EPS values confirms that the metabolic activity is high for C/N/P ratio of 100/10/1 with its EPS value of

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approximately 40 mg L^{-1}, as shown in Fig. 6. The organic matter of protein-based EPS (see EPSp) does not pass through the membrane filter due to all EPSp molecular sizes larger than the pore sizes of the membrane are stopped at its surface.

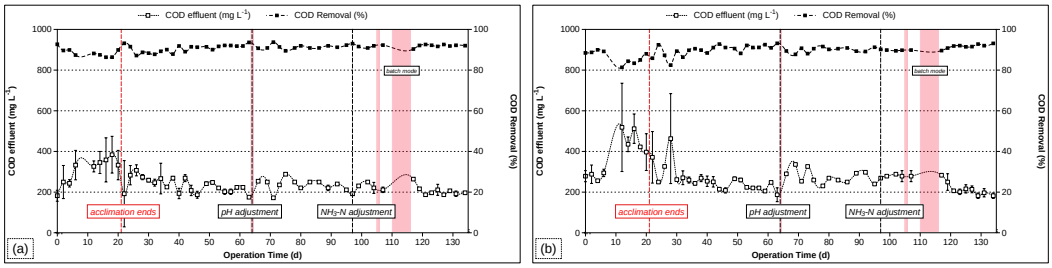

Figure 5. Variations of effluent COD and COD removal efficiency for: (a) MBR-A with a salt concentration of 10 gNaCl L^{-1} and (b) MBR-B with a salt concentration of 17.5 gNaCl L^{-1}

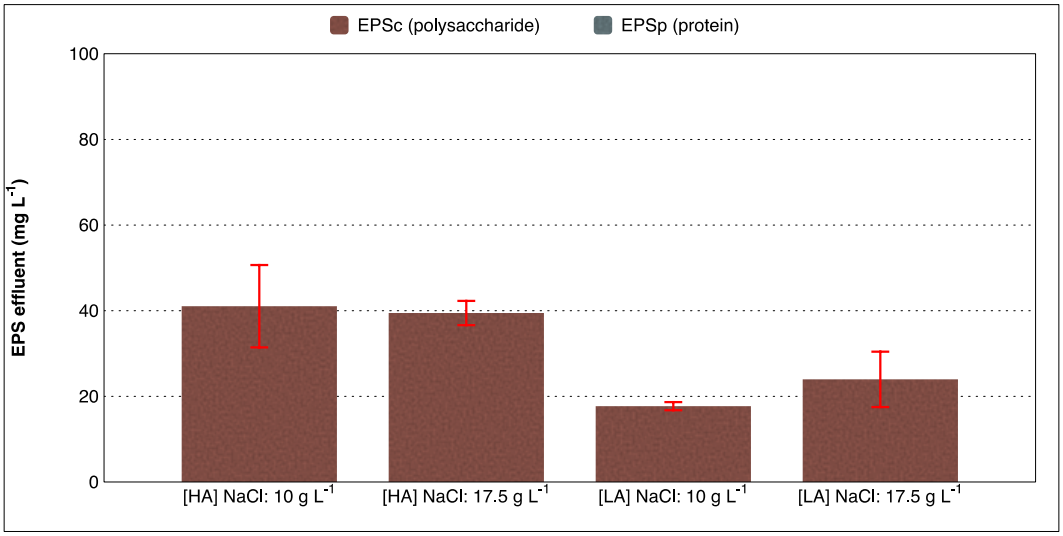

Figure 6. Average of EPS concentrations in effluent;
HA means C/N/P of 100/10/1 and LA means C/N/P of 100/2/1.

The performances (Table 4) of MBR-A and MBR-B to remove O&G, COD, NH_3, and PO_4^{3-} under steady state conditions show that: (1) the removal efficiencies of O&G, COD and NH_3 are all higher than 90% and (2) the removal efficiency of PO_4^{3-} can reach 47.26 and 46.49% for C/N/P ratio of 100/10/1 and that can reach 62.21 and 62.20% for C/N/P ratio of 100/2/1. Even though the change in C/N/P ratio from 100/10/1 to 100/2/1 does not affect the efficiency of O&G removal, it can lead to slightly increase the efficiency of COD removal i.e., from 90.93 to 91.87% for MBR-A and from 89.90 and 91.09% for MBR-B, to moderately increase the efficiency of NH_3 removal i.e., from 93.46 to 99.50% for MBR-A and from 96.30 to 99.47% for MBR-B, and to increase the efficiency of PO_4^{3-} removal quite highly i.e., from 47.26 to 62.21% for MBR-A and from 46.49 to 62.20% for MBR-B. An increase in the efficiency of COD removal could be due to the adjustment of pH, close to neutral, can complement organic matter degradation rate. Because of the low N element added into the reactor when adjusted for the C/N/P ratio of 100/2/1, the bacterial colonies of having a stable and complete nitrification that grow optimally at around the neutral pH can use the NH_4^+ ions from produced water for their metabolism. An increase of approximately 15% in the PO_4^{3-} removal was verified due to the change in C/N/P ratio from 100/10/1 to 100/2/1 can lead to the observed growth yield in the presence of the filamentous bacteria that can more efficiently absorb and incorporates P into their cell membranes.

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Table 4. Average of pollutants removal efficiency; HA means C/N/P of 100/10/1 and LA means C/N/P of 100/2/1.

Parameter	Removal (%)			
	MBR-A		MBR-B	
	HA	LA	HA	LA
COD	90.93	89.90	91.87	91.09
O&G	95.93	94.63	94.77	94.48
NH_3-N	93.46	96.30	99.50	99.47
$PO4_3^-$	47.26	46.49	62.21	62.20

3.3 Characteristics of Mixed Liquor

The mixed liqour characterization can be performed analysing the SVI value for understanding the settling properties of activated sludge and activated sludge floc morphology for understanding the microbial population and floc structure in the MBR [25]. The variations (Fig. 7) of the SVI value from the 44th day to 57th day of the experiment showed that the flocculation [26] of activated sludge flocs by stimulation of the aerobic biological activity for the C/N/P ratio of 100/10/1 is better for the experimental run in the MBR-A with a salt concentration of 10 gNaCl L^{-1}, compared to that in the MBR-B with a salt concentration of 17.5 gNaCl L^{-1}. The high salt content in solution can affect the change in ability of flocculation through several mechanisms, such that: (1) the implication of the increasing salt content can increase the density of water and thus leads to increased buoyant force, (2) the growth rate of filamentous bacteria decreases to having a crucial impact on the way of maintaining floc structural integrity, and (3) the high content of salt in solution that interrupt cell membrane can cause cell lysis due to the loss of osmotic pressure balance between the cell and its surroundings. As a conclusion, the change in C/N/P ratio from 100/10/1 to 100/2/1 can improve the flocculation of activated sludge.

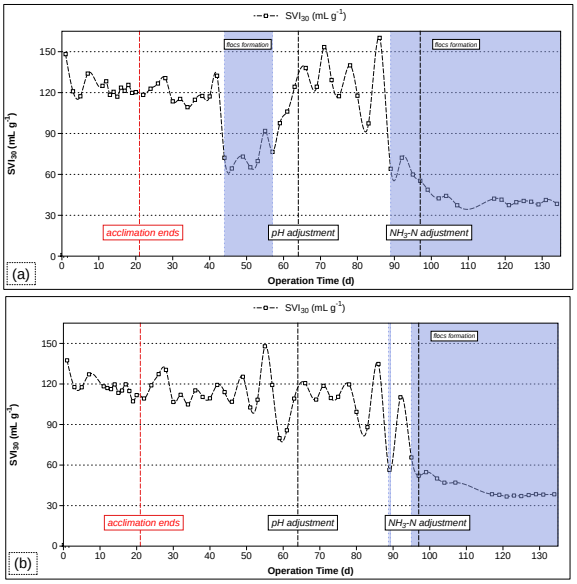

Figure 7. Variations of SVI_{30} value for: (a) MBR-A with a salt concentration of 10 gNaCl L^{-1} and (b) MBR-B with a salt concentration of 17.5 gNaCl L^{-1}

The presence (see Figs. 8b, d) of filamentous bacteria in the MBR-A and the MBR-B for the C/N/P ratio of 100/2/1 has been investigated in this work due to the lack

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of N element in the solution. Since the cell surface area for filamentous bacteria could be larger than that for bacillus bacteria, they have the ability to adsorb more minerals from produced water to grow faster when the nutrient elements in solution is low.

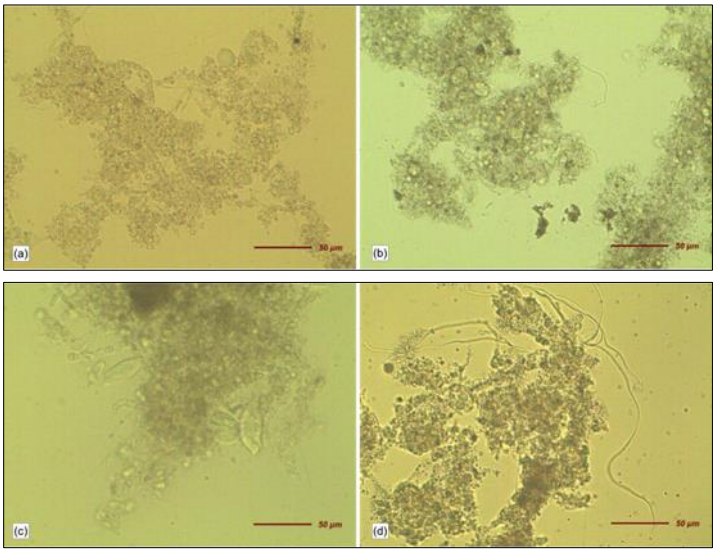

Figure 8. Floc morphology of activated sludge; (a) MBR-A with a salt concentration of 10 gNaCl L^{-1} and C/N/P ratio of 100/10/1, (b) MBR-A with a salt concentration of 10 gNaCl L^{-1} and C/N/P ratio of 100/2/1, (c) MBR-B with a salt concentration of 17.5 gNaCl L^{-1} and C/N/P ratio of 100/10/1, (d) MBR-B with a salt concentration of 17.5 gNaCl L^{-1} and C/N/P ratio of 100/2/1.

4. CONCLUSIONS

This study used two submerged MBR of added non-halophilic bacteria to remove to remove O&G, COD, NH_3, and PO_4^{3-} from synthetic produced water. The change in salt concentration from 10 to 17.5 g L^{-1} does not affect the performance of the MBR to remove O&G, COD, NH_3 and PO_4^{3-}. The change in C/N/P ratio from 100/10/1 to 100/2/1 does not affect the efficiency of O&G removal and can lead to the slightly increasing of COD removal efficiency, to moderate increasing of NH_3 removal efficiency, to the increase in PO_4^{3-} removal efficiency quite highly. The flocculation phenomena and fouling membrane were verified to contribute to the use of MBR technology for wastewater treatment in the future.

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Design of Wastewater Treatment Plant using Anaerobic Baffle Reactor and Anaerobic Biofilter Media for Sedati Fish Auction

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Abstract

Sedati fish auction is an important purchase-sale place in East Java. Although its presence is very important, the sanitation aspect inside there is unmaintained and unhealthy. Fish wash or another purchase-sale waste is discarded into the drain, causing odour and disease vector because of the characteristic of fish wash waste which is organic waste. One way to improve that particular condition is by constructing wastewater treatment plant (WWTP) with anaerobic process. Alternative processes proposed for this plant are Anaerobic Baffle Reactor or Anaerobic Biofilter with bioball media.

Between the two alternatives, selection is based on the smallest land area and building volume, the highest efficiency removal of BOD and COD, and the cheapest budget of building construction and maintenance. In this planning, the primary data consists of influent flow of wastewater, characteristic of BOD, COD, TSS, and value of pH and temperature. These data are used for calculating dimension of both building.

Before it was being discharged in the main unit, wastewater flew through a screen. This screen was made of stainless steel mesh wire. Based on the calculation, the appropriate type of WWTP in Sedati fish auction is *Anaerobic Baffle Reactor* with four baffles. This unit has total 5,6 metres long, 1,8 metres wide and 1,5 metres high, with a building blocks made of 5 mm mild steel. Total cost to build is IDR 64.319.391,00.

Keywords: Fish Auction, Anaerobic Baffle Reactor, Anaerobic Biofilter

1. INTRODUCTION

Fish auction is a system of fish port that has a functional facility as a fish market. Fish auction can increase economic value in surrounding area, because it used free trading system to improve the purchase-sale process. This kind of free trading system is an open-binding process, so that both player can determine the real cost of fish.

One of the fish auction in East Java is Sedati fish auction. There are a lot of marine catches in TPI Sedati, such as fishes, crabs, shrimps, and scallops. After marine catches placed in TPI, seller will wash those catches before they are sold. The problems that occur after washing are, some of fish wash waste will directly throw to the border river or floor stall. Thus, TPI Sedati filled with puddles and became smelly. Moreover, TPI Sedati is prone to be a disease vector for both seller and buyer.[1]

Based on that problems, it is necessary to constructing wastewater treatment plant (WWTP). Processing technology that have ever develop was a mechanical separation process and microscreen settling either fixed or rotating[2]. Wastewater in Sedati fish auction can be classified as an organic waste with high BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand) and TSS (Total Suspended Solid). Therefore, several anaerobic treatment can be chosen based on its characterictic, such as

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Anaerobic Baffle Reactor, Anerobic Biofilter, or Rotating Biological Contractor. But the most suitable treatment that can be applied in TPI Sedati, based on its characterictic, human culture, and location are Anaerobic Baffle Reactor and Anaerobic Biofilter.

2. REGION PLANNING OVERVIEW

Sedati fish auction is located in the Gisik Cemandi Village, Sedati subdistrict, Sidoarjo. Gisik Cemandi Village is a fisherman village that immediately adjacent to the village of Banjar Kemuning and Gisik Kidul, and right behind Juanda International Airport.

TPI Sedati adjacent to a river that flows directly into the Java Sea. Subdistrict Sedati has a height of 4 meters above sea level. But, because TPI Sedati located in the West End district of Sidoarjo and directly adjacent to the sea, the altitude at TPI Sedati lower than other areas in Sidoarjo. TPI Sedati land area of 1919.13 ha.

3. PLANNING METHOD

The method in this planning are data collection and building calculation. There are two types of data collected, primary data and secondary data. Primary data includes influent wastewater discharge, as well as the characteristics of the BOD, COD, TSS, pH, and temperature of the waste water obtained by the results of sampling in the field and then analyzed in the laboratory. Secondary data include wastewater quality standards by the Governor Regulation Number 52 Year 2014 and the unit price of Basic Worker Unit Price (HSPK) in Surabaya in 2015.

Table 1. Water Quality Standards

Parameter	Value (mg/L)
BOD	50
COD	100
TSS	200

Source : Data Calculation

In building calculation, wastewater treatment plant designed according to DEWATS module [5] with design criteria as shown in Table 3.

Table 2. Design Criteria

Anaerobic Baffled Reactor	Anaerobic Biofilter
Organic loading : < 5 kg COD/m^3.day	Organic Loading : 0,4 -5 kg COD /m^3.day
HRT at Sedimentation Tank : 2 hours	HRT at Sedimentation Tank : 2 hours
HRT : ≥ 8 hours	HRT at AF tanks : 24 – 48 hours
BOD removal : 70 – 95%	BOD removal : 70 – 90%
SS/COD ratio : 0,35 – 0,45	SS/COD ratio : 0,35 – 0,45
Velocity upflow : 1,4- 2 m/hours	Specific area of media: 80 - 180 m^2/m^3
	Filter void mass : 30 – 45 %
	Velocity upflow : < 2 m/hours

Source : Sasse, 1998[5]

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4. RESULT AND DISCUSSION

4.1. Determination Of Wastewater Quality And Quantity

Overall, the total sellers in TPI Sedati as many as 25 people. On weekdays, the number of sellers can be reduced up to 15 people, for a large number of visitors rely more on weekends than when the working day.

The use of water for operations at TPI Sedati from water PDAM Sidoarjo. However, water is not directly channeled to the seller. The seller must buy the second party through a jerry can. Total amount of clean water usage is 1262 L/day or 1,2 m^3/day.

Wastewater quality data obtained based on the primary data. The data is obtained from direct sampling in location ditch then it is analyzed in a laboratory. The result wastewater quality data can be seen in Table 3.

Table 3. Wastewater Quality

Parameter	Value (mg/L)
BOD	894
COD	1443
TSS	280

Source : Data Calculation

4.2. Alternatives Processes

In this planning, there are two alternatives for wastewater treatment unit. Details treatment alternatives can be seen in the following chart:.

- Alternatives 1

  Sewage ⟶ Screen⟶ Equalization tank ⟶ Anaerobic Baffle Reactor

- Alternatives 2

  Sewage ⟶ Screen⟶ Equalization tank ⟶ Anaerobic Biofilter

The difference between the first and the second alternative, that the first alternative is used anaerobic process with suspended growth systems, while the unit is used anaerobic biofilter system Attached Growth with Bioballs media. Selection of the main unit is based on the high efficiency of the two units in processing organic waste as well as operation and maintenance tend to be easy to apply in Sedati fish auction.

4.3. Screen

This planning needs to be installed on a screen or filter wastewater. It is intended to filter out scrap materials of fish wash. The remaining components are mixed in the wastewater could potentially include fish scales, fatty fish, fish bones, and others - others. Filter waste water appropriately used are *stainless steel mesh filter*. The advantages of this material include not easy to rust, easy to apply, and have varying diameter holes.In order to screen the waste water to function optimally, then use mesh filter diameter being. That is, the diameter of the mesh can filter the rest of the ingredients, but also not impede the flow of waste water.

Wastewater screen is placed in the ditch toward equalization. Before it is placed in the channel, *mesh filters* must be mounted on a metal frame / timber. It aims to facilitate

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the installation of mesh filter. Furthermore, the *mesh filter* installed above the concrete cover so as to facilitate maintenance mesh filter. Based on the dimensions of the existing sewage systems, it can be determined dimensional mesh filter for:

Wide = 30 cm

High = 50 cm

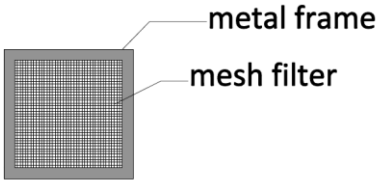

Figure 1. Screen design

4.4. Equalization Tank

Equalization tank's function is to keep the quality and quantity of discharge before heading to the next processingunit, for waste water from TPI Sedati not flow for 24 hours. After calculating the fluctuations in wastewater discharge, the greatest cumulative volume of 0.42 m^3. Volume value is then reduced, and serve as an equalization tank volume. Equalization tank volume is 0.56 $m^{3.}$ The depth of the equalization tank unit planned depth of 0.5 m, so, the equalization tank dimensions are:

Table 4. Dimension of Equalization Tank

	Equalization tank
Area (m2)	0,76
Length (m)	0,71
Height (m)	0,5
Width (m)	0,71
Freeboard (m)	0,3

Source : Data Calculation

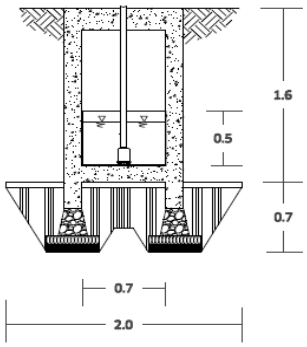

Figure 2. Equalization Tank Design

4.5. Anaerobic Baffle Reactor Design

Anaerobic Baffle Reactor is a kind of suspended growth treatment that utilizes baffle in a stirring to enabling the contact between the wastewater and biomassa. Anaerobic Baffle Reactor is a reactor that uses a series of walls (baffles) to make wastewater containing organic pollutants to flow downward and upward (through) the wall from the inlet until outlet. Basically, Anaerobic Baffle Reactor is the development of an upflow anaerobic sludge blanket reactor (UASB).[3] Anaerobic Baffle Reactor's simple structure makes it possible to design altered depending on the characteristic of wastewater

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to be treated. Hybrid design can be done to improve the performance of the reactor to a specific liquid waste.[3]

ABR advantages compared with other wastewater treatment system are high retention time of solid without media or space deposition of microorganisms attached, less sludge formation, and it doesn't require the microorganisms with particular settling ability, low hydraulic retention time, steady against shock loading hydraulic and organic, a long operation time without disposal of sludge, Functioning effectively in a range of influent flow and the amount of load that is wide enough, no mechanical mixing, small blockage and expansion risk, and low operating costs. [4]

Anaerobic Baffle Reactor calculations based on modules DEWATS[5]. Here are the results of calculating the dimensions of ABR:

Table 5. Dimension of Anaerobic Baffle Reactor

	Sedimentation Tank	Anaerobic Baffle Reactor
Area (m^2)	0,5	0,3
Length (m)	0,71	0,6
Height (m)	0,5	1
Width (m)	0,71	0,5
Freeboard (m)	0,3	0,3

Source : Data Calculation

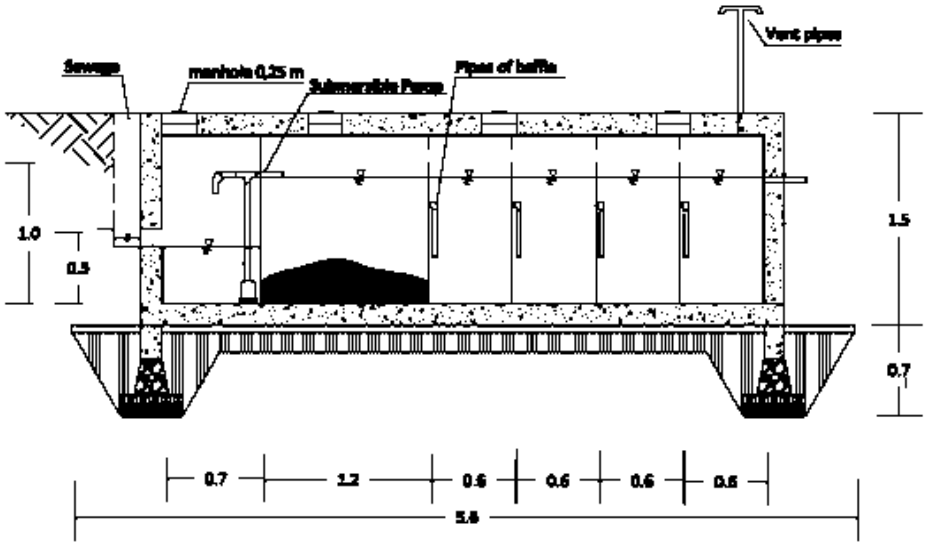

Figure 3. Anaerobic Baffle Reactor Design

4.6. Anaerobic Biofilter Design

Anaerobic Biofilter also known as fixed bed. The unit performs processing for which can't be precipitated solids and dissolved solids. Principles of *Anaerobic Biofilter* is solids processing which can't be precipitated or dissolved, by bringing these solids to contact with active mass bacteria.[3] The period of active bacteria need food so that the bacteria digest organic material dispersed and dissolved in the brief retention times.[3]

Anaerobic Biofilter is one type of submerged biofilters. Biofilters itself is a biological treatment process with the use of principles attached to the microbial growth medium. In this planning used Bioballs media. Media Bioballs widely used because it has a large enough specific media. Inside media bioballs formed biofilms. Biofilms are a

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collection of cells of microorganisms attached to a surface of the media and covered by adhesive polysaccharide excreted by the cells - the cells of these microorganisms. Biofilms formed by microorganisms derived from wastewater, that will conduct a process called *desloughing* . *Desloughing* is the decay process of biofilm due to the accumulation of microorganisms that cause facultative bacterial voluntary to non-active facultative. the calculation is based on the Anaerobic Biofilter DEWATS module[5]. Here are the dimensions of Anaerobic Biofilter :

Table 6. Dimension of Anaerobic Biofilter

	Sedimentation Tank I	Sedimentation Tank II	Anaerobic Biofilter
Area (m^2)	0,55	0,3	0,65
Length (m)	1,1	0,6	1,3
Height (m)	1,1	1,1	1,1
Width (m)	0,5	0,5	0,5
Freeboard (m)	0,3	0,3	0,3

Source : Data Calculation

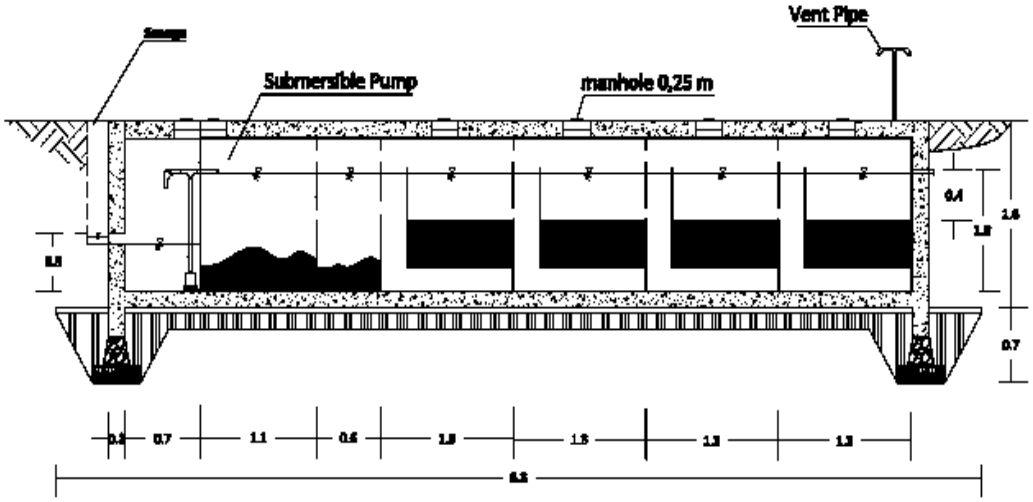

Figure 4. Anaerobic Biofilter Design

4.7. Total Cost Building

As the result of budget plan calculation, showed that the Anaerobic Baffle Reactor, including both Equalization tank and sedimentation tank needs IDR 64.391.391. Meanwhile, the Anaerobic Biofilter construction costs is IDR 124.851.161. These costs consist of preparation stages and soil, concrete work and foundation, wastewater treatment plant, finishing work, and operation and maintenance costs.

5. CONCLUSION

Total dimensions of Anaerobic Baffle Reactor is 5,6 length, 1,8 width and 1,5 height with 4 baffles while Anaerobic Biofilters with Bioballs media need 8,8 m length, 1,8 m width, and 1,6 height. Total cost for Anaerobic Baffle Reactor is IDR 64.391.391. In this results of calculation, Anaeroic Baffle Reactor have advantages from small area needs and cheaper total cost than Anaerobic Biofilter. Therefore, it can be concluded that Anaerobic Baffle Reactor is more suitable and profitable for build in Sedati fish auction.

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Removal of Trivalent Chromium using *Azotobacter S8* and *Bacillus subtilis*

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Abstract

The application of chromium in various industries have caused pollution to the environment. Chromium mostly found in wastewater in the form of Cr^{3+} and Cr^{6+}. Bacteria has known capable to remove chromium heavy metals that can be used as bioremediation agents. *Azotobacter S8* and *Bacillus subtilis* were bacteria that capable to remove chromium heavy metals. The purposes of this experiment were to determine the optimum bacteria composition and chromium removal percentage by *Azotobacter S8* and *Bacillus subtilis* either single or consortium culture. Methods used in this experiment were bacterial growth rate test, minimum inhibitory concentration test, and chromium removal by bacteria test. Chromium removal test was done by shaking chromium solution containing bacteria using shaker for 4hours test period with 50mg/L $CrCl_3$ initial concentration. The result of this experiment showed that the highest removal percentage was done by a single bacteria culture of *Azotobacter S8* with 10.53% removal on 50 mg/L initial concentration with 4 hours testing time and pH was 8.35.

Keywords: Azotobacter S8, Bacillus subtilis, Bioremediation, Cr^{3+}, Heavy metals

1. INTRODUCTION

The use of chromium in various manufacturing processes like leather tanning industry has caused the release of chromium heavy metal to the environment [1]. Leather tanning industry is an industry that produces chromium liquid waste with a concentration of between 40-25000 mg / L [2]. Chromium waste is often found in water bodies in the form of Cr^{3+} and Cr^{6+} [3]. Cr^{6+} is a form of chromium that is less stable because it easily reacts with other particles in the air to form Cr^{3+} [4], so we need further processing to treat the chromium Cr^{3+} [5].

Chromium metal contained in water bodies ranging from 0.1 to 117 g/L [4]. Chromium levels are allowed for drinking water is less than or equal to 0.05 mg / L [6], while for the waste water is 0.5 mg/L [7]. Chromium in large quantities can cause irritation to the nose, allergies, respiratory and reproductive problems [8].

Bioremediation is one of technologies used to treat wastewater containing chromium [4]. Bioremediation is one of biotechnology that can be used to improve the ecosystem, transforming pollutants into the substrate, producing biodegradable material of natural resources and developing environmentally friendly manufacturing production [9]. The principle of bioremediation is utilizing metabolic reactions of microorganisms to degrade the contaminants found in the environment [10].

Bacillus subtilis [11] and *Azotobacter S8* [12] are bacteria that can be used as an agent for bioremediation of chromium heavy metal. *Bacillus subtilis* capable of removing

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95.19% Cr^{6+} in waste water effluent with an initial concentration of 100 mg/L [11] and capable of removing chromium up to 100% with an initial concentration of 10 mg/L for 24 hours [13]. While *Azotobacter* resistant to chromium heavy metals to a concentration of 300 mg/L [12]. *Azotobacter* has a polymer component of extracellular namely exopolysaccharide (EPS) that has binding properties of metal pollutants [14]. *Azotobacter* is able to produce EPS in culture with heavy metals Fe, Zn, and Cr [15]. *Azotobacter* has a level of resistance and bonding of Pb and Cd which is greater than the *Bacillus megaterium* [16].

The removal efficiency of chromium heavy metals may change with the type and composition of bacteria used [17]. Consortium between *Pseudomonas aeruginosa* and *Bacillus subtilis* capable of removing heavy metals chromium up to 99.6% with an initial concentration of 570 mg/L [2]. While the optimum composition of *Pseudomonas putida* and *Bacillus subtilis* were used as consortium bacteria was 50:50, which has a removal efficiency up to 85% with an initial concentration of 50 mg/L [18].

Based on the above, the purpose of this study was to determine the optimum composition and chromium heavy metal removal percentage by the *Bacillus subtilis* and *Azotobacter S8*, either on single or consortium culture.

2. MATERIAL AND METHOD

2.1 Bacteria Growth Rate Test

The bacteria growth rate test was conducted to determine the bacterial growth curve and obtain the exponential phase of which will be used as a time determinant in the chromium heavy metal preliminary test stage. In the single bacteria growth rate test, 24 hours old bacteria inoculum inoculated in *Nutrient Broth* media then shaken using *shaker* (Innova 2000, USA) for 24 hours with 150rpm rotation speed [3]. In the consortium bacteria growth rate test, the inoculum which has been shaken for half an exponential time normalized at OD_{600} = 0.5 [19]. Inoculum was added to the *Nutrient Broth* media as much as 10% of the total volume of media [8].

During the time of the test, observations of pH, temperature and o*ptical density* (OD) were carried out. Sampling for the observations were made every 2 hours for 24 hours. Value of *optical density* was measured with a spectrophotometer (Genesys 20, USA) with a wavelength of 600 nm [11], while the pH and temperature were measured with a pH meter (CyberScan 510, USA) and a thermometer (EC 10 PHonLab, USA).

2.2 Minimum Inhibitory Concentration Test

Minimum Inhibitory Concentration (MIC) is the minimum concentration that can inhibit the growth of microorganisms. The method used in this research *screening* method using *Nutrient Agar* as media in petri dishes [20]. The concentration of chromium that used to determine the MIC value were 0; 5; 50; 100; 250; and 500 mg/L which has been sterilized. Observation on bacterial growth was done after 24 hours of incubation. The growth of bacteria in media containing chromium compared with the growth of bacteria in blank media that do not contain chromium.

2.3 Chromium Heavy Metal Removal by Bacteria Test

Chromium removal by bacteria test was carried by inoculating bacteria in 50 mg / L chromium solution. Bacteria that have been shaken for half an exponential time, normalized at OD_{600} = 0.5 [19]. Inoculum was added to chromium solution as much as 10% of the total volume of media. The composition of bacteria used is 100% *Azotobacter* S8; 75% *Azotobacter* S8 : 25% *Bacillus subtilis;* 50% *Azotobacter* S8:

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50%*Bacillus subtilis;* 25% *Azotobacter* S8: 75% *Bacillus subtilis and* 100%*Bacillus subtilis.*

Bacterial cultures were shaken on a *shaker* for exponential time with 150rpm speed [3]. Parameters measured were *optical density*, pH, temperature, and total chromium. Three ml samples were taken from each reactor at the beginning, middle, and end of the study to know the value of the specified parameters. The *optical density* value was measured with a spectrophotometer using 600nm wavelength [11], pH and temperature were measured with a pH meter and thermometer, while the total chromium was measured using AAS *(Atomic Absorption Spectrophotometer)* (Rayleigh WFX 210, Beijing).

3. RESULT AND DISCUSSION

3.1 Bacterial Growth Rate Curve

Bacterial growth rate test was conducted to determine bacterial growth phase based on the bacterial growth curve. The bacterial growth curve was made to determine the bacterial growth phase including lag, exponential, stationary and death phase [21]. Results of measurement parameters for each bacterial composition can be seen in Figure 1 until Figure 3.

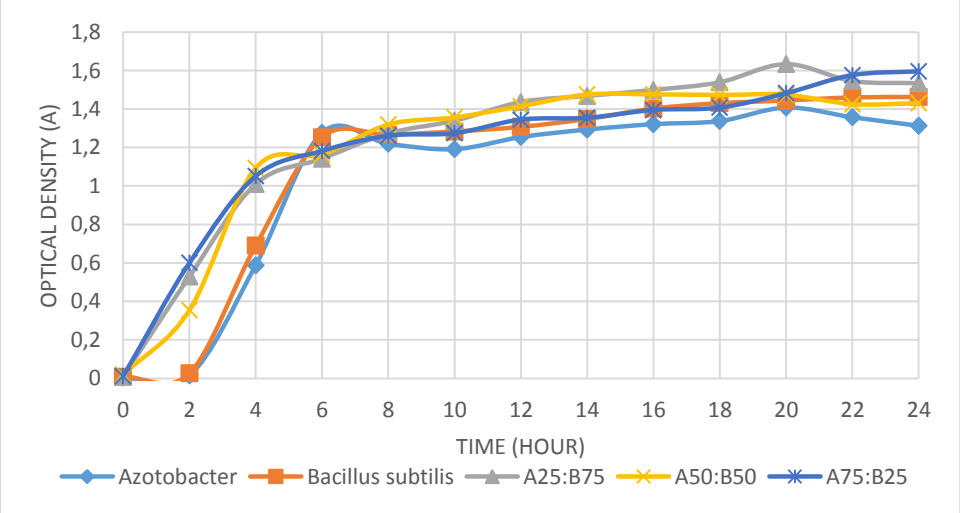

Figure 1. Single and Consortium Bacterial Growth Curve

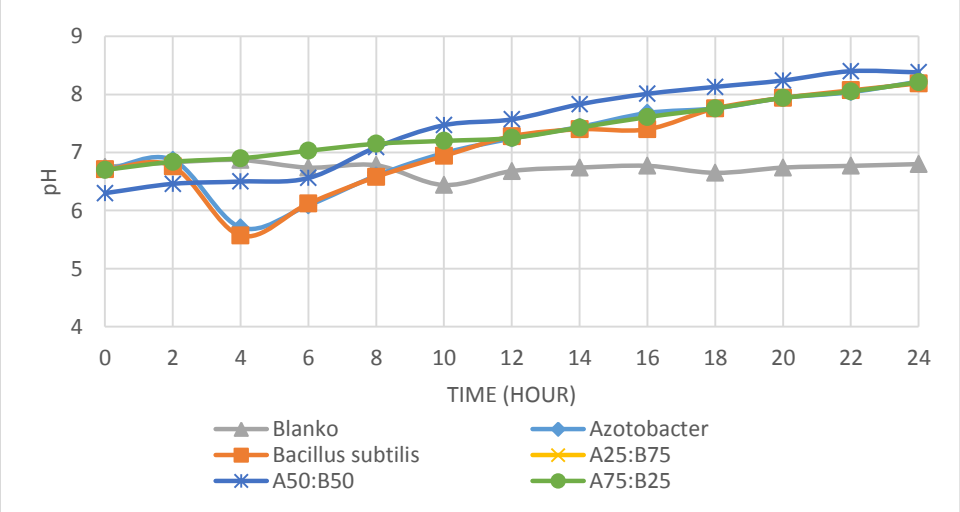

Figure 2. pH on Single and Consortium Bacterial Growth Rate Test

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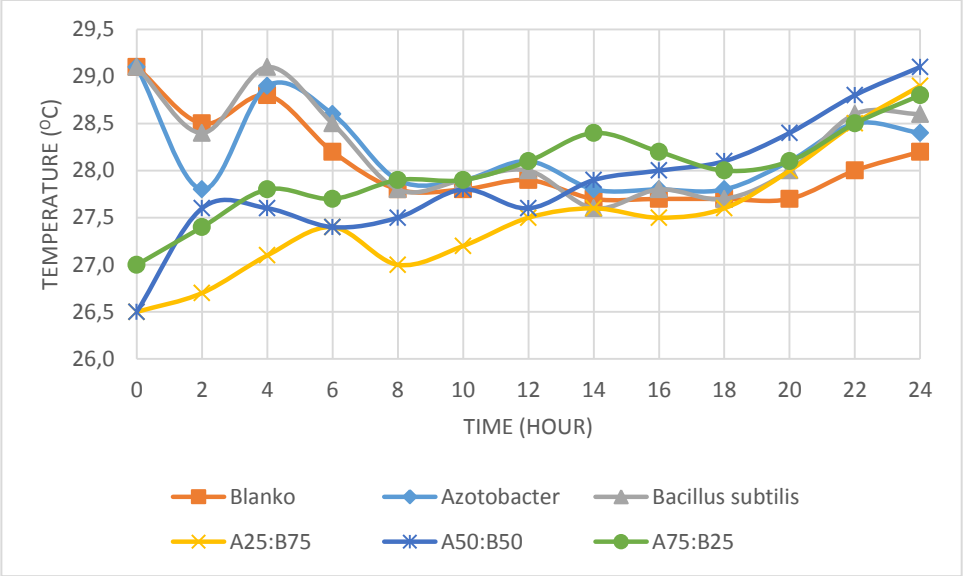

Figure 3. Temperature on Single and Consortium Bacterial Growth Rate Test

Based on Figure 1 it was known that a single bacterial lag phase to occur at 0 up to 2 hour test period, then 2 up to 6 hour test period was the exponential phase. The exponential phase of consortium bacteria occurred at 0 up to 4 hour test period. This was indicated by the addition of a significant absorbance values. The exponential phase is the phase in which the cells of microorganisms in stable condition and able to divide themselves multiple [22].

Stationary phase for a single bacteria culture occurred at 6 until the end of test period, while the stationary phase for consortium bacteria occurred at 4 until the end of test period. This was indicated by the addition of a relatively small absorbance values. In Figure 1, there was no visible bacterial death phase. It was known that the absorbance values continue to rise. The bacterial growth test method by OD measurement is one of the indirect method [21]. In this method, it can't distinguish between live bacteria and dead bacteria, so the OD measurement results will tend to rise until the end of test period.

Factor that affect the rate of bacterial growth is pH [23]. Based on Figure 2, the temperature measured for a single bacteria *Azotobacter S8* ranged from 5.71 to 8.22 while *Bacillus subtilis* ranged from 5.57 to 8.19. A decrease in pH in a single bacterial culture indicates the activity due to the growth of bacteria. These activities led to the formation of simple acid and carbon dioxide due to the breakdown of organic compounds [24]. The increase in pH on single bacteria after 4hour of test period show that bacterial growth had started optimum. The pH for all three consortium bacterias were not declined until the end of the test period. The increase in pH indicate that bacterial growth had approached the stationary phase. *Bacillus subtilis* can live in pH ranging between 5 – 9 [13]. *Azotobacter S8* can live pH ranging between 4.8 - to 8.5 with the optimum pH is 7 to 7.5 [24]. So, the pH measurement was still included in the tolerance range for bacterial growth.

In addition to pH, the factor that affect bacterial growth rate is temperature [23]. *Azotobacter S8* and *Bacillus subtilis* are mesophilic bacteria that can live in temperature ranged between 20-40^{o}C. Based on Figure 3, it was known that the temperature measured for single *Azotobacter S8* ranged between 27.8^{o}C to 29.1^{o}C. While the temperature measured for *Bacillus subtilis* ranged between 27.6^{o}C to 29.1^{o}C. Temperature measured at all consortium bacteria were ranging between 26.5^{o}C to 29.1^{o}C. The temperature measured was unstable because of the metabolic activities of microorganisms. So, the measured temperature range for both single and consortium

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bacteria was still included in the temperature needed for the bacteria to grow. Reference states that both bacterial growth optimum temperature is 37°C [13].

Based on the bacterial growth rate curves, specific growth rate and generation time of each bacterial composition can be determined with kinetic equations [30].

$$\mu = \frac{\ln(Xt) - \ln(Xo)}{t_t - t_0} \quad \text{.....(1)}$$

$$t = \frac{0{,}693}{\mu} \quad \text{.....(2)}$$

Note :

μ : Specific growth rate ($hour^{-1}$)

Xt : *Optical density* value at t – test period

Xo : *Optical density* value at 0 – test period

t_t : t – test period

t_0 : 0 – test period

t : Generation time (hour)

According to Equation (1) and (2), specific growth rate and generation time for all bacteria can be seen in Table 1.

Table 1. Bacterial Specific Growth Rate and Generation Time

Bacterium	**μ (h^{-1})**	**t (h)**
Azotobacter S8	1.11	0.62
Bacillus subtilis	0.97	0.72
A25: B75	1.18	0.59
A50: B50	1.01	0.68
A75: B25	1.14	0.61

Based on Table 1 it was known that the higher the specific growth rate of bacteria, the lower generation time of bacteria. The bacterial growth rate was specific to each different bacteria. This was because the enzyme content of each bacteria that affect the metabolism system of bacteria. The highest specific growth rate of consortium bacteria was 25% *Azotobacter S8:* 75% *Bacillus subtilis* with 1.14/hour and generation time was 0.61 hours. The lowest specific growth rate obtained by a single *Bacillus subtilis* was 0.97/hour and generation time was 0.75 hours.

3.2 Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is the minimum concentration that can inhibit the growth of microorganisms. The method used was screening method [20]. The purpose of the screening test was to determine the concentration used in chromium heavy metal removal test. The results of the screening test after incubation for 24 hours can be seen in Figure 4.

Based on Figure 4 it was known that the higher the concentration of chromium exposed into the media the less bacterial growth shown on media. In media which do not contain chromium and the concentration of 5 mg / L $CrCl_3$, both bacteria show excellent

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growth. At the concentration of 50 mg/L both bacteria showed fairly good growth, while at the concentration of 100 mg/L, showed poor growth of bacteria. This shows that both bacterial growth begun stunted by the rising of chromium levels. Both bacteria did not show any growth at a concentration of 250 mg/L $CrCl_3$ and 500 mg/L $CrCl_3$. *Azotobacter S8* has a high tolerance to chromium heavy metals at levels between 0-50 mg/L [12] and *Bacillus sp* also have a high tolerance to chromium heavy metals at levels between 0-50 mg/L [13].

The results of MIC observation were done by scoring the results obtained. MIC scoring was determined based on the surface area of bacterial growth and color change compared to control bacterial growth. However, the preferred criteria is the surface area of bacterial growth. It is defined as the spread of bacteria in the media indicates that bacteria can still survive. The MIC scoring result can be seen in Table 2.

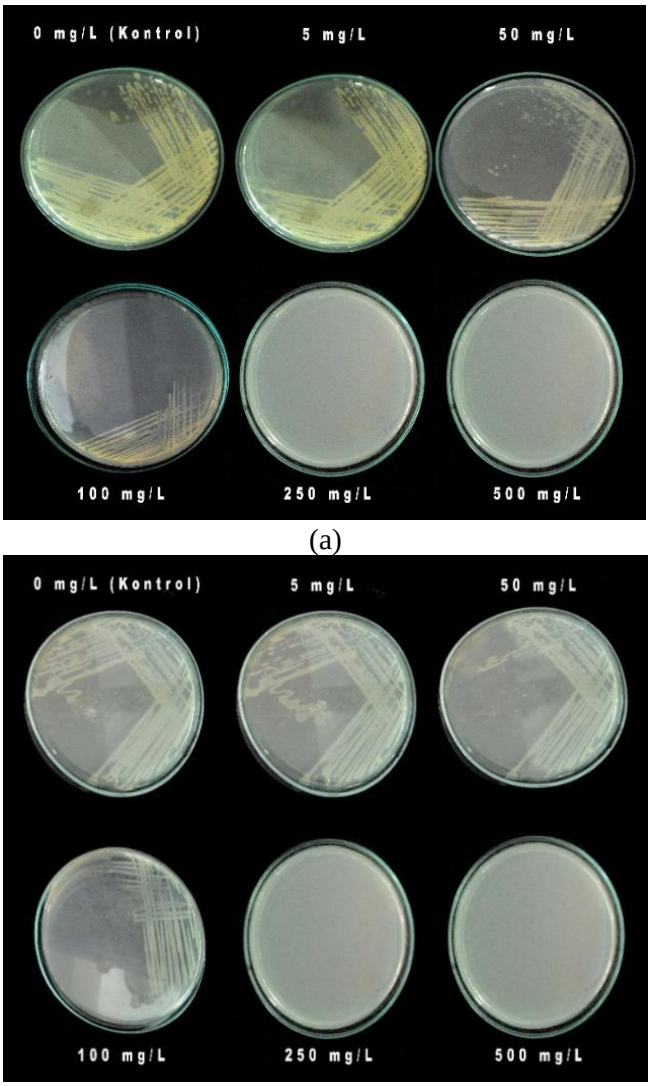

Figure 4. The Result of MIC Test using Screening Method for Azotobacter S8 (a) and Bacillus subtilis (b)

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Table 2. Growth of Bacteria Test - MIC Scoring

Bacterium	The concentration of chromium (mg / L)					
	0	5	50	100	250	500
Azotobacter S8	+++++	+++++	++++	++	-	-
Bacillus subtilis	+++++	+++++	++++	+++	-	-

Note:

+++++ = Extensive growth of bacteria was 81-100% compared to the blank and/or no color changing

++++ = Extensive growth of bacteria was 61-80% compared to the blank and/or no color changing

+++ = Extensive growth of bacteria was 41-60% compared to the blank and/or no color changing

++ = Extensive growth of bacteria was 21-40% compared to the blank and/or no color changing

\+ = Extensive growth of bacteria was ≤20% compared to the blank and/or there was color changing

\- = No bacterial growth at all

Based on Table 2, the MIC value with *screening* method for both bacterial tested were ranged between 100-250 mg/L $CrCl_3$, so the concentration used in the chromium removal test by bacteria was 50 mg/L $CrCl_3$. This concentration was chosen by considering the changes in both bacteria living media. In the MIC test, media used for both bacteria's living was *Nutrient Agar* media. Whereas in chromium removal test, media used for bacteria's living was $CrCl_3$ solution.

3.3 Chromium Heavy Metal Removal by Bacteria

Chromium removal test was the major step in this research. Time used in this test was the time of exponential growth of bacteria, that was 4 hours based on the bacterial growth curves. At this stage, main parameters including o*ptical density* (OD), temperature, pH, and total chromium were carried out during the test.

Based on Figure 5 it was known that the absorbance value of either single or consortium bacteria did not increase or decrease significantly until the end of the test period. This was because the process of bacteria acclimatization to the media containing chromium. OD parameter cannot distinguish between live bacteria and dead bacteria, so the OD measurement results will tend to rise during the time period.

Factor affecting the growth of bacteria was pH. At optimum pH, process of heavy metals removal will be more optimal [25]. Based on Figure 6 it was known that the pH value for single and consortium bacteria ranged between 8.1 to 8.9. Meanwhile, the control media pH measured was ranged between 8 - 8.2. *Bacillus subtilis* can live in pH ranging from 5 – 9 [13]. *Azotobacter S8* can live in pH ranging between 4.8 - to 8.5 with the optimum pH were 7 to 7.5 [24].

The chromium tends to make pH more alkaline [13]. The increase in pH of the chromium solution containing bacteria caused by bacterial activity and the accumulation of bacteria dead cells. The increase in pH also indicates that the growth phase of bacteria has approached the stationary phase [26].

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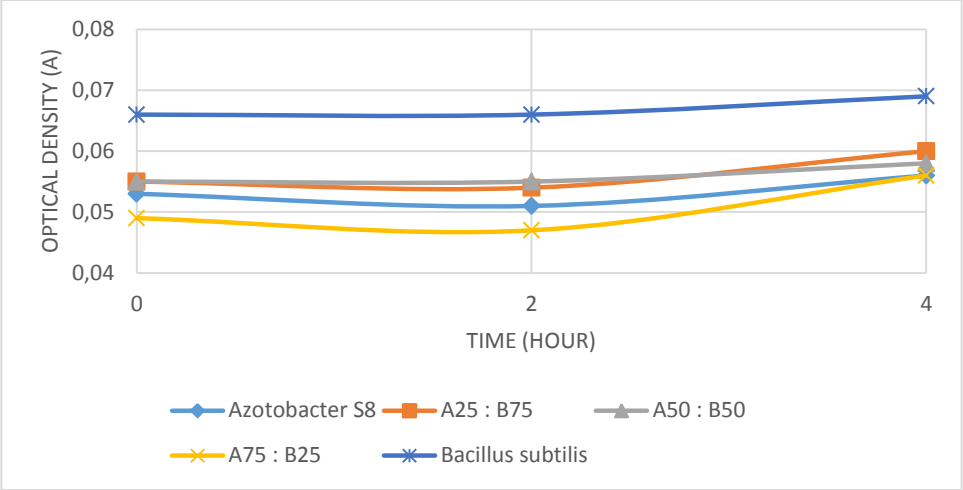

Figure 5. Optical Density on Chromium Heavy Metal Removal by Bacteria Test

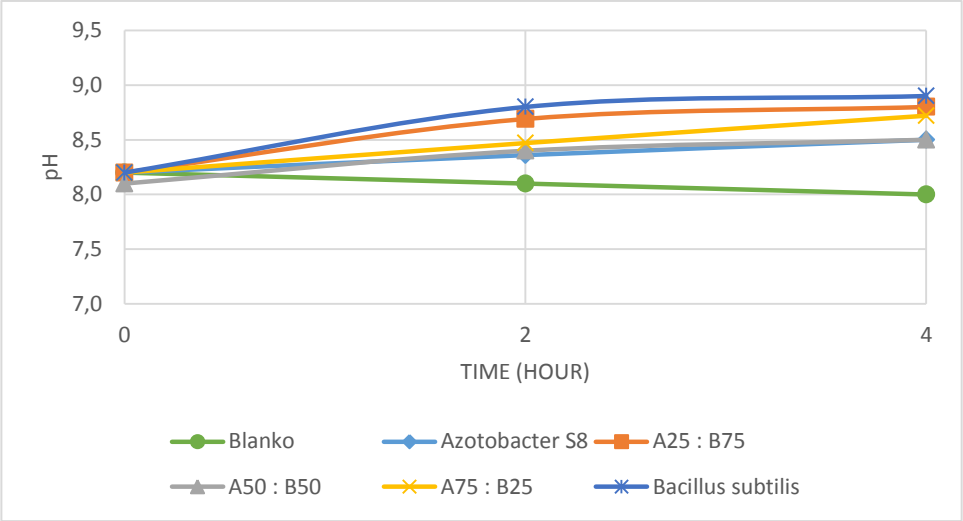

Figure 6. pH on Chromium Heavy Metal Removal by Bacteria Test

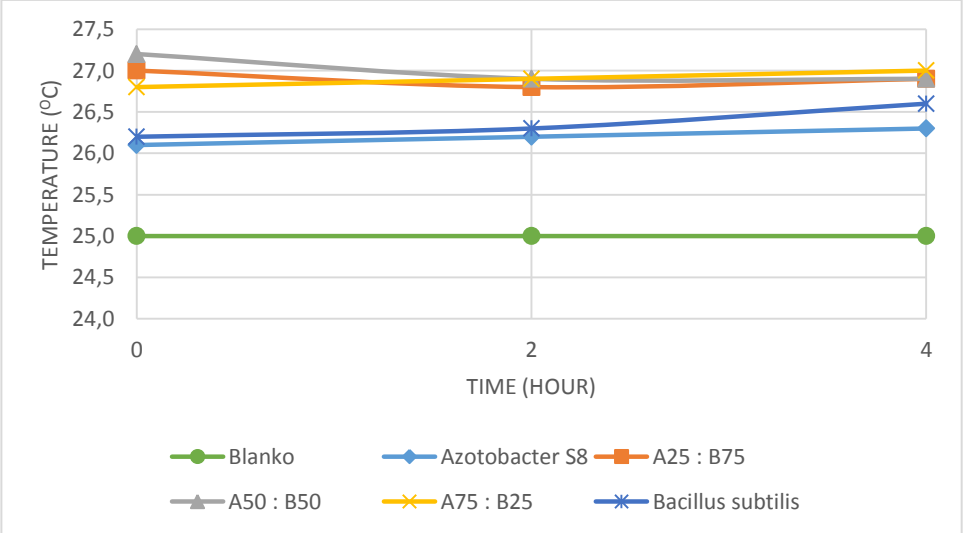

Figure 7. Temperature on Chromium Heavy Metal Removal by Bacteria Test

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Temperature was also a factor that affect the process of heavy metal elimination [23]. At the optimum temperature, bacteria can eliminate heavy metals more efficient because the bacteria metabolic processes can work well at the optimum temperature [25]. *Azotobacter S8* and *Bacillus subtilis* were mesophilic bacteria that can live in temperature ranged between 20-40^{o}C. Based on Figure 7, it was known that temperature measured on chromium solution with all bacterial composition ranged between 26.1^{o}C - 27.2^{o}C, while the temperature of control media was 25^{o}C. The measured temperature range for all tested bacteria composition were still included in the temperature needed for the bacteria to grow.

Total chromium parameter measured at the beginning and end of the study. The goal of chromium testing was to determine the chromium removal percentage by bacteria. The percentage of chromium removal for each bacterial composition can be seen in Figure 8.

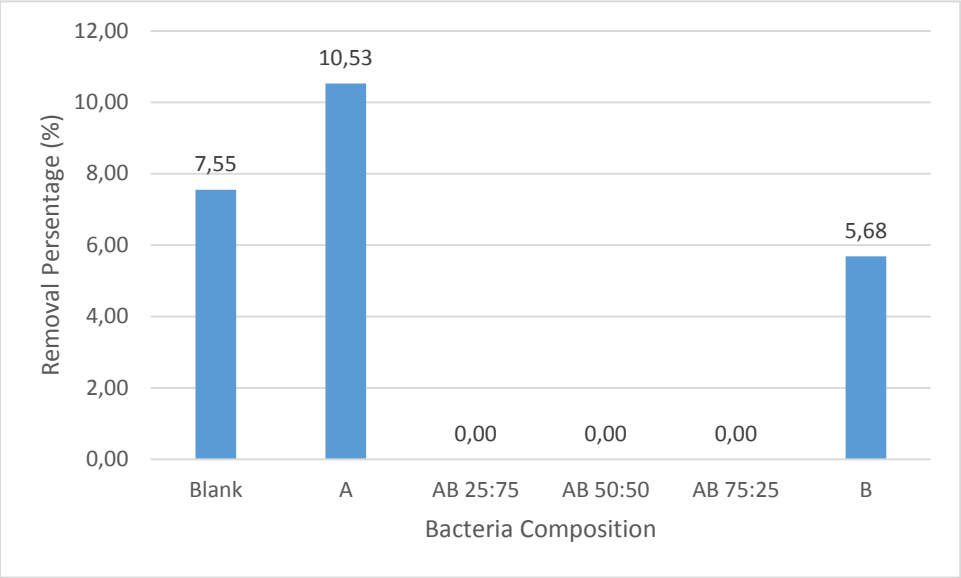

Figure 8. Chromium Heavy Metal Removal Percentage by Bacteria

Based on Figure 8, it was known that chromium concentration in chromium solution containing *Azotobacter S8* and *Bacillus subtilis* decreased. This proves that both bacteria can eliminate chromium heavy metal. Bacteria require chromium as a nutrient in small amounts to remain alive [2]. The highest chromium removal percentage was 10.53% by *Azotobacter S8* and the lowest percentage was 0% by all consortium bacteria with 4hour test period and the removal percentage for *Bacillus subtilis* was 5.68%. All removal percentage were far behind literature study, this might be due to the short time of test period. Reference [11] said that *Bacillus subtilis* capable of removing up to 95.19% chromium with an initial concentration of 100 mg/L in 24hours incubation time at optimum pH 7. In general, the length of time used for heavy metal removal by bacteria are 24 hours [11], 72 hours [12], and 96 hours [8].

Azotobacter S8 has higher chromium removal percentage than *Bacillus subtilis*. It was occurred because *Azotobacter S8* has exopolysaccharide (EPS) which can adsorb metals, then forming ligands with EPS. Environmental condition is what makes the heavy metals can be adsorbed by *Azotobacter*'s EPS [14]. *Azotobacter S8* was able to perform extracellular detoxification mechanisms due to the interaction of chromium with hydroxyl groups on the cellulose that coats cell walls of bacteria. *Azotobacter* can also produces catalase and reductase enzymes [28]. The enzyme's function is to break down any

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harmful substances that enter into the bacterial cell as well as lower levels of toxic heavy metal.

Consortium bacteria shown 0 removal percentage for all composition, it means that there is no removal process for chromium heavy metals in that reactor. This might be due to both bacteria do not have any mutualism symbiotic [29], as well as the influence of pH and temperature to the ability of bacteria for removing heavy metals [23]. The incubation time was also too short. In general, the time given for incubating bacteria for heavy metals removal are 24 hours [11], 72 hours [12], and 96 hours [8].

4. CONCLUSION

Based on the discussion, the conclusion in this study were the MIC value with a *screening* method for *Azotobacter S8* and *Bacillus subtilis* ranged between 100-250 mg / L $CrCl_3$. The optimum composition of bacteria in Cr^{3+} removal process was single *Azotobacter S8* culture with 10.53% removal percentage during 4hours test period. Removal percentage for each bacteria were: 10.53% for *Azotobacter S8*; 0% for 75% *Azotobacter S8:*25% *Bacillus subtilis*; 0% for 50% *Azotobacter S8:*50% *Bacillus subtilis*; 0% for 25% *Azotobacter S8:* 75% *Bacillus subtilis* and 5.68% for *Bacillus subtilis*.

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Tempe Industrial Wastewater Treatment by using Combined Anaerobic Baffled Reactor and Biofilter Processes

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Abstract

Tempe industries have become one of key industries in many cities in Indonesia which increase concerns on wastewater treatment to meet environmental quality standards, as a consequence of its industrial activities. Tempe industrial wastewater contains high concentrations of organic contaminants thus requires to be treated prior to discharge to the environment. This research aims to assess the performance of innovative combined processes of anaerobic baffled reactor (ABR) and biofilter. This research was conducted using two laboratory-scale reactors with total effective volume of 80.99 L, consisting of four compartments of ABR and one compartment of biofilter. The performance was assessed based on the variation of wastewater influent concentrations (i.e., high strength of 20,000 mg COD L^{-1}, medium strength of 5,000-10,000 mg COD L^{-1} and low strength of 1,000-3,000 mg COD L^{-1}) and the process variation of combined ABR-biofilter (i.e., anaerobic-anaerobic process in reactor A and anaerobic-aerobic process in reactor B). The reactor was operated in a continuous system at the rate of 43.2 L day^{-1}. Diffuser was required in aerobic condition with oxygen transfer capacity of 4.5 L O_2 min^{-1}. Bioballs were used in the biofilter reactor as attached media for the microbial growth. Both reactors A and B were able to degrade organic substances with the highest removal percentage reached was 89.49% and 94.44% for COD, 95.25% and 97.56% for BOD_5, respectively. The results showed that the higher concentration of organic contaminants treated, the higher organic removal efficiency in ABR-biofilter processes will be obtained.

Keywords: aerobic biofilter, anaerobic baffled reactor, anaerobic biofilter, tempe wastewater

1. INTRODUCTION

Tempe industry is an example of growing industries in big and small cities in Indonesia. Some activities involved in tempe processing generated wastewater containing high concentrations of organic pollutants. As the consequences of these industrial activities, it is essential to improve wastewater treatment facility in order to meet environmental quality standards. The wastewater is generated from soybeans washing, equipments washing, cooking, and soaked soybeans solution. The amount of wastewater generated is approximately 15-20 L kg^{-1} of soybeans raw material [1]. The wastewater from soybean industry contains Biochemical Oxygen Demand (BOD) concentration of 5,000-10,000 mg L^{-1} and Chemical Oxygen Demand (COD) concentration of 7,000-12,000 mg L^{-1} [2].

Various attempts have been made to reduce the concentration of organic pollutants contained in tempe wastewater. The alternative treatments that can be done is by using Anaerobic Baffled Reactor (ABR). The anaerobic treatment has an efficiency of 50-70% with the condition of the effluent still contains high levels of organic pollutants and odors [2]. Therefore, it is essential to increase the removal efficiency by combining with aerobic process.

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The combination of anaerobic and aerobic processes has a potential for obtaining high removal efficiency. Combination is done by combining Anaerobic Baffled Reactor (ABR) with Anaerobic/Aerobic biofilter (AF) for the treatment of tempe wastewater. This system also considers the amount of organic loading rate (OLR) of the wastewater. If the value of OLR is too small then the processing is not able to operate optimally. Therefore, it is necessary to study and analyse the effects of wastewater concentration variations in the application of ABR-AF combination. In addition, this study also determines the removal efficiency of using ABR-AF combination applied for the treatment of tempe wastewater by means of anaerobic-anaerobic and anaerobic-aerobic processes.

2. MATERIALS AND METHODS

2.1 Preliminary Research

The preliminary research was done to analyse characteristics of tempe wastewater derived from Kampung Tempe in Tenggilis sub-district area, Surabaya. The characteristics include the main parameters such as BOD_5, COD and Total Suspended Solid (TSS) as well as additional parameters such as ammonia, N, P, pH and alkalinity. The tempe wastewater used in this study was soybeans soaked wastewater and soybeans rinse wastewater as the diluent, i.e., to simulate the targeted wastewater concentration.

2.2 Reactor Design

This research was conducted using two laboratory-scale reactors with combination of ABR and AF in a single reactor. Reactors was made of glass material with dimensions of 89 cm × 26 cm × 37 cm. Media for microbial growth used in this reactor was bioball that made of PVC material. Two reactors were used, namely Reactor A with combination of anaerobic-anaerobic processes and Reactor B with combination of anaerobic-aerobic processes, both with the designated rate of 43.2 L day^{-1}. The design of reactor A and reactor B can be seen in Figure 1 and Figure 2.

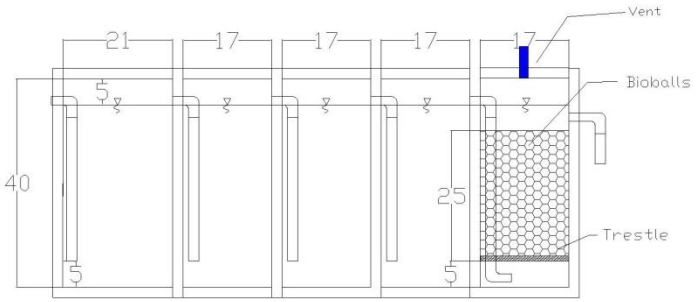

Figure 1. Reactor A with anaerobic-anaerobic processes

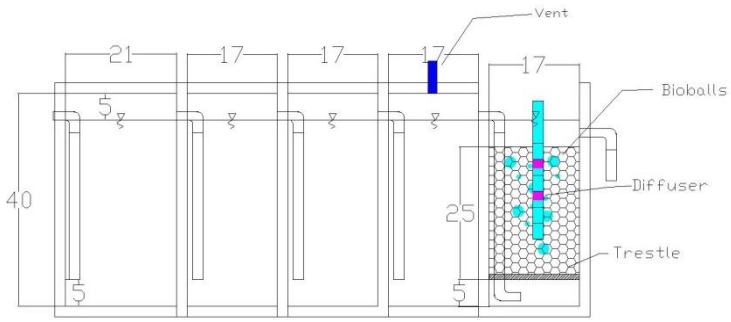

Figure 2. Reactor B with anaerobic-aerobic processes

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2.3 Seeding and Acclimatization Processes

Seeding and acclimatization processes were very essential during initial stages in order to ensure the sustainability of tempe wastewater treatment process. Seeding process was aimed to increase the population of microorganisms growth by adding adequate amount of activated sludge into the reactor. The activated sludge was taken from the return activated sludge secondary clarifier unit in the sludge treatment plant.

During seeding process, substrate (i.e., sugar) was continuously added as a carbon source to support the growth of microorganisms [3]. The next process was acclimatization as microorganisms adaptation process with the wastewater condition as to growth in specific engineered conditions. The acclimatization process was reached when the stability of microorganisms efficiency in wastewater processing or decreasing of COD parameter has removal deviation of ≤ 5% from the previous measurement.

2.4 Measurement of Wastewater Concentration Variations

The study was conducted by flowing wastewater with various concentrations of organic substances. There were three concentration ranges of organic contaminants, i.e., high strength with ≥ 20,000 mg COD L^{-1}, medium strength with 10,000-5,000 mg COD L^{-1} and low strength with COD of 1,000-3,000 mg L^{-1} which refers to the concentration of existing wastewater treated. The experiment was started by flowing 100% of high strength wastewater with determining 5 routine analysis, namely COD, BOD_5, TSS, alkalinity and pH. Monitoring tests were carried out every 2 days. Sampling point was taken from the influent compartment, ABR compartment prior AF unit and the effluent compartment.

3. RESULTS AND DISCUSSION

3.1 Preliminary Characteristics Analysis of Industrial Waste Tempe

Tempe industrial wastewater used in this study was physically containing high turbidity and odor levels. The results of preliminary research can be seen in Table 1.

Table 1. Characteristics of Tempe Industrial Wastewater

Parameter	Unit	Soaked Wastewater	Rinse Wastewater
COD	mg COD L^{-1}	21,564	6,763
BOD_5	mg BOD_5 L^{-1}	12,100	1,101
NH_3-N	mg L^{-1}	2.08	1.50
pH	-	3.70	4.29
Alkalinity	mg L^{-1}	93	110
TSS	mg L^{-1}	1,190	450
N	mg L^{-1}	711.65	692.86
P	mg L^{-1}	94.31	76.45

3.2 Analysis of Hydraulic Loading Rate (HLR) Test

In HLR test, actual detention time can be determined when the concentration of fluorescent achieved its stability or reached steady state condition. This HLR test was performed using spectrophotometric method for determining the optimum wavelength and calibration of fluorescent solution. The test was conducted in each effluent point of reactors in every 15 minutes [4]. The sampling time started when the fluorescent was detected flowing out from the reactor at the first time. The rate that used in HLR test of each reactors was 43.2 L day^{-1}. The test results were used to compare the calculation of HLR detention time with the actual detention time which can be achieved by fluorescent in the effluent point of reactor. The concentration of fluorescent as a trace color which

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used was 30.69 mg L^{-1}. The calculation of detention time in the reactor A and B with HLR 0.474 m^3 m^{-2} day^{-1} were 44.15 hours. The results of HLR test for reactor A and B can be seen in Table 2.

Table 2. The Results of HLR Test

Parameter	Reactor A	Reactor B
Q	43.2 L day^{-1}	43.2 L day^{-1}
HLR	0.474 m^3 m^{-2} day^{-1}	0.474 m^3 m^{-2} day^{-1}
Td calculation	44.15 hours	44.15 hours
Td actual	49 hours	49.75 hours
Td difference	4.85 hours	5.6 hours
% Td changes	10.99%	12.68%

In Table 2, it can be seen that detention time was increased in the reactor A by 10.99% and 12.68% in the reactor B. The increasing of actual detention time that occured in each reactor was caused by the unstable of fluorescent upflow rate due to the presence of dead space in the reactor. The values of dead space at ABR was less than 8% of hydraulic dead space on empty reactor without biomass, while the value of dead space in AF reached 50% -93% of the reactor volume [5]. The presence of dead space on the ABR and AF was also due to the fluctuating changes of the influent flow rate [6].

The decline of upflow rate in the ABR and AF affects the actual detention time of reactor. The smaller value of upflow rate, the longer detention time or HRT will be. The presence of bioballs as filter media in AF unit significantly affects the upflow rate, i.e., becomes smaller. Hydraulic efficiency affects the influent capacity which is mixed inside the reactor.

3.3 Analysis of Seeding and Acclimatization Processes

The seeding process was done by filling the activated sludge in the reactor about 30% of the reactor volume in a batch condition. The actual volume of sludge used in each reactor was about 30.5 L for 5 compartments, thus 6.1 L each compartment. The seeding process was flowed by wastewater and sugar with concentration ratio of 50 : 50 (e.g., 5,000 mg COD L^{-1} wastewater : 5,000 mg COD L^{-1} sugar) for three days in a batch system. Therefore, the reactor was operated continuously for four days by the same wastewater. The seeding process was done after 7 days and with no sludge bulking was observed.

In the seeding process, initial measurement of mixed liquor suspended solid (MLSS) concentration was done for the activated sludge used in each reactor. MLSS value indicates the amount of solid mixture from sludge combination and wastewater influent in the reactor [7]. MLSS is the total amount of suspended solid in the form of organic material and minerals, including the microorganisms [8]. This indicates that the increasing of MLSS concentration as the increasing of microorganisms population in the reactor. The result of seeding process can be seen in Table 3.

Table 3. The Results of Seeding Process

Parameter	Reactor A	Reactor B
Initial MLSS	1,180 mg L^{-1}	1,373.33 mg L^{-1}
Final MLSS	3,920 mg L^{-1}	5,900 mg L^{-1}
Increasing of MLSS	2,740 mg L^{-1}	4,526.67 mg L^{-1}

Acclimatization process in this research was performed for 25 days by continuously flowing medium strength tempe wastewater with COD concentration of 10,000 mg COD L^{-1} until COD removal percentage reached the steady state condition. The COD concentration was measured daily to determine the level of microorganisms

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adaptation in the wastewater treatment process, especially for organic pollutants. As can be seen in Figure 3, the COD removal percentage during the acclimatization process contained seemed to be stable after day 25.

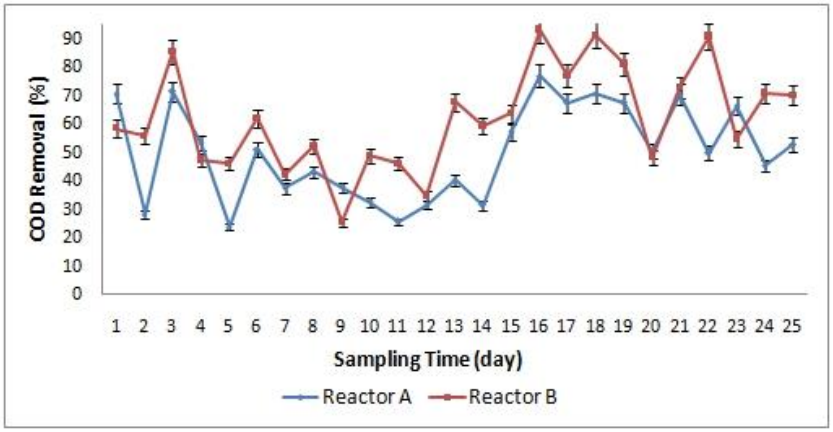

Figure 3. Removal Percentage of COD in Acclimatization Process

The highest percentage of COD removal in the acclimatization process was occured in the reactor with anaerobic-anaerobic process, i.e., 76.89% (Figure 3). In reactor B, COD removal by anaerobic-aerobic process was 93.18%. Reactor B has higher percentage of COD removal and thus it is considered to be more capable in degrading organic pollutants compare to reactor A.

The actual detention time obtained from HLR test affects the contact time of tempe wastewater with the growing microorganisms in the reactor. Low detention time has a great value of upflow rate, thus decreased contact time between wastewater with microorganisms. This decreases mass transfer rate which results in the decreasing of reactor performance efficiency to degrade organic pollutants in wastewater [9].

The average COD removal percentage for acclimatization process in reactor A was 49.97% ± 16.65 and reactor B was 61.69% ± 18.13. Reactor A has smaller standard deviation from reactor B, thus it could be concluded that reactor A was more stable in removing COD during the acclimatization process. This is due to the higher hydraulic efficiency of reactor A with smaller difference of actual detention time than in the vase of reactor B. Hydraulic efficiency greatly affects the process of microorganisms adaptation to degrade tempe wastewater that flows into the reactor, thus affects overall the reactor performance in degrading COD.

3.4. Analysis of Concentration Variations Test

Analysis of concentration variation was conducted to evaluate the loading rate in which the biological processes of ABR-AF combination can be achieved. The experiment was started by evaluating the high strength wastewater, then subsequently evaluate the medium and low strength wastewater. This is done so that microorganisms can adapt to the wastewater treatment process with high concentration thus when it reached steady state condition, the process become more stable and capable in treating wastewater with lower concentrations. Steady state condition indicates the current state of constant organic substances reduction on the specific organic and hydraulic load [10]. The steady state condition occurs when the degradation efficiency of organic substances is less than 5% for three consecutive days [11].

3.4.1 Analysis of COD and BOD_5

The decreasing of COD and BOD_5 concentration determines the reactor efficiency to oxidize the organic matter contained in tempe wastewater during both

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anaerobic and aerobic processes. The overall COD removal efficiency for high strength, medium strength and low strength wastewater was in the range of 35.38%-89.49% in reactor A and 67.16% -94.44% in reactor B. The results of COD removal in each reactor for all wastewater concentration variations can be seen in Figure 4.

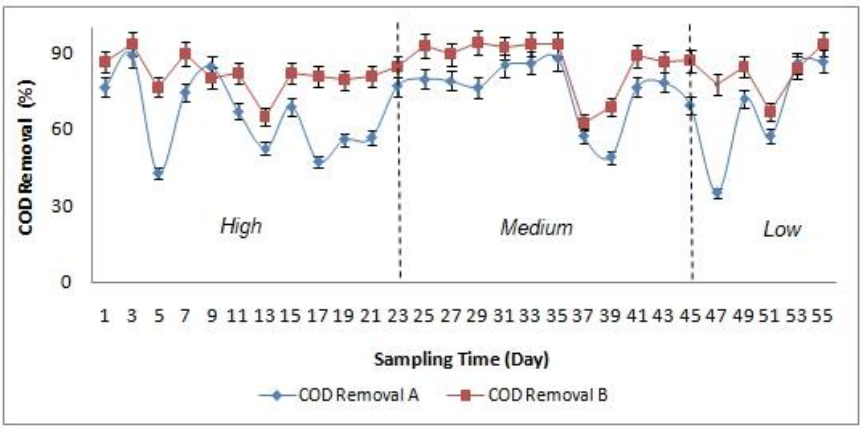

Figure 4. Removal Percentage of COD

In Figure 4, it can be seen that reactor B has more stable COD removal efficiency concentration than reactor A. The average COD removal percentage in the reactor A was 70.22% ± 15.28 and reactor B was 83.88% ± 9.07. The average COD concentration in ABR-AF effluents in high strength and medium strength wastewater concentrations were still relatively high, i.e., in the range of 1,179.14-6948.53 mg COD L^{-1}. On the other hand, in the low strength wastewater concentration, the concentration of COD effluent for reactor A and reactor B was 181.82 mg COD L^{-1} and 113.64 COD L^{-1}, respectively. These concentrations have met the regulated stream standards, i.e., 300 COD L^{-1} [12].

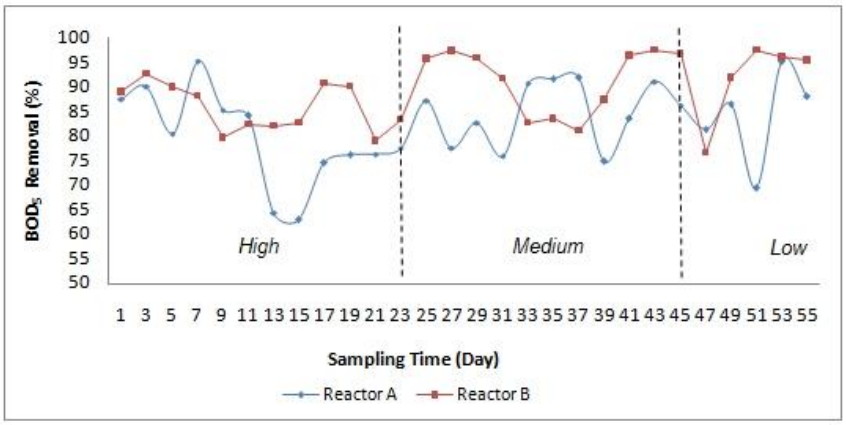

Figure 5. Removal Percentage of BOD_5

Reactor performance was also evaluated by means of BOD_5 removal efficiency. The overall BOD_5 removal efficiency for all concentrations (i.e., high strength, medium strength and low strength) was in the range of 62.95%-95.25% in reactor A and 76.64%-97.56% in reactor B (Figure 5). The average BOD_5 removal percentage for high strength concentration in reactor A and reactor B was 79.61% and 85.85%, respectively. The average effluent concentration achieved in reactor A and reactor B was approximately 1,705.03 BOD_5 mg L^{-1} and 1,172.45 BOD_5 mg L^{-1}, respectively. The combination of

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ABR-AF for both reactors showed the highest efficiency in reducing BOD_5 concentration at the medium strength wastewater load. Both reactors were capable of degrading BOD_5 concentration with the highest removal percentage of 91.97% for reactor A and of 97.56% for reactor B.

Reactor A and reactor B tend to decrease BOD_5 concentration in the low strength concentration variation. The BOD_5 removal percentage obtained in reactor A and reactor B was 84.06% and 91.50%, respectively. This was due to the low concentration of organic substances utilized by microorganisms with approximately 540.66 mg BOD_5 L^{-1} in reactor A and 573.80 mg BOD_5 L^{-1} in reactor B. The less organic substances used by microorganisms, the smaller BOD_5 removal percentage can be obtained in the tempe wastewater.

Similar to COD concentration, the average concentration of BOD_5 in ABR-AF effluents were still relatively high for high strength and medium strength concentrations, i.e., 167.61 and 3,279.11 mg BOD_5 L^{-1}, respectively. The BOD_5 concentration of the effluent in the case of low strength concentration wastewater was 25.94 mg BOD_5 L^{-1} in reactor A and 12.13 mg BOD_5 L^{-1} in reactor B. Likewise, the BOD_5 concentration of ABR-AF effluents in the reactor A and B have met the regulated quality standards, i.e., 150 mg BOD_5 L^{-1} [12].

3.4.2 Analysis of BOD_5/COD

The ratio of BOD_5/COD indicates the value of wastewater biodegradability that processed in the reactor. There were decreasing of *BOD_5/COD* in the concentration variations of medium strength, high strength and low strength in each reactor. The value of BOD_5/COD were in the range of 0.17 to 0.42. This indicates that the process occurred were on biodegradable zone. Biodegradable zone is the amount of organic substances that can be degraded by microorganisms in natural conditions and the processing conditions which have been determined [13]. The relationship between the ratio of BOD_5/COD in each reactor contained in Figure 6.

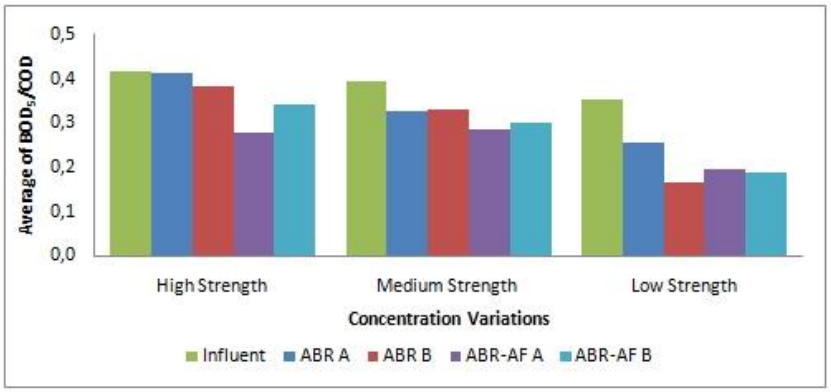

Figure 6. The ratio of BOD_5/COD

The low ratio of BOD_5/COD indicates the preference of organic substances degraded in wastewater was more biodegradable, so that showing higher capability in reducing BOD_5 than COD concentration. The ratio of BOD/COD for biological processes in the range of biodegradable, i.e 0.2-0.5 [13]. The ratio of BOD/COD between 0.2 and 0.5 can be processed with a biological process, but the process of decomposition is slower because microorganisms required acclimatization with the wastewater [14].

3.4.3 Analysis of Total Suspended Solid (TSS)

TSS analysis aims to determine the level of suspended solid in the wastewater represented by three predetermined sampling points in each reactor. The analysis of TSS

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concentration also served to determine the stability of active sludge in the reactor [4]. The TSS concentration in the wastewater influent for each reactor was ranged from 205-1,660 mg L^{-1}. The amount of TSS concentration directly proportional to the concentration of tempe wastewater. The removal efficiency of TSS concentration at each concentration variations was presented in Figure 7.

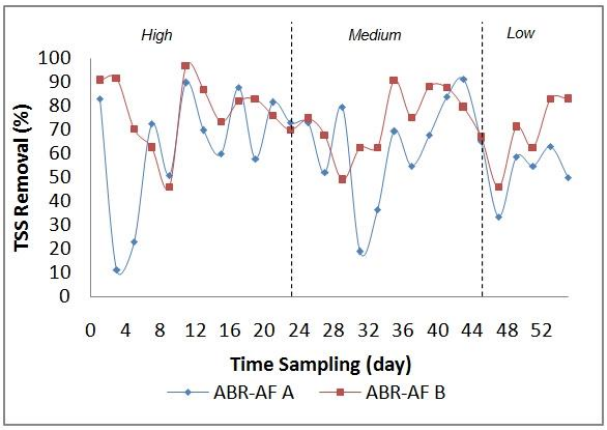

Figure 7. Removal Percentage of TSS

In Figure 7, it can be seen that reactor B tends to be more stable in reducing the TSS concentration compared to reactor A. This was influenced by the actual detention time of reactor B which was longer than reactor A. It was due to the settling time suspended solid wastes needed more time than the reactor A. The smaller retention time in the biofilter reactor, the smaller removal efficiency of reactor achieved [8]. The highest percentage of TSS concentration in each reactor was 91.18% in reactor A and 96.87% in reactor B.

The average of TSS concentration in the ABR-AF effluent for high strength and medium strength concentration variations were still relatively high, which were in the range of 339,17 and 149,09 mg L^{-1}, respectively. In the concentration variation of low strength, the effluent of ABR-AF could reach up to 120 mg L^{-1} in reactor A and 50 mg L^{-1} in reactor B. The effluent of ABR-AF in the reactor B for low strength variation has met the standard, i.e., 100 mg L^{-1} of TSS [12].

3.4.4 Analysis of pH and Alkalinity

Anaerobic wastewater treatment process requires specific environmental condition to operate the reactor. Several things must be considered in the design of anaerobic treatment for reaching the equilibrium of microorganisms activity in the processing phase of organic matter. It is important because its performance sensitivity to the fluctuations of quantity and quality wastewater influent [7]. One of important factor in the anaerobic treatment was the pH of wastewater. The pH of influent in each reactor at each various concentrations was ranged from 4.81 to 6.96. There was increasing of pH in each of processing both for reactor A and B.

The range of pH in the ABR-AF effluent in each reactors have met the environmental quality standard of pH parameter, i.e., in the range of 6-9 [12]. The pH in the ABR-AF effluent for reactor A and B has the smallest value of 7.2 and the highest value of 9.04. The value of pH were in the neutral range to support the environmental condition of reactors to do optimum treatment in anaerobic or aerobic condition. Another additional parameter which played important role in anaerobic or aerobic condition was alkalinity. Alkalinity is the ability to neutralize the acid without decreasing the value of pH solution. The results of alkalinity and pH in all concentration variations can be seen in Figure 8.

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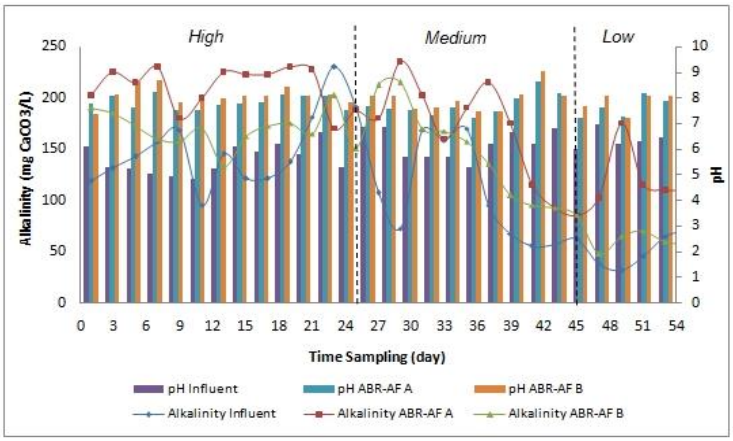

Figure 8. The Relations of alkalinity concentration and pH

In Figure 8, it can be seen that the concentration variations of high, medium and low strength increased alkalinity concentration followed by the increasing of pH in the effluent of reactor A and B. The alkalinity is useful for maintaining the pH of tempe wastewater treated. The alkalinity concentration in the ABR-AF effuent for each concentration variations was in the range of 85-235 mg $CaCO_3$ L^{-1} in reactor A and 47.5-215 mg $CaCO_3$ L^{-1} in reactor B. The water quality standard for natural alkalinity concentration never exceeded 500 mg $CaCO_3$ L^{-1} [16]. It can be concluded that based on the results of alkalinity analysis, the alkalinity concentration in the ABR-AF effluent for all concentration variations have met the environmental quality standards of alkalinity parameter [16].

3.5. Reactor Performance Efficiency

The performance of ABR-AF combination with the process of anaerobic-anaerobic and anaerobic-aerobic conditions for the treatment of tempe industrial wastewater was capable of achieving high removal efficiency of organic pollutants. The average performance of reactor A and B with combination of ABR and AF in each concentration variations was presented in Table 4.

Table 4. Performance of ABR-AF Combination

Parameter	Unit	*High strength*		*Medium strength*		*Low strength*	
		A	**B**	**A**	**B**	**A**	**B**
COD	%	66.49	82.17	75.42	86.70	67.70	81.76
BOD_5	%	79.61	85.85	84.88	91.50	84.06	91.50
TSS	%	63.32	77.41	62.89	73.08	51.93	69.02

Reactor A with combination of anaerobic-anaerobic processes has high percentage removal of organic matter. However, reactor A was not able to further process ammonia-nitrogen contained in the wastewater, instead increase the concentration of ammonia-nitrogen because the nitrification process did not occur. The advantage of reactor A was capable of decreasing the nitrates concentration contained in wastewater by combination of anaerobic processes in the AF unit. Reactor B has high percentage removal in overall parameters except the nitrate-nitrogen parameters for high, medium and low strength concentration variations. Another disadvantage of reactor B was adequate energy

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requirement to operate AF unit in aerobic process and the necessity of advanced post-treatment to further conversion of nitrates.

4. CONCLUSIONS

Anaerobic-anaerobic reactor (A) and anaerobic-aerobic reactor (B) were able to degrade organic substances with the highest removal efficiency reached 89.49% and 94.44% for COD, 95.25% and 97.56% for BOD_5 and 91.18% and 96.87% for TSS parameter, respectively. Reactor B was more effective than reactor A with the measured concentration of effluent reached 113.82 mg COD L^{-1} and 12.13 mg BOD_5 L^{-1} for the low strength influent.

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Mixture of Organic Solid Waste for Its Conversion to Protein by Utilising Black Soldier Fly (BSF) Larvae

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Abstract

Organic solid waste conversion to protein is an alternative of solid waste treatments which can be performed by utilising Black Soldier Fly (BSF) larvae as *Hermetia illucens* species. This method is applicable to treat organic solid waste that has less contamination from other anorganic wastes, i.e., waste generated from centralised market place. However, the waste, which are dominantly fruit waste (e.g., papaya, watermelon, etc.), has higher water content. This decreases the capability of BSF to reduce the organic solid waste as well as the growth of BSF larvae. Therefore, it requires a method to obtain efficient reduction of organic solid waste by BSF larvae. Protein content of BSF larvae as a result of solid waste conversion was also determined. Both mixtures were varied in three composition ratio of fruit waste and food waste or chicken manure, i.e., 90:10; 80:20 and 70:30. The results show that mixture of fruit waste and food waste with composition ratio of 70:30 obtained the highest percentage of waste reduction, with the percentage reduction of 82.87%. In addition, protein content of larvae on food waste mixture 70:30 was 41.49%. Application of the mixture of fruit waste and food waste into pilot scale was also investigated.

Keywords: BSF larvae, protein content, solid waste mixture, waste reduction.

1. INTRODUCTION

Waste reduction by utilising *Black Soldier Fly* (BSF) is a technology of waste biodegradation by larvae from *Hermetia illucens* (*H. illucens*) species [1]. BSF technology is significantly developed as it is considered to be the quickest way to reduce waste with more beneficial result compared to composting technology. Many countries are already implementing this technology as a solution to waste problem such as Argentina, United States, Australia, Hong Kong and New Zealand [2]. On the other hand, some developing countries use it to feed fish, chicken, pig and the other animals which make them cost higher [3].

BSF technology has been recently investigated in pilot scale. The waste treated by BSF technology is only from fruit waste such as papaya and watermelon. It is possible because *H. illucens* larvae can perform optimally if its food are in the form of *slurry* or having 70.2% of water content. The water content factor sometimes makes a lot of larvae *H. illucens* died and cannot degrade the waste to the maximum reduction [4]. Moreover, the residue which can be composted is unable to be used because the water content is too high and the C / N ratio was at the lower limit of the range of C / N ratio [5] at 10.

The ideal weight of BSF larvae which can be harvested is 252 mg [6]. This was in contrast to the actual condition in which the weight of harvested BSF larvae is only 100-140 mg. Waste composition in Puspa Agro Market should be arranged in order to obtain reduction improvement as well as gaining the weight of larvae. The composition that should be arranged are those which come from fruit waste and the other kind of waste

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such as food waste or chicken manure. Chicken manure is a good option as it contains better element compared to the other kind of animal manures. It has less fermentation period and also preferable for BSF larvae [7]. In other case, the selection of waste from food waste as a waste mixture is able to support the national medium term of development plan set by the government in 2019. The mixture of fruit waste with additional waste aims to increase the percentage of waste reduction as well as gaining the weight of larvae. The mixture is also determined from the result of laboratory scale research. The chosen mixture in laboratory scale will be applied in pilot at Puspa Agro Sidoarjo market.

2. MATERIALS AND METHODS

The research was conducted for 14 days based on BSF larvae phase. It consists of two stages i.e laboratory scale and pilot scale research. Pilot scale research is a field application research in Puspa Agro Sidoarjo market. The larvae feeding on laboratory scale was done everyday by giving 40 mg per larvae in dry weight, while the pilot scale carried out three times during the research phase. The research was conducted by using 2 variables. The first variable was based on the composition of waste and the second one was based on the composition ratio. The control treatment was the same as the test sample. The data of reactor can be seen in Table 1.

Tabel 1. The treatment towards composition variety and the type of waste

Reactor	Waste	Larva Amount	The Value of Repetition
Laboratory Scale			
K1	Fruit waste (control)	200	1
K2	Chicken manure (control)	200	1
K3	Food waste waste (control)	200	1
Fruit waste : Food waste			
SM1	90:10	200	2
SM2	80:20	200	2
SM3	70:30	200	2
Fruit waste : Chicken Manure			
KA1	90:10	200	2
KA2	80:20	200	2
KA3	70:30	200	2
Pilot Scale			
1	Fruit waste (Control)	10.000	12
2	Fruit waste : Waste waste = 70:30	10.000	12
3	Food waste waste (control)	10.000	12

2.1. The Measurement of Larvae Weight

The growth of larvae measurement can be determined when it gains weight whether in wet or dry condition and also from the protein contained inside the larvae body after reduction process. The measurement of larvae weight was conducted on the day 0, 5, 10 and 14. It was done by taking 10 BSF larvae. Afterwards, they were placed in an oven with the temperature of 105^{o}C for 24 hours. The next process was placing them in desiccator for 15 minutes and measured by analytical balance.

The measurement of protein was conducted at the end of research. The measured larvae of laboratory scale are those which own the highest reduction level per composition in the reactor. In addition, the measurement of protein for pilot scale was conducted towards the larvae in all reactors. The method used was the determination of protein content by total nitrogen method [19].

2.2. The Measurement of Waste Reduction Level

The determination of waste reduction was based on the residue that produced at the end of the research on day 14. Before the residue was measured, the mixed larvae

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were separated first. The percentage of waste reduction was counted based on the final weight and the total weight of the sample in the reactor. As the result, the level of waste reduction can be found by applying the formula below [6].

$$\text{WRI} = \frac{D}{T} x100 \quad (1)$$

$$\text{D} = \frac{W-R}{W} \quad (2)$$

In which:
WRI = Wate Reduction Index
D = Waste Degradation Level
T = Time needed to degrade the waste
W = The amount of waste before degradation
R = The amount of Residue

2.3. The Measurement of Waste Residue Characteristics

The measurement of waste residue characteristics was based on several parameters such as water content, temperature, pH and C/N ratio. The measurement for laboratory scale was conducted every three days, while the measurement for pilot scale was conducted on the day 0, 5, 10, and 14. The measurement of water content was done by taking ± 10 g sample from the reactor. Then, the sample was heated for 24 hours by using oven [8]. The data of temperature were obtained from the measurement which was done every day for laboratory scale by applying thermometric method [9]. The measurement of pH was conducted every day to find out the effect of BSF larvae application towards the changing of pH meter [8]. The analysis of C and N was done both at the beginning and at the end of the research. The content of C organic was analyzed by using Walkey and Black methods, while the total content of N organic in compost was analyzed by applying semi micro Kjeldahl method [10].

3. RESULTS AND DISCUSSION

3.1. Laboratory Scale

The laboratory scale research was conducted for 14 days in Department of Environmental Engineering ITS. The reactor was placed in the Workshop. It was conditioned in a dark place, covered by black cloth as BSF larva is a photophobia animal.

3.1.1. Measurement of Larva Growth

The data on weight gain was obtained from observing its water content. So that the weight gain is more valid without the influence of the water content.

In Figure 1, it can be seen that all of mixture composition gain the larvae weight. The mixture of fruit waste and food waste with the composition of 80:20 gain the highest weight of larvae. It also was observed in the mixture of fruit waste and chicken manure with the composition of 70:30. The weight gain was significantly measured on the day 9. During day 12, the weight gain did not show any significant improvement. It shows that BSF larvae have less consumed the waste as they continue to the next phase, called prepupa. The amount of larva weight gain can be seen through the percentage of its weight gain difference.The data of weight gain measurement are presented in Table 2.

The larvae weight gain on the mixture of food waste in this research was higher than the previous research that reach only 18 mg per larva each day [12]. The lack of final weight of the larvae in fruit waste control could possibly be explained by high pH level, exceeding the optimum pH level in which the larvae can live. The food wastes and chicken manure without actually mixing fruit waste can also be a food option to see the weight gain of larvae up to ± 50 times from the initial weight. According to Diener [6], the ideal weight of larvae that can be harvested is 252 mg per larva. This research suggests that only larva in the mixture of fruit waste and food waste as well as food waste control that can meet the ideal weight of larvae to be harvested.

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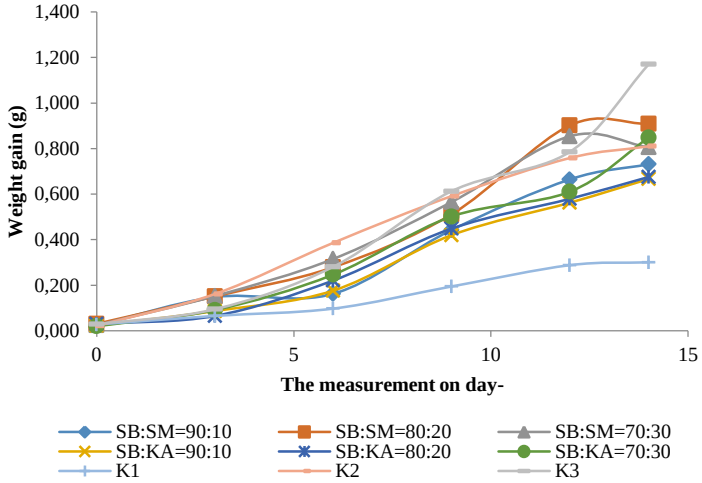

Figure 1. The graphic of larva weight gain

Table 2. Larvae weight gain

The Kind of Waste	Initial Weight of Larva (mg/each)	The Final Weight of Larva (mg/each)	Weight Gain (times)	Weight gain Amount (mg/day)
The Mixture of Fruit Waste and Food Waste				
90:10	7	261	37	18,2
80:20	6	297	50	20,7
70:30	5	260	52	18,2
The Mixture of Fruit Waste and Chicken Manure				
90:10	4	238	60	16,7
80:20	6	220	37	15,3
70:30	4	274	69	19,3
Control 1	5	137	27	9,4
Control 2	4	191	48	13,3
Control 3	5	276	55	19,3

The measurement of protein content of the larva was conducted at the end of the research. The measured larvae are the ones that own highest reduction level in each composition. The protein content of larvae can be seen in Table 3.

Table 3. The protein content of larvae on selected composition

Sample	Protein Content (%)
Larvae of Fruit Waste:Food waste = 70:30	41.49
Larvae of Fruit Waste:Chicken Manure = 80:20	34.15

According to Diener [6], the larvae that can be an alternative of animal food are those which contain protein more than 40%. It means that the larva on the mixture between fruit waste and food waste of 70:30 can be used further as animal food or fish pellets.

3.1.2. Measurement of Waste Reduction Level

The percentage of reduction is based on the amount of samples which are given and taken on dry weight. The calculation of waste reduction by BSF larvae are given in Table 4.

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Table 4. The percentage of waste reduction on laboratory scale

Sample	Reduction Percentage (%)
Fruit waste 100	69.1
Chicken Manure 100	50.6
Food Waste 100	52.2
Fruit waste: food waste = 90:10	73.8 ± 4.2
Fruit waste: food waste = 80:20	72.1 ± 0.8
Fruit waste: food waste = 70:30	82.9 ± 2.5
Fruit waste: Chicken manure = 90:10	62.0 ± 1.7
Fruit waste: Chicken manure = 80:20	62.1 ± 1.0
Fruit waste: Chicken manure = 70:30	61.8 ± 1.4

The mixture of fruit waste and food waste with ratio 70:30 achieves the highest reduction percentage value with 82.9%. The mixture of fruit waste and chicken manure with ratio 80:20 has the highest reduction percentage value with 62.1%. The percentage of mixture of fruit waste and chicken manure was lower than the reduction percentage of fruit waste control. Therefore, it can be concluded that the mixture of fruit waste without chicken manure is able to be reduced well by BSF larvae.

The reduction percentage obtained in this study was higher than in the research conducted by Zakova an Borkovcova [13], i.e., the reduction percentage of household waste and garden waste with 64% by BSF larvae in laboratory scale. Furthermore, another result from Newton et. al. reports reduction percentage value with 56% [14] by using cow manure and with al least of 50% reduction in various kind of manure. Another study reports reduction percentage on the mixture between chicken manure and coconut oil with up to 49.5% [15].

3.1.3. Measurement of Waste Residue Characteristics

The parameters consist of temperature dan waste pH, the water content of waste and C/N ratio. The measurement of temperature and the pH of waste was conducted every day. The measurement was done towards the residue of the reactor before feeding process. According to the measurement of temperature, the maximum temperature was 31^{o}C of chicken manure composition. The composition of 100% fruit waste and the mixture of fruit waste and food waste with ratio of 70:30 have minimum temperature, i.e., 28^{o}C. Overall, the sample of waste in the reactor has similar temperature with room temperature. Therefore, the BSF larvae are in preferable condition of living. The maximum temperature of BSF larvae can live is 45^{o}C [16].

The pH on the first day was decreasing. After several days, it increases constantly to 7 of pH. The increasing number of pH was caused by the synthetic process of protein done by microorganism. On the other hand, the decreasing number of pH was caused by the release of protein content, peptide and amino acid from degradation process [17]. The optimum degradation process by larva is in the range of pH 5.0-8.0 [16]. The composition that has pH number out of the optimum range is still possible to have degradation process. The larva can survive on the lowest pH (pH= 2) and the highest pH (pH= 9) [12]. The pH condition during the research have no influence towards the life phase of larvae, proven by the capability of the larvae to survive.

The fruit waste control have the higher water content compared to food waste control. The highest water content is on fruit waste control with 97% on the day 14. On the other side, the lowest water content was on the food waste control with 50% on the day 5. The water content on the day 5 and 14 in several reactors was in fluctuation. İt happened as the water content was too low on each food samples.

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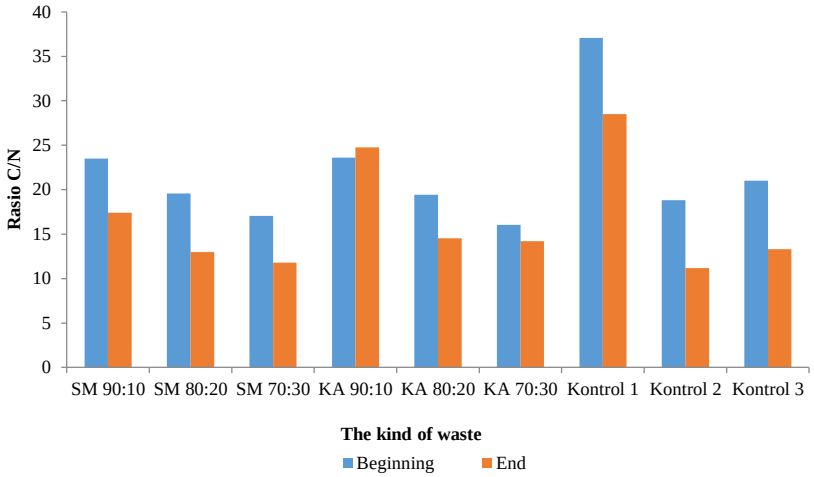

Figure 2. The comparison graphic of C/N ratio from start to final

The result of C/N measurement shows that all reactors significantly undergo the decreasing of C/N ratio because of waste conversion process as the existence of BSF larva and bacteria activities in the waste samples [6]. According to Surtinah [18], the lowest C/N ratio of compost is in 9.97 with 10.5 % of C-organic percentage and 1.05% of TKN percentage. This final result signifies that the C/N ratio meets the standard to make the residue of BSF larvae as compost. However, it is needed a further process to decrease the water content.

3.2. Pilot Scale

According to the result of laboratory scale research, the mixture of fruit waste and food waste with ratio 70:30 produces the highest waste reduction percentage. This suggest that the composition can be further studied in pilot scale research in Puspa Agro market, Sidoarjo. The amount of the reactors were 36, divided in 12 reactors for fruit waste, 12 reactors for the mixture of fruit waste and food waste 70:30 and 12 reactors for food waste.

3.2.1. Measurement of Larva Growth in Pilot Scale

The 14 days research shows that the larva in the reactor gain more weight in dry condition. The result of larva growth measurement can be seen in Table 5.

Table 5. The Weight Gain of Larvae in Pilot Scale Reactor

Types of Waste	The Initial Weight of Larvae (mg/larvae)	The Final Weight of Larva (mg/Larvae)	Weight Gain (times)	Weight Gain (mg/day)
Fruit waste	6	174	29	14,0
Mixed wste	6	268	45	18,7
Food waste	6	303	51	21,2

According to Table 4, the larvae from all reactors tend to gain weight more than ten times from the initial weight. BSF larvae in food waste reactor have the higher weight gain with 51 times from the initial weight and 21.2 mg day^{-1} for each larvae. There was approximately 68% of larva in food waste reactor that have turned to prepupae phase. This is because food waste have more protein content compared to the other types of waste [15]. From the three types of larvae, there was only one from fruit waste that obtain weight less than 252 mg. Therefore, it can be said that the larvae of food waste and mixed waste are classified to the ideal weight of larvae to be harvested (>252mg per larva) [5]. The result of protein content shows that the larvae from food waste have the highest protein content with 50.9%. On the other hand, the lowest protein content measured by

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the larva from fruit waste with 28.1%. The result of data measurement can be seen at Table 6.

Table 6. The protein content of larva in pilot scale

Types of Reactor	The Protein Content of Larvae (%)
Fruit waste	28,1
Mixed waste	42,6
Food waste	50,9

3.2.2. Measurement of Waste Reduction in Pilot Scale

Based on Table 6, the mixture of fruit waste and food waste achieve the highest reduction percentage with 57%. Fruit waste (100%), as the control, obtains the lowest reduction percentage with 24.3%. It can be concluded that the use of food waste as larva food option have more effective result.

Table 7. The percentage of waste reduction in pilot scale

Types of Waste	Reduction Percentage (%)
Fruit waste 100	24,3
Fruit waste:Food waste = 70:30	57,3
Food waste 100	51,3

3.2.3. Characteristics of Waste Residue in Pilot Scale

The measurement of pH and temperature was conducted for 4 days based on the feeding schedule. The measurement result of temperature and pH reactor in pilot scale can be seen in Figure 3 and 4, respectively.

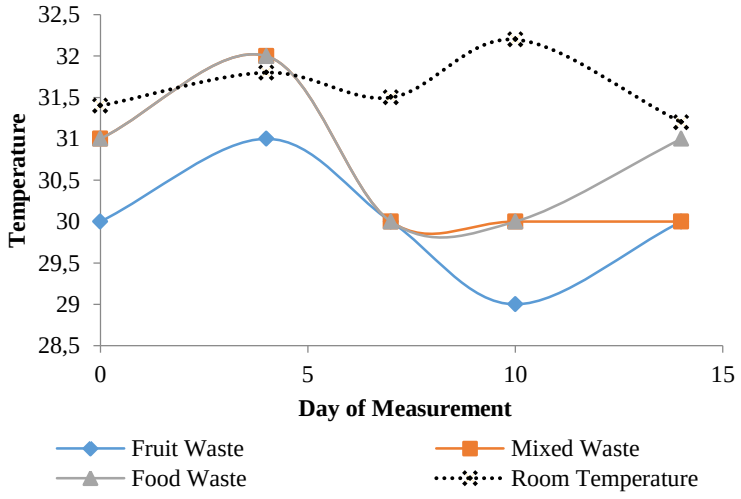

Figure 3. The graphic of temperature measurement in pilot reactor

Based on the measurement of day 10, fruit waste was on the lowest temperature with 29°C. On the day 4, both the mixed waste and food waste were on the highest temperature with 32°C. The temperature of all reactors are the same with room temperature. Based on Figure 4, the pH of three wastes increase, from pH 4-5 to pH 6-7. The pH changes because of the presence of some compounds such as NH_4^+ and humic

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acid. Humic acid decreases the pH, while on the other hand NH_4^+ increases the pH [19]. It can be said that the increasing pH occurs when NH_4^+ dominates the content of waste. In composting process, pH is set no more than 8.5 in order to keep the existence of Nitrogen in the form of ammonia [17]. The measurement of water content of waste in the reactor was conducted on day 0, 4, 7, 10 and 14. The *trend* result of water content measurement of waste in the reactor can be seen in Figure 5.

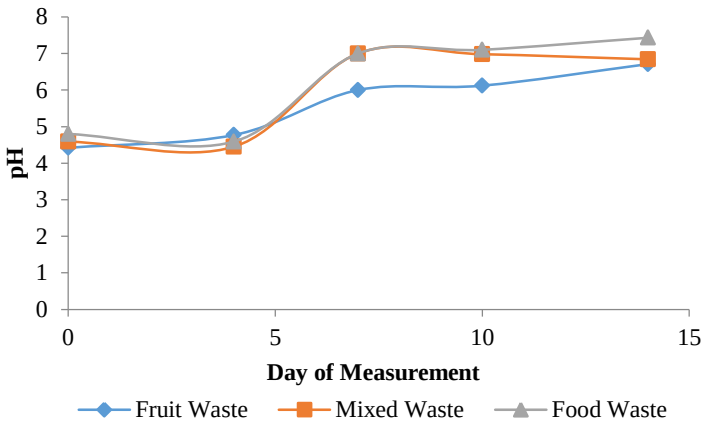

Figure 4. The graphic of pH measurement in pilot reactor

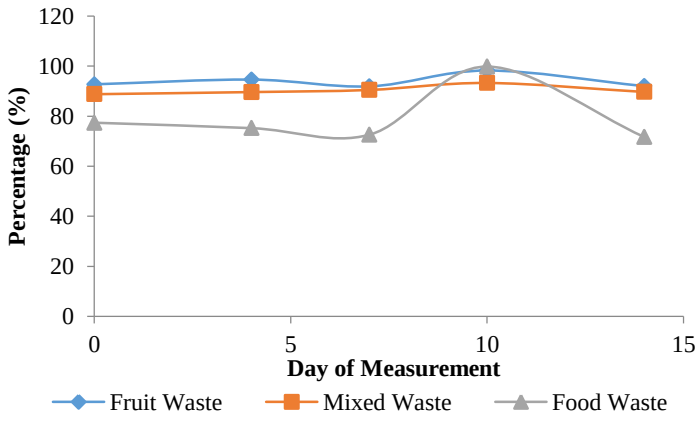

Figure 5. The trend graphic of water content measurement in pilot scal

The residual waste that has high water content creates the harvesting process more easily compared to waste with low water content waste. Residual waste with high water content can easily cause the larvae fall to the crop, while in the low water content, the larvae do not descend and remain hidden beneath the residue. The result of comparison between the initial C/N ratio and C/N residue ratio can be seen in Table 7.

Table 8. The result of C/N ratio measurement in pilot reactor

Types of Waste	The Initial C/N Ratio	C-organic (%)	TKN (%)	The Final C/N Ratio
Fruit waste	12.7	50.6	2.8	18.2
Mixed waste	15.3	46.1	3.7	12.4
Food waste	16.7	42.3	3.9	10.9

The final C/N ratio from mixed waste was lower compared to the C/N ratio measured in the case of laboratory scale, i.e., 12.4. It happens because laboratory scale have better control on the temperature condition as well as the humidity than in pilot scale

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study. However, it is still possible that the residue can be used as compost by conducting further treatment to decrease the water content of the residue.

4. CONCLUSIONS

The addition of mixture on laboratory scale research resulted in the highest reduction percentage as well as the weight gain on the mixture of fruit waste and food waste (70:30) with 82.9% each. The weight gain was also up to 52 times from the initial weight. Furthermore, the result of protein content measurement of remained larvae inside the reactor wa 41.49%. Therefore, it can be concluded that the addition of food waste is an effective way to improve waste reduction.

The selected composition was then applied for pilot scale study in Puspa Agro market, Sidoarjo. The percentage of waste reduction reached up to 57.3% and the larva weight gain becomes 45 times bigger than the initial weight with the protein content of 42.64%. The reduction percentage and the weight gain in the pilot scale was lower than the results in the laboratory scale.

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Evaluation of Anaerobic Digester Facility for Biogas Production in SIMANTRI Cattle Farm in Seririt District, Buleleng, Bali, Indonesia

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Abstract

SIMANTRI, which stands for integrated farming system, is a program of Bali Province Government that integrates agricultural and cattle farm activities. One of the integrated activities is the processing of solid waste from the cattle farms into biogas. The anaerobic digester capacity in each SIMANTRI project was not large enough for treating all of the daily solid waste generated by each cattle farm. Therefore, evaluation of the anaerobic digester facility of the SIMANTRI project is required. This study aimed to evaluate anaerobic digestion facility and to recommend a more proper anaerobic digestion design recommendation that could be implemented in the SIMANTRI project in Seririt District.

Data of faecal waste generation rate, digester capacity and design, and operational problems encountered during biogas production were collected in the cattle farms facilitated by the SIMANTRI projects. These data were used for evaluating the performance of the digester, and to recommend a proper design of digester for the cattle farms.

The faecal waste generation rate of each cattle farm was 160.16 kg per day or 7.28 kg per cow.day. The anaerobic digester was redesigned for low solid faecal waste condition. The redesign resulted in a hemispherical form of digester with a dimension of 2.48 m diameter and 1.24 m height. The outlet part was proposed to have a dimension of 0.8 m diameter and 0.24 m height, and to be connected with a pipe to the digester. A total of 4 new designed digesters should be provided for treating the faecal waste of each cattle farm with 22 cows.

Keywords: Anaerobic digester, cattle farm, faecal waste, redesign, SIMANTRI

1. INTRODUCTION

Biogas is a mixture of gases, which is produced from biodecomposition of faecal waste by microorganisms in the absence of oxygen or anaerobic process [1, 2]. Biogas productivity from cow manure fermentation is 0.24 m^3/kg of faecal waste, and 0.20 to 0.30 methane gas m^3/kg volatile solids [3-4].

Common technology for obtaining biogas fermentation of faecal waste is by using anaerobic digester [5]. The biogas formation process in anaerobic digesters consists of four stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis [6, 7].

There are several types of biogas reactors that have been developed, namely, fixed dome, floating drum, balloon, horizontal, soil hole, and ferrocement types of reactors. From the six types of biogas digesters, the fixed dome and floating drum types are widely used. However, in recent years, the balloon type has been developed and become widely used as a simple small scale reactor [8].

Integrated Agricultural Systems, which is known as SIMANTRI, has been a model of regional agricultural development in the Province of Bali, Indonesia.

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Implementation of SIMANTRI programs is aimed to conduct agricultural activities with zero waste approach, by producing food, feed, fertilizer and fuel (4F). One of the integrated activities is the processing of solid waste from the cattle farms into biogas. There are 13 SIMANTRI projects in Seririt District of Buleleng town. Each SIMANTRI project manages one cattle farm with 22 cows in average. Only 4 of the anaerobic digesters from all SIMANTRI projects worked. The biogas product has been currently used for limited cooking and lighting for one householder only. Besides, the anaerobic digester capacity in each SIMANTRI project was not large enough for treating all of the solid waste generated daily by each cattle farm. Therefore, evaluation of the anaerobic digester facility of the SIMANTRI project is required. This study aimed to evaluate anaerobic digestion facility and to recommend a more proper design anaerobic digestion recommendation that could be implemented in the SIMANTRI project in Seririt District.

2. MATERIAL AND METHODS

Stages in this study comprised research preparation, data collection, and evaluation ofexisting biogas production. Each stage of this research is described below.

2.1. Research preparation

The initial survey, which was aimed to identify existing condition of biogas reactors, was conducted inSeririt District. Then5 representative cattle farms receiving SIMANTRI Project were selected from a total 13. These selected cattle farms were: Suka Jaya Makmur, MayongPengulkulan, EkaSanthi, Sari Mekar, and Madu Amerta. A questionnaire for data collection on existing faecal waste treatment for biogas processing and the encountered problems was prepared. The toolsfor conducting this research, which comprised scales, plastic bags, gloves, shovels, and 40 L volume measure, were also prepared in this stage.

2.2. Data Collection

The faecal waste generation rate was measured according to the Indonesian National Standards (SNI) 19-3964-1994 concerning method for sample collection, measurement urban waste generationand composition [9]. The faecal waste generation rate was conducted in eight consecutive days in each selected cattle farm. Amount of the faecal waste was measured using a weighing balance. The waste density was calculated according to the SNI method.

Data of existing design and capacity of the existing anaerobic digester, biogas processing performance, and the encountered problems were obtained from each farm personnels and fom direct observation.

2.3. Evaluation of Existing Biogas Processing

The estimated maximum gas production per kg of faecal waste is 0.04 m^3/kg [10]. The raw material feed per day was calculated by using equation (1), whereas the maximum gas production per day was calculated by using equation (2) [10].

$$\text{Daily feeding} = \frac{\text{Active Slurry Volume}}{\text{HRT}} \times \text{Density of feeding} \quad (1)$$

$$\text{Maximum gas production per day} = \text{feeding per day} \times \text{gas produce per kg faecal waste} \quad (2)$$

Data of faecal waste generation in each farm per day were used to redesign the existing digester. The type of process in the new design was low solid anaerobic digestion. The type of anaerobic digester that was applied in the farm was fixed dome anaerobic digester. The redesigned digester consisted of an inlet tank, an anaerobic digester and outlet tanks. This redesign was adapted to be able to overcome the problems

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occured in the application of existing digester. The redesigned biogas digester was recommended to be applied in SIMANTRI cattle farms in Seririt District.

3. RESULTS AND DISCUSSION

3.1. Faecal Waste Generation Rate

Measurements of faecal waste generation rates were done from 24 February to 30 March 2016.The average faecal waste generation rates in Suka Jaya Makmur, Mayong Pengulkulan, Eka Santhi, Sari Mekar, and Madu Amertacattle farms were: 7.50; 7.37; 7.10; 6.99; and 7.45 kg/cow.day respectively. Based on thethese results, the average faecal waste generation rate from the cattle farms was 7.28 kg/cow.day. The estimated total volume of each cattle farm varied in Figure 1. Considering that the average number of cows in each farm was 22 cows, the assumed faecal waste generation rate in each cattle farm was 160.16 kg/day.

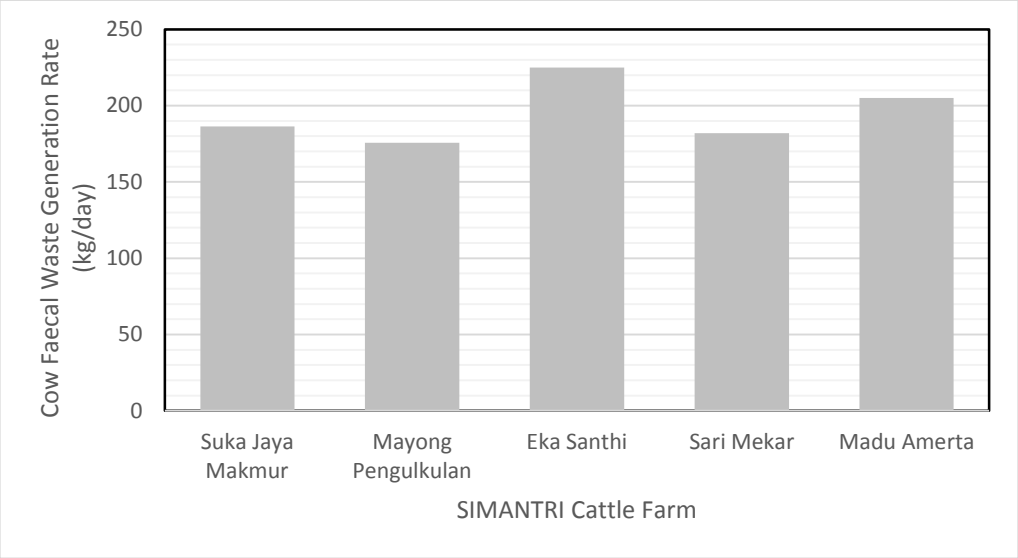

Figure 1. The Estimated Total Volume of Each Cattle Farm

3.2. Evaluation of Existing Biogas Processing

Suka Jaya Makmur and Mayong Pengulkulan cattle farms are the only farms which operated the biogas digesters. The other cattle farms did not use the digesters. The biogas product was used for stove need, with operation durations of 1 hour 15 minutes and 1 hour 21 minutes respectively in Suka Jaya Makmur and Mayong Pengulkulan cattle farms. The biogas was also used for lighting purpose withan average duration of1 hour 44 minutes. The main reasons of Eka Santhi, Sari Mekar and Madu Amerta cattle farms in not producing biogas were the limited processing capacity of the digesters, lacked in biogas storage facility, and poor condition ofinfrastructure.

Supporting biogas processing facilities in each cattle farm consisted of pipeline, a desulfurizer tube, a biogas stove, and a biogas lamp. Hydrogen sulfide gas might impurify the biogaswith 0-0.5% v/v. This gas might cause corrosion to biogas equipment and pipeline, which were made of metal [11]. Therefore, a desulfurizer unitshould be installed in the end of the pipeline.

The existing biogas digester in each farm had an average capacity of 4 m^3. The dimensions of existing anaerobic digester were 2.2 m diameter, 0.8 m height, and 0.65 m dome height.Type of the existing anaerobic digester was fixed dome anaerobic digester. The existing active slurry volume was 3.042 m^3 and the gas storage volume was 1.338 m^3. Thus, the anaerobic digester capacity at existing condition was 4.380 m^3. The feeding rate was 76 kg per day, with a composition of 38 kg of faecal waste and 38 kg of water.

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With a maximum gas production 0.04 m^3/kg faecal waste and daily feeding of 38 kg of faecal waste, a maximum gas production of 1.52 m^3 could be generated. Therefore, with the current faecal waste generation rate of 160.16 kg/day and faecal waste feeding rate of only 38 kg/day for a digester, the existing digester facility could not accommodate all faecal waste generated per day. The digester was equipped with an inlet pipe of 110 mm in length, and the outlet tank of 1.48 length and 1.2 m width.

A new design of digester was proposed with a capacity of 4 m^3, which would be sufficient for treating faecal waste of 5 cows. İf there were 22 cows in each cattle farm, at least four digesters should be provided. The proposed digester processing was fixed dome type. With average faecal waste generation rate of 7.28 kg/cow.day, the daily faecal waste feeding rate would be 5 cows x 7.28 kg/cow.day = 36.4kg/day. Since low solid anaerobic digestion system would be applied, it was necessary to add water in order to meet total solid (TS) concentrations of 4-8% [12]. The amount of water, which should be added for reaching total solids concentration of 8% was 36.4 kg/day. Thus, the feeding rate of faecal waste and water mixturewas 72.8 kg/day.

Using hydraulic retention time (HRT) of 40 days in mesophilic condition, and feeding mixture density of 1000 kg/m^3, the digester would have an active slurry volume of 2.912 m^3. With faecal waste feeding rate of 36.4 kg/day and biogas production of0.04 m^3/kg faecal waste, the estimated biogas production generation rate would be 1.456 m^3/day. The biogas storage capacity should be designed for more than 50% of maximum gas production generated per day [10]. Thus, with gas storage capacity of 60% of the maximum daily biogas production, the volume of the gas storage space would be 0.874 m^3. In addition, a vacuum space in an anaerobic digester should be provided with 0.2 - 0.3 m^3 volume [10]. This vacuum space was needed for anticipating the high water level (HWL) of slurry, so that the slurry would not overflow and clog the gas pipes [10]. The proposed volume of vacuum space in the digester was 0.25 m^3. Thiscouldcope with the gas flow blockage problem in the pipeline as occured in the existing condition. The total capacity of anaerobic digestercapacity was 4.036 m^3.

The new design digester was of hemispherical form with 2.48 m diameter and 1.24 m height. Inlet tank of 0.5 m diameter and 0.6 m height was connected using an inlet pipe of110 mm diameter tothe anaerobic digester. Height ofthe inlet tank for HWL condition and overflow outlet was 0.15 m. Accordingly, height of the inlet pipe from the bottom of the digester was 0.3 m with a slope of 60^0. The outlet part was proposed to have a dimension of 0.8 m diameter and 0.24 m height, and to be connected with a pipe to the digester. This redesigned digester facility was expected to ease slurry displacement from the digester to the outlet in the biogas pressure of 0.26 m water column. A total of 4 new designed digesters should be provided for treating the faecal waste in each cattle farm with 22 cows. The new design of fixed dome anaerobic digesteris shown in Figure 2 - 4.

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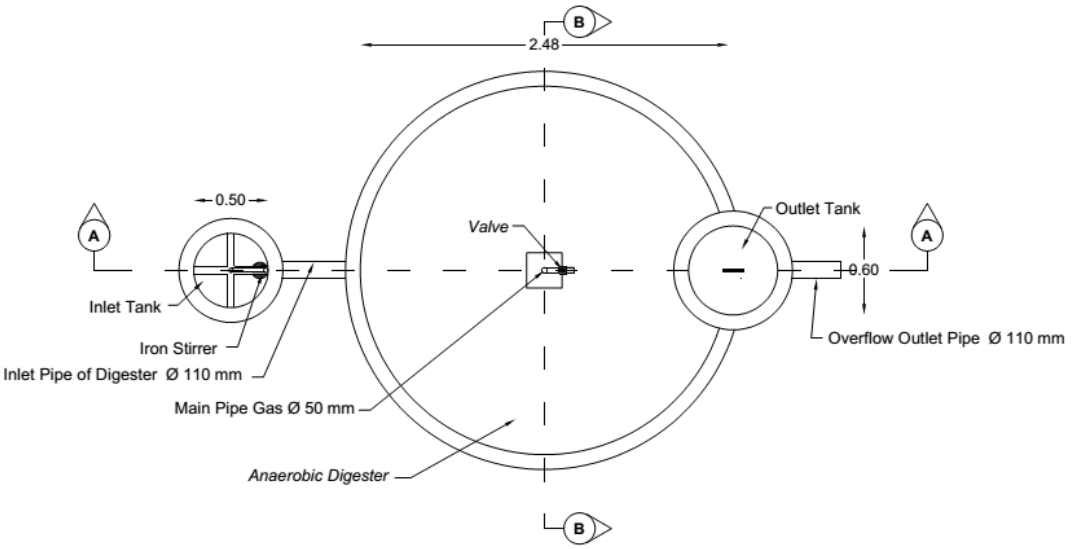

Figure 2. Layout of Redesigned Anaerobic Digester

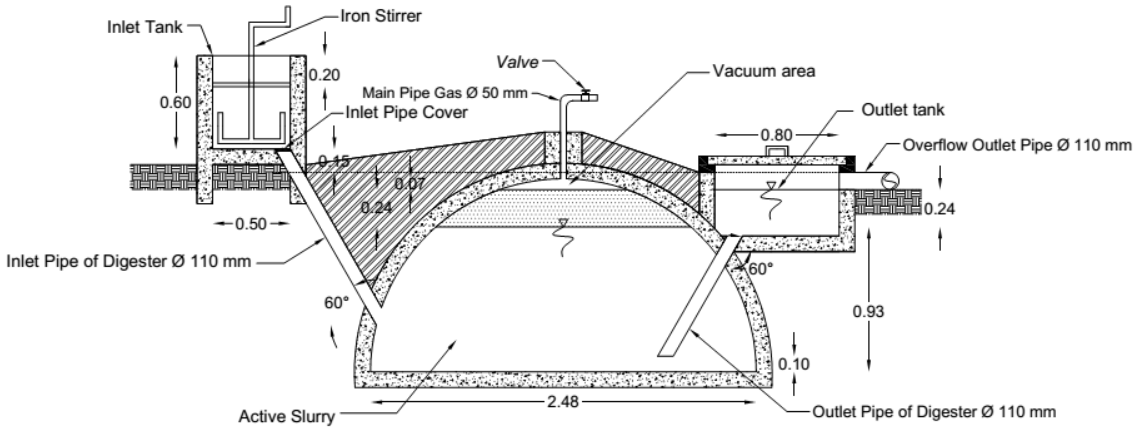

Figure 3. A-A Section of Redesigned Anaerobic Digester

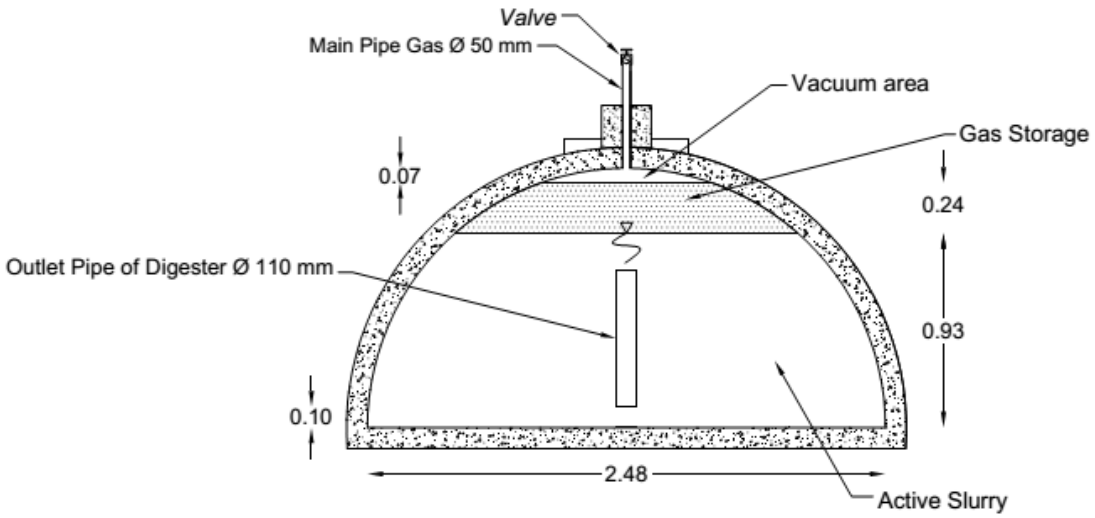

Figure 4. B-B Section of Redesigned Anaerobic Digester

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4. CONCLUSION

The average faecal waste generation rate of each cattle farm was 160.16 kg per day, or 7.28 kg per cow.day. The currently used digester had a dimension of 2.2 m diameter, 1.45 m height, or 4.38 m^3 capacity. The digester was equipped with an inlet pipe of 110 mm length, and outlet tank of 1.48 length and 1.2 m width. The anaerobic digester was redesigned for low solid faecal waste condition. The redesign resulted in a hemispherical form of digester with a dimension of 2.48 m diameter and 1.24 m height. The outlet part was proposed to have a dimension of 0.8 m diameter and 0.24 m height, and to be connected with a pipe to the digester. This redesigned digester facility was expected to ease slurry displacement from the digester to the outlet in the biogas pressure of 0.26 m water column. A total of 4 new designed digesters should be provided for treating the faecal waste in each cattle farm with 22 cows.

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