Review Article **Asian J Agri Biol, 2013, 1(3):155-163.**

MARINE MACRO ALGAE *ULVA*: A POTENTIAL FEED-STOCK FOR BIO-ETHANOL AND BIOGAS PRODUCTION

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ABSTRACT

Present industrialization and global mobility era is an energy intensive period of the human history with an ever increasing energy demand. Unfortunately, fossil fuels are no more sustainable due to growing gap between the demand and supply. Biofuels are considered as promising alternative liquid fuels in recent global energy scenario. Food crops and ligno-cellulosic plant biomass have been widely studied as an alternative feedstock for biofuels production. After decades of research, the competition of fuel with food and recalcitrant nature of plant biomass, these feed stocks are losing their popularity. Marine macroalgae have come forward as another potential feedstock for biofuels production. Marine algae have several advantages over the traditional energy crops including absence of lignin, higher growth rates and no competition with human food. Moreover, along with several environmental benefits, they can be grown using saline and waste water and have higher abilities to sequester the atmospheric CO_2 than traditional energy crops. Although there are several challenges associated with the algal biomass conversion to bioenergy yet these problems can be overcome using integrated biorefinery approach.

Keywords: Ligno-cellulosic biomass, Marine algae, Alternative feed stock, Bioenergy production

INTRODUCTION

The present instability and increasing prices of petroleum based fuels have assertively driven the development of alternative energy sources (Goldemberg and Guardabassi, 2009). Ligno-cellulosic plant biomass is widely studied alternative and is composed of mainly cellulose, hemi-cellulose and lignin (Wright, 2006). However, the extensive production of bio-ethanol from cellulosic biomass is slowed down by several scientific and environmental issues, such as deforestation, loss of biodiversity, lower energy output/input-balance and recalcitrance nature of lignin present in plant biomass (Hill et al., 2006; Goldemberg 2007). Researchers have focused to marine biomass because the oceans are home to 90 % of global photosynthesis, as an alternative feed stock. Moreover, marine biomass has no competition with agricultural food and feed production (Ray and Lahaye, 1995; Demirbas, 2007). Interestingly, algae have the advantage of having no lignin and very low hemi-cellulose levels, which results in an increased hydrolysis and/or fermentation efficiency (Kloareg and Quatrano, 1988). The use of marine biomass as an alternative feed stock for bio-ethanol and biogas production could also reduce environmental problems in the sea because some sea pollutants could be utilized as bio-ethanol biomass (Choi et al., 2012).

Macroalgae have shown to contain more than 2400 natural products of pharmaceutical, biomedical and nutraceutical importance. Moreover, they have been widely used in human and animal food preparations due to the presence of higher proportions of polyunsaturated fatty acids (PUFAs), sugars, vitamins, minerals, and dietary fibers (Munro and Blunt, 1999; Chandini et al., 2008; Kumari et al., 2010). Recently, the interest in algal resources have been renewed all over the world for their highest yield potential (Georgianna and Mayfield, 2012) and even higher hydrolytic efficiency than agricultural

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plant biomass (Choi et al., 2012), that may also triumph over the hullabaloo of ''*fuel versus food*''. They are believed to have relatively higher production turnover, easy depolymerization of biopolymers (mainly carbohydrates) along with greater carbon sequestration potentials than terrestrial crops (Lee et al., 2011; Kumari et al., 2011). Although, algal potential genetic pool is much larger than that of animals or land plants yet this pool has only recently been explored for fuel production, which includes green algae, diatoms and cyanobacteria (Georgianna and Mayfield, 2012). This mini-review presents the most recent studies on marine algae (seaweed) exclusively the species studied from genus *Ulva* for the evaluation of its potential as an alternative feedstock.

Ethanol production

Higher growth rate, lower cost and biomass properties (moisture, ash, alkali and sugar contents) are the principal selection criteria for selection of promising energy crops (McKendry 2002; Bruhn et al., 2011). Although the strains vary among different regions yet the *U. lactuca* is common from tropical to polar climates. Regardless of reports growth rates (under natural conditions) up to 30% d^{-1} in northern temperate regions (Pedersen and Borum, 1996), cultivation has become possible only in warmer regions of lower latitudes. Few species have previously been harvested from natural populations in shallow coastal areas (Cecchi et al., 1996) or cultivated in land based systems (Gao and McKinley, 1994; Neori et al., 1991; Msuya and Neori 2008; Robertson-Andersson et al., 2008).

The biochemical composition of macroalgae depends strongly on season and the growth conditions (Black 1950; Lamare and Wing 2001). *U. lactuca* has a total solid (TS = dry matter) content between 9.6% (Msuya and Neori, 2008) and 20.4% (Lamare and Wing, 2001). Whereas, the TS comprises of 62% carbohydrates, 27% protein and 0.3% lipids (Ortiz et al., 2006), but the protein content can surpass 40% if external nitrogen loads are high (Msuya and Neori, 2008). Conversion of *U. lactuca* into biofuels has been described to a limited extent and primarily as substrate for anaerobic digestion to biogas. Fermentation of *U. lactuca* carbohydrates into bioethanol for automobiles would be advantageous as the transport sector has problems with reducing its CO_2-emmisions. However, preliminary results on fermentation of *U. lactuca* and nine other species of green macroalgae to ethanol have shown relatively poor yields (Isa et al., 2009).

The green alga *U. lactuca* (Chlorophyceae) has been evaluated as a potential feedstock for energy production in USA since 1978 because of its high growth rate and high sugar contents (Bruhn et al., 2011). Later, it was concluded that use of *U. lactuca* as aquatic energy crop is not economically sustainable. However, the climate change agenda has caused a growing interest in renewable and CO_2 neutral energy sources, which has increased the pressure on traditional biomass resources. Land based resources are limited and have been used for food production and energy leading towards the identification of alternative, sustainable energy resources such as aquatic biomass. It brought macroalgae (particularly *Ulva* sp. due to their higher sugar contents) such as *U. lactuca* back in focus as an alternative energy source (Bruhn et al., 2011). In a recent study, *U. lactuca* has been used to produce butanol (4 gL^{-1}) in the fermentation broth (Potts et al., 2012).

The green alga *U. pertusa* Kjellman is another example. It is a major sea pollutant in the far-east and southeast areas. The alga contains approximately 47.0 % total carbohydrates (on dry mass basis), in the form of several types of polysaccharides, and low levels of cellulose (Kloareg and Quatrano, 1988; Percival, 1979; Zhang et al., 2003; Wang et al., 2008) . So, the hydrolysis process for marine biomass is different from conventional pretreatment techniques for the saccharification of cellulosic materials depending on the type and composition of biomass. Thermochemical treatment using dilute acid with or without mechanical disruption, ammonia pretreatment followed by cellulase treatment are the most widely used hydrolysis processes for lingo-cellulosic biomass (Palmqvist and Hahn-Hagerdal, 2000). The acid and/or alkali treatment at moderate temperatures (100–120 °C) can digest only hemicelluloses, so the cellulose must be further hydrolyzed by cellulase treatment for efficient conversion of polysaccharides to fermentable sugars (i.e.

glucose) (Palmqvist and Hahn-Hagerdal, 2000; Zhu et al., 2009). However, it has been found that these treatment methods cannot effectively hydrolyze several kinds of algae due to either their different cell wall composition or due to the presence of complex sulfated polysaccharides (Kloareg and Quatrano, 1988; Percival, 1979; Choi et al., 2012). It is interesting to note that high-temperature liquefaction (HTP) coupled with high pressure has shown relatively efficient hydrolysis of algae into glucose (Choi et al., 2012). Subsequently, it can help to increase fermentation efficiency to produce alcohols (Klinke et al., 2004; Choi et al., 2012). This strategy is believed to achieve an approximately 90 % of the maximum theoretical ethanol yield (Choi et al., 2012). So, the perception "*macroalgae are not suitable for ethanol production*" may be re-considered. Most recently, hydrocarbon producing genes were transferred from *Botryococcus braunii* into kelp *Macrocystis pyrifera* to get higher biofuels yield, successfully (Petcavich, 2009; Kumari et al., 2011).

Biogas production

Although liquid biofuels are mainly used for transportation yet gaseous fuels (natural gas) are also an alternative fuel option for vehicles (Smyth et al., 2010). Macroalgae can be converted to biofuels by various processes including thermal treatment and fermentation (Lam et al., 2010) but the most direct route to obtain energy from macroalgae is via its anaerobic digestion (AD) to biogas (~ 60% methane). The biochemical conversion pathway is an anaerobic digestion of biomass (usually in the form of liquid or paste-like substrates) by methanogenic bacteria, producing a mixture of gases containing approximately two-thirds CH_4, one-third CO_2, water vapors and some impurities. This process is well established and is commercially available (Demirbas, 2009; Ryckebosch et al., 2011). Regarding the utilization of algae, it is possible to use it as a substrate because feeding wet biomass to digestion is the one general advantage in using algae as a substrate. Thus, algal biomass is required to concentrate only instead of complete drying out but in wet-fermentation systems concentrations should not exceed 5% on DM biomass basis. It has shown that digestion of algae for biogas production is suitable and the yield depends on the selected algal strain and the method of pretreatment chosen (Mussgnug et al., 2010; Kroger and Franziska, 2012).

Co-digestion of algae together with other substrates is another perspective and the biogas yield may be enhanced using this integrated approach (Yen and Brune, 2007). Because, sometimes protein content of the algae used may be higher leading to production of high ammonium concentrations in the biogas sludge and may lead to toxicity inhibition to reactions (Salerno et al., 2009). So, this problem could be overcome by adding organic substrates with low protein content (Mann et al., 2009). However, the daily and seasonal fluctuations of the (phototrophic) algae production is a drawback of this combined application to the biogas production on sustained basis. The C:N ratio is also an important factor and the argument for the co-digestion of seaweeds with other more N-rich substrates, for instance waste food or agricultural slurries. Biogas yield also depends on wide range of other variables such as inoculum, digester system and feed stock composition. Overall, the biogas production process has an advantage in the utilization of the whole algal cell and algal blooms from polluted or wastewater (Kroger and Maller-Langer, 2012).

Anaerobic digestion of *U. lactuca* to methane seems more suitable and yields have been reported in the range of 180–330 mL CH_4 g^{-1} of Volatile Solids (VS) depending on the treatment procedure (Bruhn et al., 2011). In 2006, the most realistic estimate of industrial potential of methane production using macroalgae as feedstock was studied (Lewis et al., 2000). A commercial scale 4 stage anaerobic digester was used in this study for over 150 days, with a daily input between 0.2-1.0 tonnes of seaweed and a retention time of 15 to 25 days. An average production of 22 m^3 of methane per tonne wet weight of brown seaweed (*Laminaria* sp.) was measured (Lewis et al., 2000). However, recent studies suggest that there is still potential for further optimization of co-digestion of macroalgae with a more nitrogenous substrate, manipulation of the microbial composition of the inoculums (Xu and Mi, 2011; Hsu and Robinson, 2006; Huber et al., 2007), suitability of the selected strain and improved pretreatment technologies (Kroger and Maller-Langer, 2012).

Non-fermentation technologies for energy production

In addition to fermentation technologies, a number of non-fermentation options for energy production using macroalgae are also available. These include direct combustion to produce heat energy and gasification using pyrolysis where the biomass is converted to gas or liquid (tar) before further downstream processing. The products of such systems can be either utilized in engines or turbines for electricity production or as biofuels for transportation and in bio-refineries to produce high value products (Bruhn et al., 2011). Compared to traditional biomass (such as wood and straw), the thermo-chemical conversion of aquatic biomass is less studied and thermal behavior of macroalgae for combustion and pyrolysis has been described for a few species of brown algae only, primarily *Laminaria* and *Fucus* (Ross et al., 2008; Ross et al., 2009; Bruhn et al., 2011). It was found that ash and alkali contents of macroalgae (such as *U. lactuca*) are the main challenges in the direct combustion. However, application of a bio-refinery concept and integrated systems could increase the economic value of the *U. lactuca* biomass as well as improve its suitability for bioenergy production (Bruhn et al., 2011).

Global scenario and environmental concerns

Keeping in view, the realistic estimate of macro-algal growth (Kraan, 2013) (200 t ha^{-1}) and biogas yield (22 m^3) yielding 171 GJ ha^{-1}, we will need to cultivate macroalgae on enormous scale to make a significant contribution to global bioenergy targets. For example if all of the brown algae currently produced in culture (~6.8 million t year^{-1} (FAO 2000)) is converted to biogas, it would yield almost 5.7 PJ (Peta Joules) of biogas, which can full fill only 0.06% of the UK's total energy demand (9518 PJ) for the year 2010 (Hughes et al., 2013). To meet 1% of UK total energy demand, it would require an area of cultivation of almost 5440 km^2 which is half of the total global aquaculture production area. However, it accounts for only 3% of the UK territorial waters (161200 km^2). Interestingly, if we use maize to meet 1% of the UK total energy, it would require a land area of 7700 km^2 (equivalent to 18% of the UK's total cropland area 45000 km^2) (Hughes et al., 2012). So it reflects that although, use of macroalgae for bioenergy production does not seems attractive yet comparative studies clearly demonstrate the potential advantages of biofuels production using marine algae instead of traditional feedstocks (Hughes et al., 2012). This perception is supported by similar calculations for China, Scotland and Japan (Yokoyama et al., 2008; Zhang et al., 2009; Hughes et al., 2012).

Environment is another vital element of concern. Large scale cultivation of algae may change local hydrodynamics and sedimentation patterns, increased organic matter supply, changes in water column nutrient availability and can from shading of the sea-floor (particularly in shallow sites). Although, some types of positive interactions are also being anticipated (Hughes et al., 2013) yet the extent and nature of interactions with fish, cetaceans, birds and overall marine environment must be studied in details (Cromey and Nickell, 2009). Moreover, in any event, nutrients taken up by algal culture for biofuels production would be far less than that produced by agricultural, urban sources and fin-fish aquaculture. The digestates produced after anaerobic digestion during biogas production are typically higher in ammonia and lower in organic nitrogen (Holm-Nielsen et al., 2009; Hughes et al., 2012) and may be used in fertilizers making its way back into the hydrological cycle. On the other side, there may also be a number of positive benefits associated with large scale cultivation. (1) The macro-algal farms can enhance effectively less destructive fisheries within the cultivation zone and can provide spill over benefits to adjacent water reservoirs (Frank et al., 2012). (2) The crop is not removed completely at the end of the cycle it will offer a sanctuary to enhance local biodiversity. (3) The digestate after anaerobic digestion may be valuable depending upon a number of factors including the contaminants and the mixing of macroalgae with other organic waste streams in the digestor. It is believed that 80% of the nitrogen in the biomass is recoverable as ammonium/ammonia from the liquid supernatant fraction after lipid extraction with 40% bioavailability when applied to soil (Langlois et al., 2012). Similarly, a detailed analysis of the fate of nitrogenous emissions after anaerobic digestion of macro-algal biomass is required. Overall, the global effect of using macro-algal culture for biofuel is likely to be positive and life cycle assessment analyses of biomethane production have shown a 69% reduction in fossil fuel utilization. Offshore cultivation of macroalgae has shown a 54% reduction in greenhouse gas emissions and an enhancement in the marine eutrophication index (Hughes et al., 2012).

Economics of bioenergy production using macroalgae

It is hard to say something about the economic feasibility of biofuels production from macroalgae. However, an analysis based on inshore production suggested that the production costs for microalgal biogas would be competitive with fossil fuels without supplementary subsidy as per current monetary value of natural gas (Hughes et al., 2012) and such production costs are difficult to achieve. In addition the identification and extraction of higher value products, prior to anaerobic digestion, is highly recommended. Added value could be achieved by processing part of the cultivated macroalgae for human food or food supplements, to improve the mineral contents of the animal feed, as an organic fertilizer and potential bioactive compounds (Foley et al., 2011; Hughes et al., 2012) of pharmaceutical importance.

Conclusion and future perspectives

The marine alga *Ulva* sp. demonstrates a high biomass yield and a high photosynthetic efficiency compared to terrestrial crops but use of the biomass for combustion represents few challenges due to high contents of moisture, ash and alkali. Anaerobic digestion of the wet biomass to produce methane seems more promising but further improvement in this conversion technology is desired. The economic and environmental sustainability of using seaweed for production of bioenergy would benefit for the bioremediation as well as extracting of high value products from the biomass prior to energy production. Large scale cultivation and biofuels production plants should be designed in different zones of the world using native strains to fully understand the impacts and performance of native macroalgae.

Fig: Different *Ulva* species

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