Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Utilisation of an aquatic plant (*Scirpus grossus*) for phytoremediation of real sago mill effluent

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ARTICLE INFO

Article history: Received 22 April 2020 Received in revised form 1 July 2020 Accepted 8 July 2020 Available online 10 July 2020

Keywords: Aquatic plant Industry effluent Phytoremediation Scirpus grossus Wastewater parameters

ABSTRACT

Phytoremediation technology is one of the most promising treatment methods for wastewater, especially from food and agricultural effluent. In this experiment, sago mill effluent (SME) was treated through phytoremediation. The present study aimed to explore the potential of a native aquatic plant, *Scirpus grossus*, to reduce the concentrations of total suspended solids (TSS), chemical oxygen demand (COD), and biological oxygen demand (BOD) in SME before installing a full-scale system, to ensure the effectiveness of the remedy. Two systems, subsurface batch (SS) and free-surface batch (FS), were applied to select a better system to treat SME using *S. grossus*. After 80 days of experimentation, the findings indicate that the plant can better survive in the SS batch system compared to the FS batch system. Furthermore, the plant can reduce TSS, COD, and BOD by 98, 88, and 93%, respectively. Therefore, it can be concluded that *S. grossus* has remarkable potential in the removal of pollutants in the phytoremediation of SME.

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1. Introduction

Malaysia contributes to the Southeast Asia economy in diverse ways in agro-industries. The sago starch industry is considered to be an important source of food and raw material used around the world. In Malaysia, 24,000 hectares are cultivated with the sago palm (*Metroxylon* sp.), with 32 processing mills (Phang et al., 2000). The annual export of raw sago starch fluctuates between 30,000 and 50,000 tons, to Singapore, Taiwan, Japan, and other countries, contributing profits of US\$3.4 million to US\$10.8 million (Bujang, 2010; Kanakaraju et al., 2019). This industry generates sago mill effluent (SME), waste rich in organic materials (Nururrahmah et al., 2018). It mostly contains macromolecules in the form of polysaccharides (starch and hemicelluloses) (Ling-Chee et al., 2019). Under normal processes of a sago mill (1000 logs per day), about 5% of solids will be generated from 400 tons of sago effluent (COD), biochemical oxygen demand (BOD)

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https://doi.org/10.1016/j.eti.2020.101033 2352-1864/© 2020 Elsevier B.V. All rights reserved.







Initial characterisation of SME.			
Parameter	Unit	Value	Environmental Quality Act (Industrial Effluent) 2009 ^{ab}
pН	_	4.9 ± 0.3	5.5–9
Т	°C	30	40
DO	mg/L	7.36 ± 0.25	-
ORP	mV	-46.4 ± 5	-
COD	mg/L	8920 ± 256	400
BOD	mg/L	965 ± 53	50
TSS	mg/L	937 ± 66	100

^aDepartment of Environment (2010).

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^bFor Unit/pH, '-' means unitless number, whereas the '-' for the Environmental Quality Act column means that no corresponding standard is set.

and total suspended solids (TSS) which are known to reduce the biodiversity and ability of aquatic ecosystems to support life (Jenol et al., 2019).

A significant concern in this regard is improper methods of SME management, which has led to intensive research to seek proper solutions for the sago industry mainly in Malaysia (Yunus et al., 2014). Numerous treatment technologies have been designed and utilised to treat SME, such as anaerobic reactors, bioremediation (Ayyasamy et al., 2008), fluidised bed bioreactors (Rajasimman and Karthikeyan, 2007; Sabri et al., 2018), electrochemical degradation (Sangeetha et al., 2015), and aerobic microbial consortia (Ayyasamy et al., 2002), which are reported to reduce the environmental impact. The main challenge faced by engineers, specifically environmental engineers, and scientists is to establish efficient yet cost-effective technologies for the remediation of industrial effluents, which do not burden industries. Phytoremediation is economical, aesthetically attractive solution for numerous environmental issues around the globe, and also a green technology that has become a prominent universal subject in recent years.

Phytoremediation is a technology that directly utilises macrophytes to clean effluents from contaminated medium. Application of phytoremediation requires identifying plants with potential capability to resist and degrade or adsorb pollutants (Abdullah et al., 2020). The plant species that are used for phytoremediation should be able to absorb high levels of organic and inorganic pollutants and survive in harsh conditions of water pollution. In addition, these plants should successfully grow and spread in wastewater (Darajeh et al., 2014). *Scirpus grossus* is an aquatic plant native to tropical regions. It grows abundantly in Malaysia and is known as 'Rumput Menderong' by local communities. It also has rapid growth and can efficiently enhance the degradation of total petroleum hydrocarbons (Al-Baldawi et al., 2013, 2017; Allamin et al., 2020), shows high removal efficiency of dyes (Almaamary et al., 2017; Abdulqader et al., 2019), demonstrates enhancement of heavy metal (Cr, Pb, Al, Fe) adsorption from the water (Tangahu et al., 2013; Kamaruzzaman et al., 2019, 2020; Ismail et al., 2015, 2019, 2020), exhibits remediation of gasoline (Almansoory et al., 2013), and the plants have also demonstrated their capability to polish recycled paper mill effluent (Yusoff et al., 2019; Ahmad et al., 2017). Thus, *S. grossus* has been widely used with various types of contaminants. In contrast, the application of phytoremediation for the treatment of SME is a new approach, as there are no records on the utilisation of this technology in SME. Therefore, in this present study, *S. grossus* was grown in SME to demonstrate its potential in reducing BOD, COD, and TSS.

2. Materials and methods

2.1. Sago mill effluent (SME) wastewater source

SME was obtained from one of a few manufacturers of sago flour, located in Batu Pahat city in Johor state, Malaysia. It was collected from the main wastewater drain of the sago starch processing mill. Analyses of BOD, COD, and TSS were performed on the basis of standard methods (Table 1) (APHA, 2012). All the parameter analyses were carried out in the environmental laboratory in Universiti Kebangsaan Malaysia (UKM) and compared with the Environmental Quality (Industrial Effluent) Regulations 2009 for wastewater in Malaysia (Department of Environment, 2010).

2.2. Plant source and tolerance study

The plant selection process was to choose a plant species with suitable features for growth under the site conditions, which also met the purposes of phytoremediation. The selected plant should optimally be a native perennial plant that can grow under regional conditions and can tolerate contaminants. *S. grossus* was obtained from Tasik Chini, Pahang, propagated in a greenhouse to produce the first generation, and then transferred into pails. A preliminary test was done with two batch systems, free-surface (FS)and subsurface (SS) batch systems. Four healthy plants of *S. grossus* were planted in each of six pails, each packed with 3 kg of fine sand (Fig. 1). The fine sand used in this experiment, obtained from the



Fig. 1. Tolerance study with Scirpus grossus.

river area, was screened, washed and dried under the sun before planting the plants. To each SS and FS batch system was added 0.8 L and 2 L of effluent, respectively. During the 15-day period of exposure, tap water was added to the plants occasionally to maintain the water close to the original levels and to make sure the plants had adequate water to grow (Al-Sbani et al., 2014; AL Sbani et al., 2015; Ismail et al., 2015). The withered plant percentage was calculated using Eq. (1) (AL Sbani et al., 2015).

Withered plants (%) =
$$\frac{\text{No. of withered plants}}{\text{No. of total plants}} \times 100$$
 (1)

2.3. Experimental set-up for batch phytoremediation run

This experimental study was organised in a greenhouse located at the university, using the SS and FS batch systems (Fig. 2). Eight glass aquariums were used (rather than pail in preliminary test in Section 2.2), and each aquarium was treated in a single exposure. Three replicates were used for each system, and two aquariums were used as controls: (1) contaminants without plants (CC) and (2) plants without SME contaminants (PC). All the aquariums were identical in terms of shape and size (dimensions: $30 \times 30 \times 30$ cm), and each was filled with 10 cm of gravel (diameter: 10-20 mm), followed by 3 cm of small gravel (diameter: 1-5 mm) and then 8 cm of sizeved fine sand.

For the experiment, 12 healthy *S. grossus* (aged 25 days) were planted in each dedicated aquarium. Amounts of 8.5 and 14 L of SME were added to the SS and FS batch systems, respectively. To create an environment similar to a constructed wetland in the SS batch system, the level of water was maintained at the sand surface level, with the height of the water being 21 cm (Al-Baldawi et al., 2013). The water level was maintained with a height of 29 cm for the FS batch system, which is 8 cm above the sand surface. The plants were watered with tap water once a week in order to maintain the wastewater level inside each aquarium, because the wastewater level became reduced as a result of evaporation of the water on hot days.

2.4. Physicochemical properties of wastewater

The batch phytoremediation study was performed for 80 days, and sampling was carried out on the first day and later repeated every seven days. For each sample, the parameters temperature (T, $^{\circ}$ C), oxidation–reduction potential (ORP, mV), dissolved oxygen (DO, mg/L), and pH were recorded to monitor the physicochemical parameter variations in the effluent. Monitoring of pH, temperature and ORP used an IQ150 Multi-probe 5 (IQ Scientific Instruments, UK), and dissolved oxygen was recorded using a Model 63 dissolved oxygen sensor (GLI International, USA) (Al-Baldawi et al., 2013).

2.5. Analysis of COD, BOD, and TSS

For the analysis of water, a sample of 100 mL was collected periodically from each aquarium during the run process. The samples were collected using a water sampling point located five cm above the bottom of each aquarium (Fig. 2). COD analysis was performed using high-range COD digestion reagents (3–150,000 ppm) (Hach, USA). All samples were incubated in the COD reactor series 8000 (Hach, USA) for two hours at 150 °C. After two hours, COD was measured using a portable data logging spectrophotometer DR/2010 (Hach, USA) (Hasan et al., 2016, 2013; Nash et al., 2019).



Fig. 2. (a) Set-up of aquarium for batch test of subsurface (SS) and (b) set-up of aquarium for batch test of free-surface (FS).

BOD₅ was assessed by preparing dilution water with a BOD nutrient buffer pillow (Hach, USA). After incubation for five days at 20 °C in a dark room, the dissolved oxygen was directly evaluated before and after the analysis in cuvettes (Consort C535) (Jouanneau et al., 2014). Meanwhile, TSS was measured at wavelength 810 nm using the DR/2010 spectrophotometer (Hach, USA). The removal efficiencies of the treatment systems (Al-Sbani et al., 2016; Yusoff et al., 2019) were assessed using the following formula, Eq. (2):

Removal efficiency (%) =
$$\frac{C_{\text{inf}} - C_{\text{eff}}}{C_{\text{inf}}} \times 100$$
 (2)

where C_{inf} is the initial parameter concentration, and C_{eff} represents the final parameter concentration.

2.6. Analyses of plant growth parameters

S. grossus was harvested on days 7, 14, 28, 45 and 80. Tap water was used to rinse one plant from each one of the replicates and then smoothly dried using tissue. Wet weight of plants and plant physiological parameters (i.e., stem height and root length) were recorded. Each plant was dried in an oven (Memmert, Germany) at 105 °C for 24 h or until a constant weight was achieved, and its dry weight was recorded (Almansoory et al., 2013). Eq. (3) was used to portray the growth responses of the plants towards contaminants (Ismail et al., 2019; Zhang et al., 2010).

Relative growth rate (RGR) =
$$\frac{\ln W_2 - \ln W_1}{Days}$$
 (3)

where W_1 and W_2 represent the initial and final dry biomasses (g) of *S. grossus*, respectively, and the units for RGR are g g⁻¹ d⁻¹.



Fig. 3. Physical parameter variations in batch tests of *S. grossus* in sago mill effluent (SME): (a) temperature; (b) pH; (c) dissolved oxygen; and (d) ORP [CC: SME without plants; SS: subsurface system (SME and plants); FS: free-surface system (SME and plants)].



 Table 2

 Physical growth of Scirpus grossus in PC, SS and FS systems during tolerance study.

2.7. Statistical analysis

Statistical analysis was conducted using Statistical Product and Service Solutions (SPSS) Version 21.0 for Windows. The significant difference between treatments with plants compared to the non-planted aquarium, for the measurements of COD, BOD, TSS and plant growth, was sought; the data were analysed using one-way analysis of variance (ANOVA) and Pearson linear correlation to determine whether there was significant difference in the parameters between SS and FS systems. Assessment of the statistical differences for all the parameters at the 0.05 probability level, unless otherwise stated, used Duncan's multiple range tests (Al-Baldawi et al., 2013; Hasan et al., 2013). All samples were taken three times to reduce errors, with the results presented as means with standard deviation.

3. Results and discussion

3.1. Characterisation of SME effluents

Table 1 lists the characterisation of SME, representing its initial analysis prior to a preliminary experiment, compared with the Environmental Quality Acts (EQA) (Department of Environment, 2010). The highly important parameter analysis results for COD, BOD and TSS concentrations were 8920, 965 and 937 mg/L, respectively, with all concentrations were very much higher compared with EQA Standard B: 400, 50 and 100 mg/L, respectively.

3.2. Variations of physicochemical parameters

As shown in Fig. 3, the physical parameters for the phytoremediation of SME were obtained in the present study. In general, our findings indicated that the mean temperature was within the range of 27-30 °C during the 80 days of the experiment, which is normal for a tropical region. Mean pH of the aquariums was 4.8-7.2, whilst it was within the range of 4.8-5.2 for the aquariums without plants. It is possible that the microbial utilisation of SME influences the acidic pH conditions (Al-Baldawi et al., 2017; Allamin et al., 2020). Changes in the dissolved oxygen levels for SS and FS system were within the range of 5.3-8.6 mg/L. While ORP fluctuated within the range -47.1 to -20.1 mV for the SS system and -54.3 to -26.3 mV for the FS system, indicating no significant difference between the treatments in this regard. However, the ORP range of CC system without plants did not vary much (-46.4 to -49 mV). As mentioned by Nivala et al. (2019), the increasing ORP values demonstrates that the conditions in the aquariums became more aerobic due to associated oxygen leakage via the roots.

3.3. Plant tolerance and response

From the tolerance study, as depicted in Table 2, *S. grossus* had shown that it could grow and survive in SME after 15 days of exposure in the SS and FS systems, compared with the plant control (PC). At the final day of the experiment, no plants were withered in the SS system, and one plant was withered in the FS system. The withered plant percentage was 0 and 25% for the SS and FS systems, respectively.

3.4. Performance of S. grossus in the removal of COD, BOD, and TSS

Measurement of the organic strength of wastewater was analysed on the basis of COD and BOD readings. BOD assessment is a widely used criterion for water quality assessment (Jouanneau et al., 2014). COD is the amount of oxygen required to oxidise organic and inorganic materials through chemical pathways. High levels of COD denote the presence of biologically resistant substances and their consequent toxicity. The COD concentration in SS and FS systems with plants was significantly decreased after 80 days (p < 0.05) compared with and without plants in both systems, as shown in Fig. 4. The COD concentration in the SS system significantly decreased (p < 0.05) when compared with the FS system (Fig. 4).

BOD is the amount of dissolved oxygen (DO) used by microbial activities for the biochemical degradation of organic matter in water within a specific period (typically five days) at a certain temperature (20 °C) in the dark (Nivala et al., 2019). According to the findings, *S. grossus* had a positive impact on the efficiency of BOD removal compared to the BOD concentrations without plants, after 80 days of exposure to SME. The significant decrease in BOD concentration from both systems with plants was achieved after 80 days (p<0.05) compared with without plants in both systems (Fig. 5). In addition, the BOD concentration in the SS system significantly decreased (p<0.05) compared with the FS system, as shown in Fig. 5. In the present study, the influent COD and BOD concentrations were 8,920 and 965 mg/L, respectively, and the highest COD and BOD removals were 88 and 93% in the SS batch system, respectively. COD and BOD decreased significantly, with p < 0.05, in the SS batch system (1076 and 60 mg/L, respectively) compared to the FS system, respectively. Throughout the exposure, the COD and BOD levels of the planted aquariums were lower than the control group (CC). By the end of the 80-day exposure, the control sets only achieved 66 and 65% removal of COD and BOD in the SS system, respectively, and 64 and 62% removal of COD and BOD, respectively, for FS control (CC). These findings highlight the beneficial effects of *S. grossus* on SME treatment. Yusoff et al. (2019) found that the COD removal was 66% with *S. grossus*



Fig. 4. Concentration and removal of COD in SS and FS batch systems [PSME: plants exposed to SME; CC: SME without plants; D-N: significant difference at p < 0.05 between with plants and without plants on the same day within each system; d-n: significant difference between SS and FS systems with plants on the same day].



Fig. 5. Concentration and removal of BOD in SS and FS batch systems [PSME: plants exposed to SME; CC: SME without plants; D-N: significant difference at p < 0.05 between with plants and without plants on the same day within each system; d-n: significant difference between SS and FS systems with plants on the same day].

and it was used to biopolish the final effluent of recycled paper. Ahmad et al. (2017) had recorded 100% for the COD removal with *S. grossus* for pulp and paper mill effluent. The COD removal between treatments with and without plants was significantly different, giving evidence that *S. grossus* has shown significant impact in SME remediation. The results by Almaamary et al. (2019) indicated that *S. grossus* plants had significant impact on the BOD removal of 55% in gasoline-contaminated water compared to the control aquariums without plants. Although there is a substantial reduction in BOD and COD, the treated effluent has yet to comply with the Standard B limit (400 mg/L and 50 mg/L for BOD and COD, respectively), as stipulated in the Malaysia Environmental Quality (Industrial Effluent) Regulations 2009 (Department of Environment, 2010). Alternatively, the phytoremediation technology could be applied as the biopolishing treatment of SME after going through some prior primary treatments.

In the current study, the overall TSS removal efficiency was 98% in the planted SS system and 98% in the planted FS system (Fig. 6). No significant difference (p>0.05) was observed between the average concentrations and removal during 80 days of exposure for TSS in both systems, and also when compared between planted aquariums and the unplanted ones. It indicates that there was a small difference between SS and FS systems, which are 98% SS system with plants, 97% SS system without plants, 98% FS system with plants and 96% FS system without plants for TSS removal. According to Rani et al. (2011), the roots and microorganisms synergetic relationship helps in the degradation



Fig. 6. Concentration and removal of TSS in SS and FS batch systems [PSME: plants exposed to SME; CC: SME without plants; D-N: significant difference at p < 0.05 between with plants and without plants on the same day within each system; d-n: significant difference between SS and FS systems with plants on the same day].



Fig. 7. Growth response parameters of wet weight of stem and root of *Scirpus grossus*, in subsurface batch system (SS), free-surface batch system (FS) [PC: plant control; PSME: plants exposed to SME contaminant; D-N: statistically significant difference at p < 0.05 between PC and PSME on the same day within each system; d-n : statistically significant difference at p < 0.05 between PSME in SS and PSME in FS on the same day].

of suspended solids. The treated SME had complied with Standard B limit (100 mg/L) for industrial effluent as stipulated by the Malaysia Environmental Quality (Industrial Effluent) Regulations 2009 (Department of Environment, 2010). No significant difference was observed between the systems, as TSS removal is mainly achieved through sand filtration and also a minor contribution by the roots of the plants.

3.5. Plant growth and its relative growth rate

Wet weight of *S. grossus* throughout the 80 days is depicted in Fig. 7. The wet weight of the roots and stems of plants in all the treatments increased during the 80 days compared with the plant control without SME (PC), in both SS and FS systems. However, the wet weight of roots and stems in the SS system increased and had shown responses to SME as a nutrient through the 80 days of treatments. Nash et al. (2019) and Kutty et al. (2009) stated that SME could increase plant biomass by nutrients from SME being absorbed by the plants. Essential nutrients exist in sago effluent include nitrogen, phosphorus and potassium which can be applied in agriculture as irrigation water (Bhaskar and Prasada Rao, 2014). From 28 days onwards, the wet weight of the roots for PSME was significantly higher (p < 0.05) than that of the PC. And the weight of plant roots in the SS system was significantly higher (p < 0.05) than that of the FS system plant roots from



Fig. 8. Growth response parameters (dry weight of stem and root, and relative growth rate (RGR)) of *Scirpus grossus*, in subsurface batch system (SS), free-surface batch system (FS) [PC: plant control; PSME: plants exposed to SME contaminant; D-N: statistically significant difference at p < 0.05 between PC and PSME on the same day in same system; d-n: statistically significant difference at p < 0.05 between PSME in SS and PSME on the same day in FS].

28 days onwards. Also, the plant stem weight in the SS system was significantly higher (p < 0.05) than that of the PC plant stems throughout 80 days in SS system. Plant stem weight in the SS system has shown significant difference (p < 0.05) from the plant stem weight in the FS system from 28 days of exposure onwards. This might due to the withering symptom of some of the plants in the FS system, like yellowing at the end of leaves, compared with the plants in the SS system and the control PC. These phenomena are in line with similar results obtained by Licata et al. (2019).

The dry weight of the plants, in Fig. 8, has shown that the plant root weight in the SS system increased from 28 days and continued to increase significantly (p < 0.05) until the end of the experiment, compared with PC root weight in the same system. The plant dry weight increased in the SS system from 32 g at 28 days to 56 g at 80 days, compared with only 37.7 g at 80 days for the plant control. Plant root weight in the SS system was significantly higher (p < 0.05) than that of the FS system plant roots after 14 days. The dry weight of the stems of these plants was significantly higher (p < 0.05) in SS than that of the PC plants from 28 days onwards. Also, the plant stem weight in SS was significantly different (p < 0.05) than that of FS from 14 days onwards.

In addition to wet and dry weights, the impact of SME on the growth of plants can be captured in the plot of RGR, in Fig. 8. PC was referred to plants that act as plant control i.e. plants that did not expose to SME while PSME was for plants exposed to SME. Increasing RGR values give indication of good health of plants. This trend was clearly observed for PSME both in SS and FS rather than in PC which shows horizontal trend towards the end of exposure period. The nutrients from SME contribute to the better growth of plants which related to wet and dry weight as mention before whereas for PC the only nutrients exist was from sand (Titah et al., 2013). RGR of the roots and stems for PC and PSME was significantly higher (p < 0.05) for SS compared to FS system after 14 days. For example, the RGR values at the end of the experiment for SS system root PC, SS system root PSME, FS system stem PSME, FS system stem PSME, FS system stem PC, and FS system stem PSME values were 1.31, 1.72, 1.08 and 1.35 g g⁻¹ d⁻¹, respectively. SS system showed higher RGR values compared to FS system. This trend also observed for dry and wet weight of the roots and stems for PC and PSME.

Thus, the increase of RGR, wet and dry weight in the SS system for root and stem signify that the growth of *S. grossus* was influenced by the system applied in this experiment. In addition, referring to Section 3.3, the results of BOD and COD removals show the higher removal rate for both planted FS and SS systems after 28 days, as presented in Figs. 4 and 5, respectively, giving evidence that healthy growing plants have beneficial impact on the removals of BOD and COD. SS system having the benefit of preventing mosquitoes and odours and also produce no residual biosolids that require post treatment and disposal (USEPA, 2000). It can be concluded that the native aquatic plant, *S. grossus* have the potential to survive and also remediate SME by enhancing the removal of BOD and COD under SS system.

4. Conclusions

After 80 days of *S. grossus* plants exposure to SME in the SS and FS batch systems, our findings indicated that *S. grossus* could survive in and tolerate in SME. In addition, it could reduce COD and BOD more significantly in the SS batch system, with removal rates of 88 and 93%, respectively, compared to the FS system, with removal rates of COD and BOD of 73 and 85%, respectively. The removal of TSS (98%) had complied with the regulation limits of Standard B in Malaysia

Environmental Quality (Industrial Effluent) Regulations (2009) after the first 14 days of exposure. Through the obtained results, the presence of native emergent plant *S. grossus* in phytoremediation has been shown to be able to play a crucial role in removing the pollutants to below the regulation limits. Therefore, it can be concluded that the SS batch system is applicable in further investigations of the use of *S. grossus* to remediate SME wastewater.

CRediT authorship contribution statement

Daniah Ali Hassoon Nash: Conceptualization, Investigation, Data curation, Methodology, Writing - original draft. Siti Rozaimah Sheikh Abdullah: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing - review & editing. Hassimi Abu Hasan: Supervision. Mushrifah Idris: Supervision. Ahmad Razi Othman: Supervision. Israa Abdulwahab Al-Baldawi: Investigation, Data curation. Nur 'Izzati Ismail: Writing - review & editing, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We extend our gratitude to the authorities and staff of Universiti Kebangsaan Malaysia (UKM), Tasik Chini Research Centre and Ministry of Higher Education of Malaysia for funding this research MI-2020-003. The authors would like to thank Kilang Tepung Sagu manufacturer for supplying sago effluent.

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