ASEAN Journal of Chemical Engineering 2022, Vol. 22, No. 1, 42 – 57

Performance of *Chlorella sp.* and Multicultural Bacteria in Removing Pollutants from Nutrient-Rich Wastewater

Mohd Edyazuan Azni [1]
Atiqah Zainal Abidin [1]
Roslan Noorain [1]
Sharifah Mariam Syed Hitam [1]
Lusi Ernawati [2]
Rosnah Abdullah [3]
Ahmad Shoiful [4]
Rozyanti Mohamad [1*]

[1] *Universiti Kuala Lumpur Branch Campus Malaysian Institute of Chemical and Bioengineering Technology (UniKL MICET), Kawasan Perindustrian Bandar Vendor, Taboh Naning, Alor Gajah, Melaka, 78000, Malaysia.*

[2] *Department of Chemical Engineering, Institut Teknologi Kalimantan, Balikpapan, East Kalimantan, 76127, Indonesia.*

[3] *Centre of Advanced Material and Energy Sciences, University of Brunei Darussalam, Gadong BE 1410, Brunei Darussalam.*

[4] *Center of Technology for the Environment, Agency for the Assessment and Application of Technology (BPPT), Kawasan PUSPIPTEK, Serpong, Tangerang Selatan, 15314, Indonesia*

[*]e-mail: rozyanti@unikl.edu.my

Submitted 28 September 2021 *Revised* 23 March 2022 *Accepted* 1 April 2022

Abstract. The most common method of treating palm oil mill effluent (POME) is by using various types of bacteria communities. However, the utilization of microalgae in consuming the high nutrient content in wastewater offer additional benefit, particularly for CO_2 sequestration. In this study, we proposed to evaluate the performance of multicultural bacteria obtained from municipal wastewater and *Chlorella sp.* for batch treatment of POME at different COD concentrations (ranges between 600 to 1,300 mg/L), microalgae species (*C. vulgaris* and *C. pyrenoidosa*) and speed of agitation (0 and 150 rpm). The results showed that between the bacteria and microalgae that are involved in POME treatment, microalgae give high removal of colour (93%) and ammoniacal nitrogen (95%). As for the COD and phosphate removal, both microorganisms show comparable performances. It was observed that *C. pyrenoidosa* was able to remove more colour compared to *C. vulgaris* where higher lipid yield production was obtained (47.6%). However, there is no significant impact of agitation on pollutant removal. This study also reveals that co-cultivation of different microalgae species does not affect the efficiency of the system. This study provides an important insight into developing an efficient and environmentally friendly method to treat wastewater by incorporating green technology in the treatment system.

Keywords: POME, *Chlorella sp.*, Multicultural Bacteria, Pollutants Removal, Lipid Production

DOI: 10.22146/ajche.69427

INTRODUCTION

Wastewater is the main waste product from human activities. It is known as water that has been used in residential, agricultural, and commercial events. Wastewater also includes rain from sidewalks, parking lots, and rooftops (Chen et al., 2019). The wastewater contains 99.9% of water and the remaining 0.1% comprises organic matters, inorganic compounds, and microorganisms. Wastewater effluents such as wetlands, streams, waterways, estuaries, and oceans, are released into a variety of environments (Tuser, 2020). Industrial wastewater is created by industrial or commercial processing methods and is typically more difficult to handle than domestic wastewater. The composition of industrial wastewater varies depending on an industry-by-industry basis (Hussain et al., 2021, Iloms et al., 2020).

Palm oil mill effluent (POME) is one example of industrial wastewater generated by the palm oil industry which has become a critical issue for the environment (Al-Amshawee et al., 2020, Wu et al., 2010). This is because POME is produced in a large amount with high oxygen content that will affect the soil and marine life if discharged without proper treatment (Najafpour et al., 2006). Although there are other types of palm oil mill waste such as empty fruit bunch (EFB), it will not affect the surface water like POME (Azni et al., 2015).

POME is a deep brownish colloidal combination of water, oil, and fine suspended solids. It is wet and has around 25,000 mg/L of biochemical oxygen demand (BOD), 55,250 mg/L of chemical oxygen demand (COD), and 19,610 mg/L of suspended solids (SS) which is 100 times more polluting than domestic sewage (Abdurahman et al., 2013). As no additives are applied to the extraction process, the effluent is non-toxic but often acidic with a pH of about 4 to 5, because it contains organic acids in complex forms that are ideal sources of carbon (Din et al., 2006). Excessive nutrients such as phosphorus and nitrogen in POME also can cause eutrophication which is harmful to marine species. This also resulted in unsustainable growth of plants and decreases in the supply of oxygen, shifting environments, and potentially endangering some animals (Lin et al., 2021, Tuser, 2020).

There are many biological pathways and techniques to treat POME such as the utilization of microalgae and bacteria. Several studies have successfully conducted the degradation of POME organic compounds using bacteria such as *Pseudomonas sp*., *Bacillus sp*., and *Rhodococcus opacus* (Hwangbo & Chu, 2020; Karim et al., 2021). Rather than using pure strain bacteria, multicultural bacteria potentially enhance the pollutant removal performance (Bala et al., 2015).

On the other hand, microalgae can be growth via photosynthesis by absorption of dissolved CO_2 from POME up to 200 times more than trees (Mohd-Nor et al., 2019, Rachmadona et al., 2020). Microalgae also help in aerobic oxidation that produces oxygen that will stimulate the POME treatment (Muhamad Maulana Azimatun Nur & Buma, 2019). In fact, increased growth of microalgae biomass and lipid was reported during POME treatment (Wang et al., 2016).

Chlorella is one of the microalgae species that has good performance in wastewater treatment. Yet, most researches were conducted in a regulated environment of 5°C (Maxwell et al., 1994; Renaud et al., 2002) which does not indicates the full capabilities of microalgae when cultivated at room temperature of 20°C to 30°C (Khadaroo et al.,

2019). A study reported by Al-Amshawee (2020) stated that the growth of microalgae was significantly high by using POME due to its high nutrient contents.

The potential of POME treatment and lipid production using multicultural bacteria and microalgae has not yet been fully explored. Hence, this study was conducted to give new insight into the great potential of using these microorganisms for POME treatment. Apparently, the use of a biological agent for high-strength wastewater treatment will not change the POME characteristic into a toxic solution.

The focus of this study is to compare the performance of multicultural bacteria, pure culture microalgae, and mixed culture microalgae in removing colour, COD, ammonium, and phosphorus. This study also emphasized the specific growth rate and lipid yield of the microalgae at different agitation speeds specifically at 0 and 150 rpm. The initial COD value of POME was designed at 600, 700, 800, and 1,300 mg/L to observe the performance of the bacteria and microalgae under different COD concentrations. The experimental works were designed to determine the optimum condition for pollutants removal, specific growth rate, and lipid yield.

MATERIALS METHOD

Materials

The POME sample was collected from Bell Kilang Sawit Linggi, Negeri Sembilan, and stored in a plastic container at 4°C to avoid further biodegradation (Samsudin et al., 2018). The characteristics of POME are summarized in Table 1. Multicultural bacteria were obtained from Indah Water Konsortium (IWK) while microalgae *Chlorella vulgaris* and *Chlorella pyrenoidosa* were cultivated in the laboratory.

Table 1. Characteristic of the POME (Kamyab et al., 2015).

Parameter	Average concentration
pH	4.25
COD	1600 mg/L
BOD	330 mg/L
Total phosphorus	350 mg/L
Total nitrogen	500 mg/L

Experimental Setup

Bacteria and microalgae were added into a 250 ml conical flask at 10% concentration ($V_{microbe}$ / V_{POME}). The initial COD concentration of POME samples is 600, 700, 800, and 1,300 mg/L. The samples were incubated for 7 days at 25°C in the incubator shaker (Kamyab et al., 2015). The agitation speed was set at 0 and 150 rpm under a fluorescent light that was supplied 12:12 hours light and dark conditions alternately (Samsudin et al., 2018).

Analytical Method

The removal of colour, chemical oxygen demand (COD), ammonium, and phosphorus of the sample were analyzed by referring to HACH methods 8025, 8000, 8155, and 8048 respectively according to Standard Methods for the Examination of Water and Wastewater (APHA, 2002, Samsudin et al., 2018, Syafiqah Hazman et al., 2018). The percentage of pollutant removal was calculated based on the average value of triplicate sets of data for day 1 and day 7 as per Eq. (1).

$$\text{Removal}(\%) = \frac{\text{Data day 1} - \text{Data day 7}}{\text{Data day 1}} \text{x} 100 \quad (1)$$

The cell of biomass was obtained from the centrifugation of the sample at 10,000

rpm for 2 minutes (Toh et al., 2016). The biomass was used to measure specific growth rate and lipid yield (Mujtaba et al., 2015) while the supernatant was then analyzed for colour, COD, ammonium, and phosphorus.

The specific growth rate, μ was calculated by using Eq. (2):

$$\mu = \frac{1}{t}\ln\left(\frac{X_m}{X_0}\right) \quad (2)$$

where X_m and X_0 are the concentrations of biomass at the end and initial, respectively and t is the duration of the experiment run (Converti et al., 2009). The total lipids of microalgae were extracted from the biomass by using a modified method from Bligh and Dyer, 1959 and Yoo et al., 2010. The lipid yield, Y was calculated by using Eq. (3).

$$Y(\%) = \frac{W_L}{W_{DA}} \quad (3)$$

where W_L and W_{DA} are the weight of extracted lipids and dry biomass, respectively (Converti et al., 2009). The pH of the initial and final samples was also measured accordingly. All the experimental works were performed in triplicates.

RESULTS

POME Treatment

The analyses were performed for all samples on day 1 and day 7 to obtain the percentage of pollutants removal. Figure 1 shows the POME samples at different initial COD concentrations before and after being treated with the bacteria and microalgae. The pollutants removal efficiency at 0 and 150 rpm are presented in Figures 2 and 3, respectively.

Based on Figure 2, the highest colour removal is obtained from microalgae *Chlorella pyrenoidosa* at designated COD of 600 mg/L which is 93% followed by the combination of microalgae at designated COD of 600 mg/L which is 86%. However, Figure 3 shows that the highest removal of colour is only up to 78% from microalgae *Chlorella pyrenoidosa* at designated COD 600 mg/L and followed by 77% which is from *Chlorella pyrenoidosa* at designated COD 700 mg/L and a combination of microalgae at designated COD 800 mg/L. From the observation, the multicultural bacteria can remove colour up to 66% at 0 rpm meanwhile at 150 rpm, it can remove up to 69%. For the microalgae *Chlorella vulgaris,* it can remove colour up to 51% at 0 rpm and 31% at 150 rpm. Then for microalgae *Chlorella pyrenoidosa*, it can remove colour up to 93% at 0 rpm and 78% at 150 rpm. For the combination of microalgae, at 0 rpm, it can remove 86% colour and 77% colour at 150 rpm.

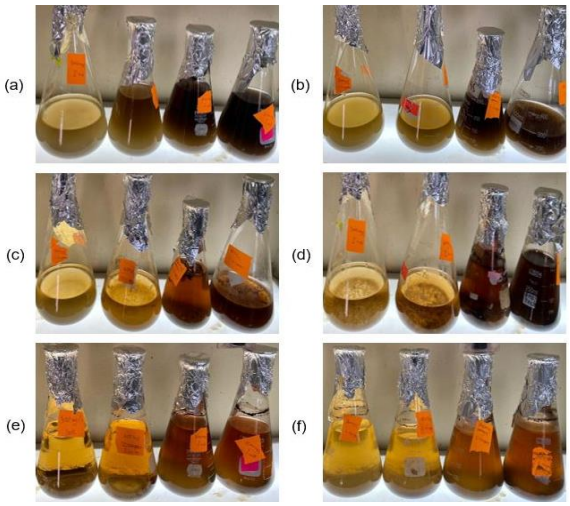

Fig. 1: POME samples with COD concentrations of 600, 700, 800, 1,300 mg/L (a) Initial samples of multicultural bacteria (b) Initial samples of microalgae (c) multicultural bacteria at day 7, 0 rpm agitation (d) microalgae at day 7, 0 rpm agitation (e) multicultural bacteria at day 7, 150 rpm agitation (f) microalgae at day 7, 150 rpm agitation.

The percentage of COD removal at 0 rpm in Figure 2 shows the highest removal is from

multicultural bacteria and microalgae *Chlorella vulgaris* both at designated COD 600 mg/L which is 79%. Then followed by the microalgae *Chlorella vulgaris* at designated COD 700 mg/L which is 76%. For the percentage of COD removal at 150 rpm in Figure 3, the highest removal of COD is from microalgae *Chlorella vulgaris* at designated COD 600 mg/L which is 79%, followed by multicultural bacteria at designated COD 700 mg/L which is 74%. From the results, the multicultural bacteria can remove 79% COD at 0 rpm and 74% at 150 rpm.

For the microalgae *Chlorella vulgaris,* it can remove COD up to 79% at 0 rpm and150 rpm. Then for microalgae *Chlorella pyrenoidosa*, it can remove COD up to 49% at 0 rpm and 48% at 150 rpm. For the combination of microalgae, at 0 rpm, it can remove 50% COD and 48% COD at 150 rpm. Then, for the removal of ammonium at 0 rpm in Figure 2, it shows the highest removal is 91% which is from microalgae *Chlorella pyrenoidosa* at designated COD 800 mg/L and a combination of microalgae at designated COD 700 mg/L. The second highest of removal ammonium is from combination microalgae at designated COD 600 mg/L which is 87%. Meanwhile, for the removal of ammonium at 150 rpm in Figure 3, the highest removal is from the combination of microalgae at designated COD 1,300 mg/L which is 95%, followed by microalgae

Chlorella pyrenoidosa and combination microalgae both at designated COD 800 mg/L which is 91%. Overall, the multicultural bacteria can remove ammonium up to 83% at 0 rpm and 90 at 150 rpm. For the microalgae *Chlorella vulgaris*, 81% of ammonium can be removed at 0 rpm and 90% 150 rpm. Then for microalgae *Chlorella pyrenoidosa*, it was able to remove 91% of ammonium at 0 rpm and 150 rpm. For the combination of microalgae, at 0 rpm, it can remove 50% COD and 48% COD at 150 rpm.

The graph of removal phosphorus at 0 rpm in Figure 2 shows the highest percent of removal is 84% and 76% from microalgae *Chlorella pyrenoidosa* at designated COD 1,300 mg/L. For the removal of phosphorus at 150 rpm in Figure 3, the highest percent removal is from multicultural bacteria at designated COD 1,300 mg/L which is 79%. Then followed by microalgae *Chlorella pyrenoidosa* at designated COD 1,300 mg/L which is 78%. The data shows that the multicultural bacteria can remove 44% of phosphorus at 0 rpm and up to 79% of phosphorus at 150 rpm. For the microalgae *Chlorella vulgaris*, it can remove phosphorus up to 84% at 0 rpm and 74% at 150 rpm. Meanwhile, for the microalgae *Chlorella pyrenoidosa*, 76% of phosphorus can be removed at 0 rpm and 78% at 150 rpm. The combination of microalgae, at 0 rpm and 150 rpm, can remove 74% of phosphorus.

Overall, the performance of multicultural bacteria, microalgae *Chlorella vulgaris*, and microalgae *Chlorella pyrenoidosa*, is good at 0 rpm compared to performance at 150 rpm. Meanwhile, for the combination of microalgae, the performance is good at 150 rpm compared to performance at 0 rpm.

Biofuel Production

The analysis of the specific growth rate, μ, and the yield of lipid need to use the dry biomass (Converti et al., 2009) and the Figure 4 shows the overall results for specific growth rate, μ and the yield of lipid at 0 rpm meanwhile Figure 5 shows the overall of results for specific growth rate, μ and the yield of lipid at 150 rpm for microalgae.

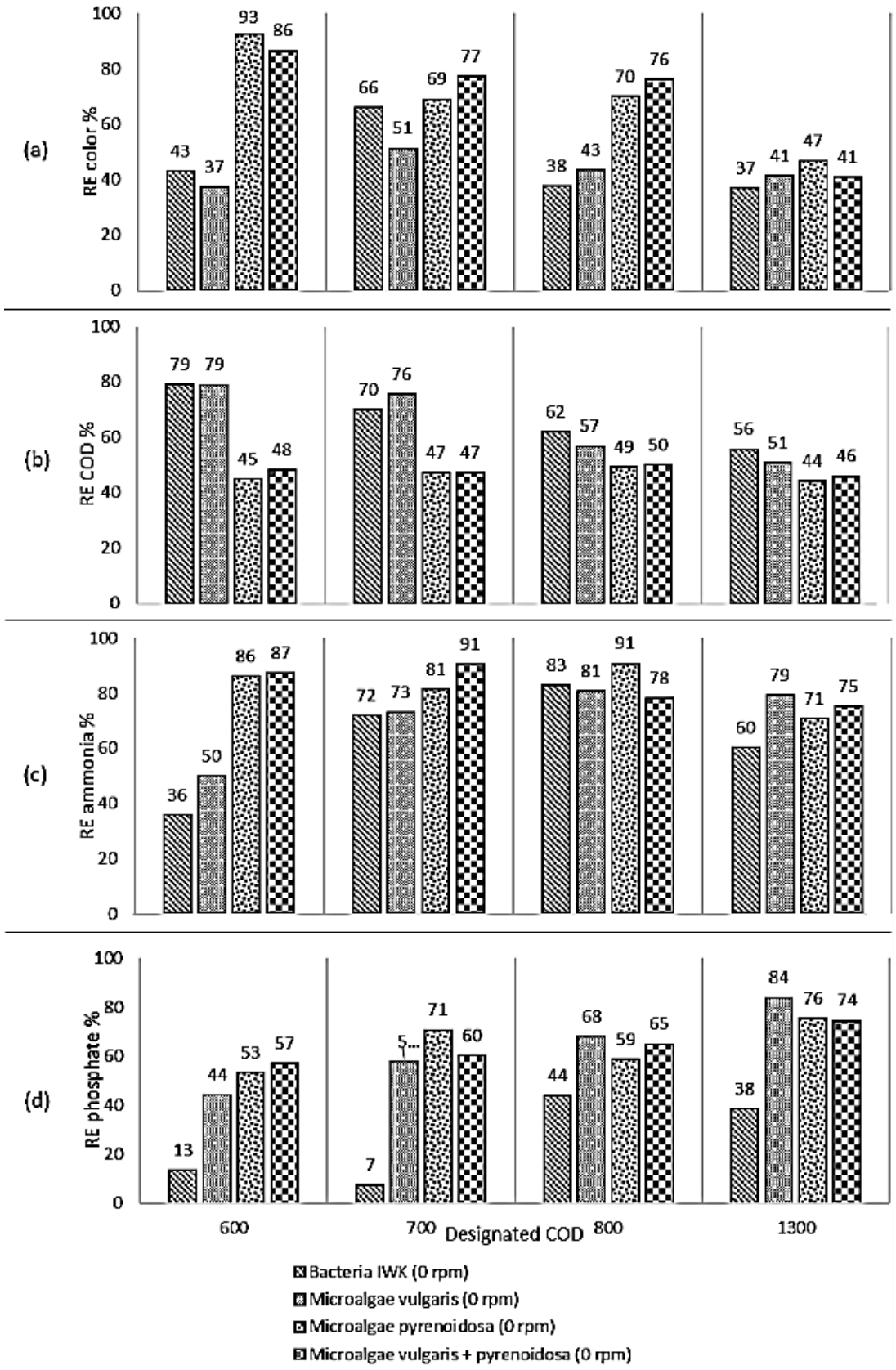

Fig. 2: POME pollutants removal efficiency percentage with different designated COD concentration at 0 rpm agitation (a) colour (b) COD (c) ammonia (d) phosphorus

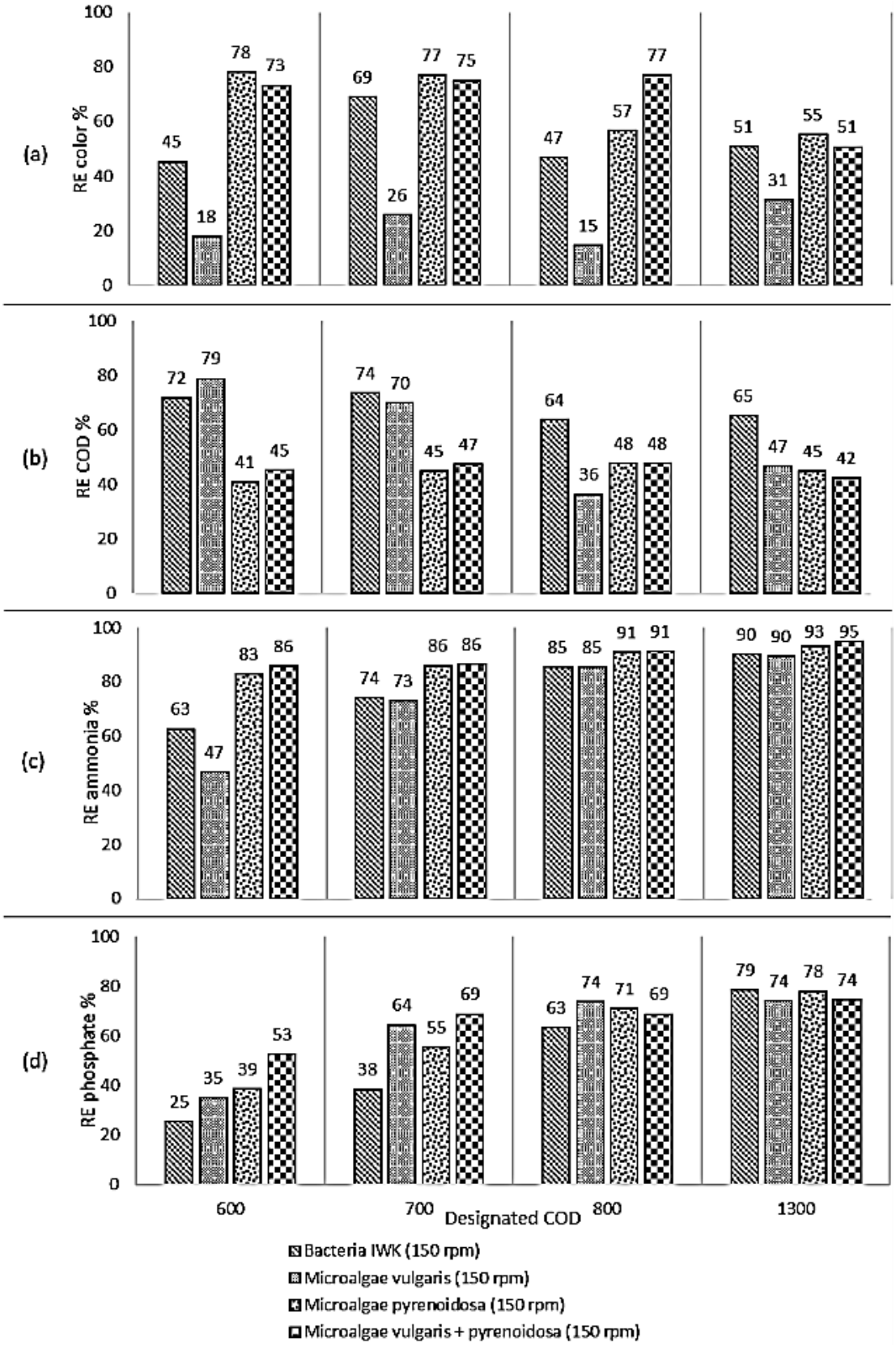

Fig. 3: POME pollutants removal efficiency percentage with different designated COD concentration at 150 rpm agitation (a) colour (b) COD (c) ammonia (d) phosphorus

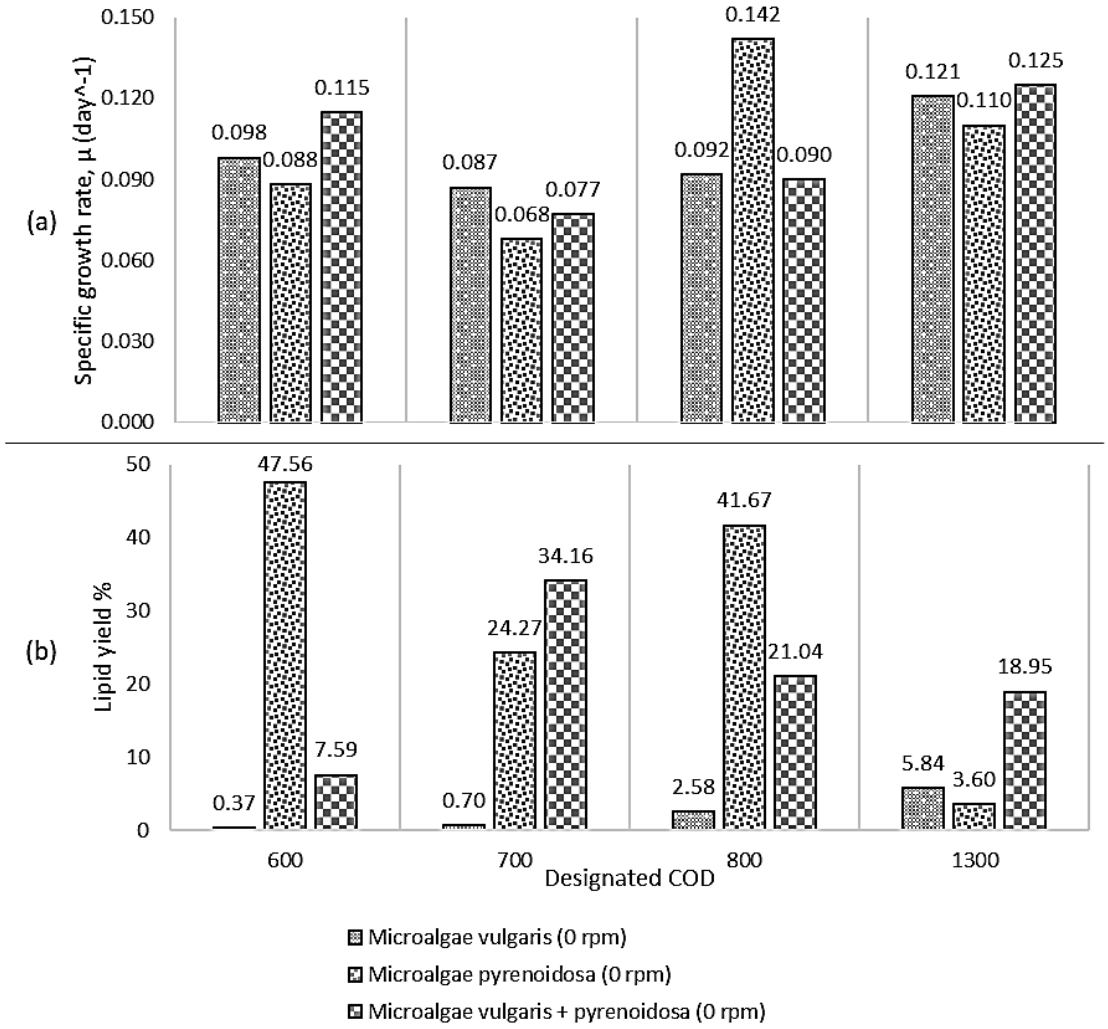

Fig. 4: Microalgae productivity at 0 rpm agitation for (a) Specific growth (b) lipid for microalgae yield

Based on the graph of specific growth rate at 0 rpm in Figure 4, the highest growth rate is 0.142 day^{-1} from microalgae *Chlorella pyrenoidosa* at designated COD 800 mg/L and followed by 0.125 day^{-1}of growth rate from the combination of microalgae at designated COD 1,300 mg/L. Meanwhile, the specific growth rate at 150 rpm in Figure 5 shows the highest growth rate is from *Chlorella pyrenoidosa* at designated COD 600 mg/L which is 0.126 day^{-1}, followed by microalgae *Chlorella vulgaris* at designated COD 600 mg/L which is 0.125 day^{-1}. The data shows that the specific growth rate for the microalgae *Chlorella vulgaris* can be up to 0.121 day^{-1} at 0 rpm and 0.125 day^{-1} at 150 rpm. Meanwhile, for the microalgae *Chlorella pyrenoidosa*, the specific growth rate is up to 0.142 day^{-1} at 0 rpm and 0.126 day^{-1} at 150 rpm. For the combination of microalgae, at 0 rpm the specific growth rate can be up to 0.125 day^{-1} and 150 rpm, 0.103 day^{-1} of specific growth rate.

The yield of lipid in Figure 4 at 0 rpm shows that microalgae *Chlorella pyrenoidosa* at designated COD 600 mg/L have the highest yield of lipid which is 47.56% and followed by 41.67% yield of lipid also from

microalgae *Chlorella pyrenoidosa* but at designated COD 800 mg/L. For the yield of lipid at 150 rpm in Figure 5, the highest is 40.19% from microalgae *Chlorella pyrenoidosa* at designated COD 600 mg/L and followed by 35.36% from the combination of microalgae at designated COD 600 mg/L. From the graph yield of lipid, microalgae *Chlorella vulgaris* can achieve up to 5.84% of lipid at 0 rpm and 6.26% of lipid at 150 rpm. For the microalgae *Chlorella pyrenoidosa*, the yield of lipid can be up to 47.56% at 0 rpm and 40.19% at 150 rpm. Meanwhile, for the combination of microalgae, the yield of lipid can be up to 34.16% at 0 rpm and 35.36% of lipid at 150 rpm.

Overall, the performance of microalgae *Chlorella vulgaris* and the combination of microalgae is good at 150 rpm compared to performance at 0 rpm. Meanwhile, the performance of microalgae *Chlorella pyrenoidosa*, is good at 0 rpm compared to performance at 150 rpm.

DISCUSSION

In this study, the multicultural bacteria, microalgae *Chlorella vulgaris*, and microalgae *Chlorella pyrenoidosa* has been used to treat POME to determine their performance to remove colour, COD, ammonium, and phosphorus. The COD concentration that was used were 600, 700, 800, and 1,300 mg/L. It can be observed from Figure 1 that POME with the highest concentration of COD has a darker brownish colour than the lesser one (Irfan et al., 2017). During oil palm mill operation, there is no chemical added to the system, therefore the brownish colour is present due to the pigments from the fresh fruit bunch (FFB) (Bello et al., 2013). The pigment exists in the form of phenolic, lignin, and carotenes (Altogbia et al., 2021). Crude palm oil (CPO) has 4,000 to 6,000 ppm of carotenes (Ng & Choo, 2016). The appearance from multicultural bacteria treatment of Figure 1a (day 0) to Figure 1c and 1e (day 7), the colour of the POME has turned to green yellowish colour after the treatment indicated that the multicultural bacteria has successfully removed carotenes from the POME. The colour removal occurs when the process of adsorption of the pigment from the biomass. It is worthy to state that the samples that agitated at 150 rpm show clearer POME compared to no agitation samples. The same trend can be seen for samples treated with microalgae. As expected, applying multicultural bacteria and microalgae in POME will remove the colour.

The nutrient removal of colour, COD, ammonia, and phosphate have been tested according. *Chlorella pyrenoidosa* (single group) and *Chlorella vulgaris* + *Chlorella pyrenoidosa* (combine group) perform better than multicultural bacteria and *Chlorella vulgaris* (single group) in removing POME colour. The agitation gives a negative impact on the colour removal for all microalgae single groups and combine groups except for multicultural bacteria. The strong agitation probably damages the cell wall of the microalgae thus lowering its colour removal capabilities (Chu et al., 2009). The various forms of functional groups in the microalgae cell wall play an important function in the adsorption of colour which in POME case the carotene (Daneshvar et al., 2017). There are about 390-1,450 ppm of carotenes in POME (Ahmad et al., 2008). Microalgae absorb pigment from the POME which will be assimilated with its cell wall to help better the photosynthesis process (Huang et al., 2010). When the media COD concentration is higher, the colour removal for microalgae

performance decrease despite the multicultural bacteria showing no effect. This phenomenon happens due to microalgae metabolism which tends to build more cell walls to reproduce in a high COD concentration medium and will start to assimilate pigment when it reaches optimum community that differs from bacteria which stay the same performance (Tan et al., 2018).

It can be highlighted that Figures 2b and 3b show the multicultural bacteria and *Chlorella vulgaris* does remove more COD than colour than *Chlorella pyrenoidosa* and the combined group. This show that multicultural bacteria and *Chlorella vulgaris* display heterotrophic characteristic which grows more by assimilating carbon content in the POME rather than carotenes (Yu et al., 2019). Lee et al. (2016) has reported that bacteria tend to assimilate better the organic compound which is COD and convert it to biogas such as methane.

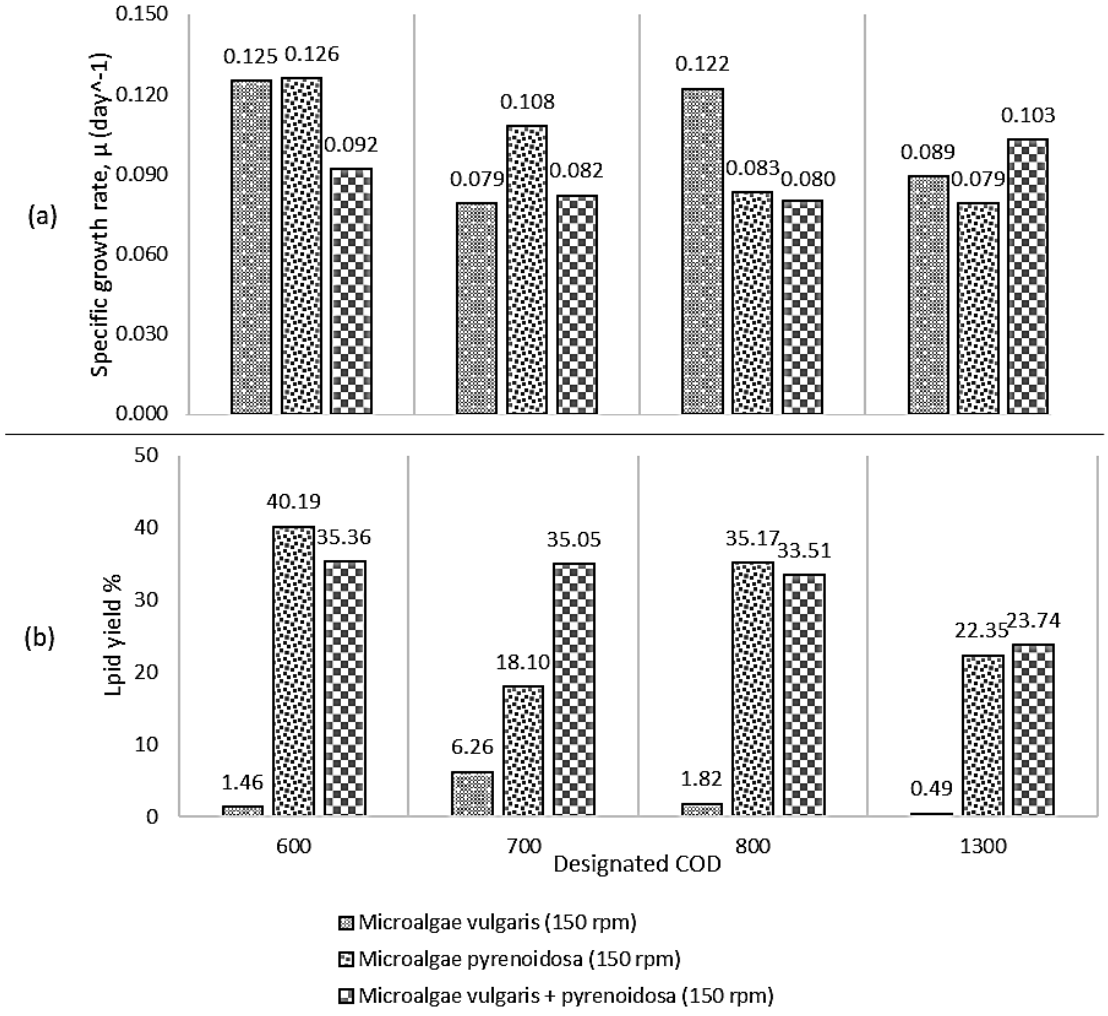

Fig. 5: Microalgae productivity at 150 rpm agitation for (a) Specific growth (b) lipid for microalgae yield

The COD removal rate decreased in line with POME which has a higher COD concentration was used. There is significant COD removal for POME with 600 and 700 mg/L COD concentration but when the COD concentration reaches 800 and 1,300 mg/L the performance decrease. This happens when the COD concentration reaches a high level, the POME becomes toxic level which decrease the COD removal (Muhamad Maulana Azimatun Nur & Buma, 2019). The same trend also come about with the agitation of 150 rpm. In the process of removal of COD, nitrogen, and phosphorus, the microalgae use light and CO_2 by photosynthesis. Generally, microalgae use photoautotrophic metabolism by utilizing light and CO_2 as energy and carbon sources to remove COD (Mujtaba et al., 2017).

It should be mentioned that the COD removal rate of POME treated with *Chlorella pyrenoidosa* (single group) and *Chlorella vulgaris* + *Chlorella pyrenoidosa* remain the same for all COD concentrations. The agitation also appears to be no effect on the COD removal as shown in Figures 2b and 3b. This is thought to be due to the POME having been filtered prior to the treatment, thus there are no lignocellulose materials that will decompose and add to the COD content during the treatment (Nwuche et al., 2014). Microalgae take up nitrogen such as ammonium (NH_4^+-N) and nitrate (NO_3^--N) to synthesize biomass (Lee et al., 2016). Organic phosphates are converted to orthophosphates by phosphatases at the cell surface of microalgae, and it occurs during orthophosphates are in short supply. Microalgae are also able to assimilate phosphorus in excess, which is stored within the cells in the form of polyphosphate (Mao et al., 2021).

Referring to Figures 2c and 3c, the ammonia removal was better for the POME treated with *Chlorella pyrenoidosa* (single group) and *Chlorella vulgaris* + *Chlorella pyrenoidosa* (combine group) due to the lower removal ratio of ammonia removal by *Chlorella vulgaris* (Bhuyar et al., 2021). Interestingly, the result suggests that higher wastewater COD concentration led to higher ammonia removal. The increase in efficiency is due to the microalgae using the nitrogen in the ammonia to build up its nucleic acids and proteins. The same trend of performance is also demonstrated by the phosphate removal percentage but at a slightly lower rate. Even though microalgae do consume ammonia and phosphate in helping their growth, they only need a lower amount of phosphate than ammonia. This is proven by the evidence that *Chlorella pyrenoidosa* has higher protein content in its cell (Taufikurahman & Shafira, 2019). Microalgae is better in terms of ammonia and phosphate removal from multicultural bacteria. This is due to the metabolites rate of microalgae being much higher than bacteria which assimilate the ammonia and phosphate faster (Joint et al., 2002).

In correlation with colour removal to the growth rate, higher colour removal does increase the growth rate of microalgae. Interestingly, the growth rate of microalgae samples that were agitated generally has a better growth rate than samples without agitation as shown in Figures 4a and 5a. This is influenced by the light intensity that can penetrate inside the POME container when the samples are clearer. This also happens during the agitation process, the majority of the microalgae will be exposed to the light thus helping in a better photosynthesis process occur (M. M.Azimatun Nur et al., 2021). Despite that, samples without agitation show nearly the same growth rate

performance for all the COD concentrations used which may be the effect of less light penetrating the POME samples (Azimatun Nur et al., 2017). The result obtained agreed with the previous work carried out by Shen et al. Overall, all the data has shown that there is a significant ability of bacteria and microalgae capable to consume organic carbon from the POME for their digestion and integrate the organic compound their carbon source (Mujtaba & Lee, 2016; Y. Shen et al., 2017).

Lipids produce more by *Chlorella pyrenoidosa* compared to *Chlorella vulgaris* as shown in Figures 4a and 5b. This is due to the level of protein content being higher in *Chlorella pyrenoidosa* (Negi et al., 2015; X. Shen et al., 2015; Zhu et al., 2014). By referring to Figure 2c and 3c, the removal of ammonia which consists of nitrogen, of *Chlorella pyrenoidosa* and the combined group was high, thus a lot of nitrogen has been removed compared to multicultural bacteria and *Chlorella vulgaris*. This phenomenon indicates that with a lower nitrogen content of nitrogen starvation, the lipid productivity increase (Yu et al., 2019). The addition of *Chlorella vulgaris* to the combined group does give a negative effect on the lipid yield. The productivity of lipid in microalgae does correlate with the nitrogen content in the sample. Several studies stated that lipid production is optimized when there is limited content of nitrogen in the wastewater (Negi et al., 2015). The total lipid represents 20–50% of the dry biomass weight. The different lipid content also appeared in many microalgae due to different species which pass through the transesterification process to produce biodiesel that largely consists of fatty acid methyl esters (Putri et al., 2011). Agitation does improve lipid production by promoting new cell growth. Concurrently, *Chlorella pyrenoidosa* does show the same performance with and without agitation thus showing better cost savings in the agitation equipment.

CONCLUSIONS

This work was devoted to accessing the removal rate of colour, COD, ammonia, phosphate, and the specific growth with lipid yield from POME treated with multicultural bacteria, *Chlorella vulgaris* (single group), *Chlorella pyrenoidosa* (single group), and *Chlorella vulgaris* + *Chlorella pyrenoidosa* (combine group). The POME samples have been prepared with COD concentrations of 600, 700, 800, and 1,300 mg/l. It was found that *Chlorella pyrenoidosa* (single group) and *Chlorella vulgaris* + *Chlorella pyrenoidosa* (combine group) demonstrate good performance in the removal colour, ammonium, and phosphorus. Alternatively, multicultural bacteria and *Chlorella vulgaris* are excellent in removing COD. Overall, the different designated COD gives different results in removal colour, COD, ammonium, and phosphorus. For the specific growth rate and yield of lipid, the microalgae *Chlorella pyrenoidosa* gives the best performance compared to the *Chlorella vulgaris*. However, there is no significant difference in the results at the agitation speed of 0 rpm and 150 rpm. Producing lipid from microalgae gives advantages such as fast-growing, high lipid content, and lower media cultivation cost. Therefore, it is recommended to conduct an investigation on similar microorganisms under continuous conditions. The combination of the biological agent and new technique of wastewater treatment might enhance the pollutant removal which is very interesting to be explored.

ACKNOWLEDGEMENT

We would like to thank the Southeast Asian Regional Centre for Tropical Biology (SEAMEO-BIOTROP) for grant no 404/DIPA-RES/III/2021, Universiti Kuala Lumpur for Short Term Research Grant (STR19039), and the Ministry of Higher Education under the Fundamental Research Grant Scheme (FRGS) FRGS/1/2020/TK0/UNIKL/02/9 for funding this project.

REFERENCES

Abdurahman, N. H., Rosli, Y. M., & Azhari, N. H., 2013. The performance evaluation of anaerobic methods for palm oil mill effluent (pome) treatment: A review. International Perspectives on Water Quality Management and Pollutant Control, InTechOpen, London, United Kingdom.

Ahmad, A. L., Chan, C. Y., Abd Shukor, S. R., & Mashitah, M. D., 2008. "Recovery of oil and carotenes from palm oil mill effluent (POME)", *Chem. Eng. J.*, *141*(1–3), 383–386.

Al-Amshawee, S. K., Yunus, M. Y., & Azoddein, A. A., 2020. "A review study of biofilm bacteria and microalgae bioremediation for palm oil mill effluent: Possible approach." *IOP Conf. Ser.: Mater. Sci. Eng.*, *736*(2).

Altogbia, W. M., Yusof, N. A., Zainal, Z., Idris, A., Rahman, S. K. A., Rahman, S. F. A., & Isha, A., 2021. "Molecular imprinted polymer for β -carotene for application in palm oil mill effluent treatment", *Arab. J. Chem.*, *14*(2), 102928.

American Public Health Association (APHA), 2002. Standard methods for the examination of water and wastewater: 20th edition. Washington DC, USA.

Azimatun Nur, M. M., Setyoningrum, T. M., & Budiaman, I. G. S., 2017. "Potency of Botryococcus braunii cultivated on palm oil mill effluent wastewater as a source of biofuel. *Environ. Eng. Res.*, *22*(4), 417–425.

Azni, M. E., Norhan, A. S., Hans, L., & Roslan, S. N., 2015. "Feasibility study on empty fruit bunch (EFB) cement board". 5th International Conference on Environment and Natural Science, Paris, France.

Bala, J. D., Lalung, J., & Ismail, N., 2015. "Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains," *Intr. J. Recycl. Org. Waste Agric.*, *4*(1), 1–10.

Bello, M. M., Nourouzi, M. M., Abdullah, L. C., Choong, T. S. Y., Koay, Y. S., & Keshani, S., 2013. "POME is treated for removal of color from biologically treated POME in fixed bed column: Applying wavelet neural network (WNN)", *J. Hazard. Mater.*, *262*, 106–113.

Bhuyar, P., Farez, F., Ab. Rahim, M. H., Maniam, G. P., & Govindan, N., 2021. "Removal of nitrogen and phosphorus from agro-industrial wastewater by using microalgae collected from coastal region of peninsular Malaysia", *Afr. J. Biol. Sci.*, *3*(1), 58–66.

Bligh, E.G. and Dyer, W. J., 1959. "A rapid method of total lipid extraction and purification", *Can. J. Biochem. Physiol.*, *37*(8).

Chen, J., Liu, Y., Gitau, M. W., Engel, B. A., Flanagan, D. C., & Harbor, J. M., 2019. "Evaluation of the effectiveness of green infrastructure on hydrology and water quality in a combined sewer overflow community", *Sci. Total Environ.*, *665*, 69–79.

Chu, W. L., See, Y. C., & Phang, S. M., 2009. "Use of immobilised Chlorella vulgaris for the removal of colour from textile dyes", *J. App. Phycol.*, *21*(6), 641–648.

Converti, A., Casazza, A. A., Ortiz, E. Y., Perego, P., & Del Borghi, M., 2009. "Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production", *Chem. Eng. Process.: Process Intensif.*, *48*(6),

1146–1151. 03.006

Daneshvar, E., Vazirzadeh, A., Niazi, A., Kousha, M., Naushad, M., & Bhatnagar, A., 2017. "Desorption of methylene blue dye from brown macroalga: Effects of operating parameters, isotherm study and kinetic modeling", *J. Clean. Prod., 152*, 443–453.

Din, M. F. M., Ujang, Z., Yunus, S. M., & Van Loosdrecht, M. C. M., 2006. "Storage of Polyhydroxyalkanoates (PHA) in fed batch mixed cultures using Palm Oil Mill Effluent (POME)". 4th Seminar On Water And Waste Management And Technologies, Granada, Spain.

Huang, G. H., Chen, F., Wei, D., Zhang, X. W., & Chen, G., 2010. "Biodiesel production by microalgal biotechnology", *Appl. Energy*, *87*(1), 38–46.

Hussain, F., Shah, S. Z., Ahmad, H., Abubshait, S. A., Abubshait, H. A., Laref, A., Manikandan, A., Kusuma, H. S., & Iqbal, M., 2021. "Microalgae an ecofriendly and sustainable wastewater treatment option: Biomass application in biofuel and bio-fertilizer production. A review", *Renew. Sust. Energ. Rev., 137*, 110603.

Hwangbo, M., & Chu, K. H., 2020. "Recent advances in production and extraction of bacterial lipids for biofuel production", *Sci. Total Environ.*, *734*, 139420.

Iloms, E., Ololade, O. O., Ogola, H. J. O., & Selvarajan, R., 2020. "Investigating industrial effluent impact on municipal wastewater treatment plant in vaal, South Africa", *Int. J. Environ. Res. Public Health*, *17*(3), 1–18.

Irfan, M., Butt, T., Imtiaz, N., Abbas, N., Khan, R. A., & Shafique, A., 2017. The removal of COD, TSS and colour of black liquor by coagulation–flocculation process at optimized pH, settling and dosing rate", *Arab. J. Chem.*, *10*, S2307–S2318.

Joint, I., Henriksen, P., Fonnes, G. A., Bourne, D., Thingstad, T. F., & Riemann, B., 2002. "Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms", *Aquat. Microb. Ecol.*, *29*(2), 145–159.

Kamyab, H., Din, M. F. M., Keyvanfar, A., Majid, M. Z. A., Talaiekhozani, A., Shafaghat, A., Lee, C. T., Shiun, L. J., & Ismail, H. H., 2015. "Efficiency of Microalgae Chlamydomonas on the Removal of Pollutants from Palm Oil Mill Effluent (POME)", *Energy Procedia*, *75*, 2400–2408.

Karim, A., Islam, M. A., Khalid, Z. Bin, Yousuf, A., Khan, M. M. R., & Mohammad Faizal, C. K., 2021. "Microbial lipid accumulation through bioremediation of palm oil mill effluent using a yeast-bacteria co-culture", *Renew. Energy*, *176*, 106–114.

Khadaroo, S. N. B. A., Poh, P. E., Gouwanda, D., & Grassia, P., 2019. "Applicability of various pretreatment techniques to enhance the anaerobic digestion of Palm oil Mill effluent (POME): A review", *J. Environ. Chem. Eng.*, *7*(5), 103310.

Lee, J., Lee, J., Shukla, S. K., Park, J., & Lee, T. K., 2016. "Effect of algal inoculation on COD and nitrogen removal, and indigenous bacterial dynamics in municipal wastewater", *J. Microbiol. Biotechnol., 26*(5), 900–908.

Lin, S. S., Shen, S. L., Zhou, A., & Lyu, H. M., 2021. "Assessment and management of lake eutrophication: A case study in Lake Erhai, China", *Sci. Total Environ.*, *751*, 141618.

Mao, Y., Xiong, R., Gao, X., Jiang, L., Peng, Y., & Xue, Y., 2021. "Analysis of the status and improvement of microalgal phosphorus removal from municipal wastewater", *Process.*, *9*(9), 1486.

Maxwell, D. P., Falk, S., Trick, C. G., & Huner, N. P. A., 1994. "Growth at low temperature mimics high-light acclimation in Chlorella vulgaris", *Plant Physiol.*, *105*(2), 535–543.

Mohd-Nor, D., Ramli, N., Sharuddin, S. S., Hassan, M. A., Mustapha, N. A., Ariffin, H., Sakai, K., Tashiro, Y., Shirai, Y., & Maeda, T., 2019. "Dynamics of microbial populations responsible for biodegradation during the full-scale treatment of palm oil mill effluent",

Microbes Environ., *34*(2), 121–128.

Mujtaba, G., & Lee, K., 2016. "Advanced treatment of wastewater using symbiotic co-culture of microalgae and bacteria", *Appl. Chem. Eng.*, *27*(1), 1–9.

Mujtaba, G., Rizwan, M., & Lee, K., 2015. "Simultaneous removal of inorganic nutrients and organic carbon by symbiotic co-culture of Chlorella vulgaris and Pseudomonas putida", *Biotechnol. Bioprocess Eng.*, *20*(6), 1114–1122.

Mujtaba, G., Rizwan, M., & Lee, K., 2017. "Removal of nutrients and COD from wastewater using symbiotic co-culture of bacterium Pseudomonas putida and immobilized microalga Chlorella vulgaris", *J. Ind. Eng. Chem.*, *49*, 145–151.

Najafpour, G. D., Zinatizadeh, A. A. L., Mohamed, A. R., Hasnain Isa, M., & Nasrollahzadeh, H., 2006. "High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor", *Process Biochem.*, *41*(2), 370–379.

Negi, S., Barry, A. N., Friedland, N., Sudasinghe, N., Subramanian, S., Pieris, S., Holguin, F. O., Dungan, B., Schaub, T., & Sayre, R., 2015. "Impact of nitrogen limitation on biomass , photosynthesis , and lipid accumulation in Chlorella sorokiniana", *J. Appl. Phycol.*, 28, 803-812.

Ng, M. H., & Choo, Y. M., 2016. "Improved method for the qualitative analyses of palm oil carotenes using UPLC", *J. Chromatogr. Sci.*, *54*(4), 633–638.

Nur, M. M.Azimatun, Garcia, G. M., Boelen, P., & Buma, A. G. J., 2021. "Influence of photodegradation on the removal of color and phenolic compounds from palm oil mill effluent by Arthrospira platensis", *J. Appl. Phycol.*, *33*(2), 901–915.

Nur, Muhamad Maulana Azimatun, & Buma, A. G. J., 2019. "Opportunities and challenges of microalgal cultivation on wastewater, with special focus on palm oil mill effluent and the production of high value compounds", *Waste Biomass Valorization*, *10*(8), 2079–2097.

Nwuche, C. O., Aoyagi, H., & Ogbonna, J. C., 2014. "Treatment of palm oil mill effluent by a microbial consortium developed from compost soils", *Int. Sch. Res. Notices*, *2014*, 1–8.

Putri, E. V., Din, M. F. M., Ahmed, Z., Jamaluddin, H., & Chelliapan, S., 2011. "Investigation of microalgae for high lipid content using palm oil mill effluent (Pome) as carbon source." International Conference on Environment and Industrial Innovation (IPCBEE), Singapore.

Rachmadona, N., Amoah, J., Quayson, E., Hama, S., Yoshida, A., Kondo, A., & Ogino, C., 2020. "Lipase-catalyzed ethanolysis for biodiesel production of untreated palm oil mill effluent", *Sustain. Energ. Fuels*, *4*(3), 1105–1111.

Renaud, S. M., Thinh, L. Van, Lambrinidis, G., & Parry, D. L., 2002. "Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures", *Aquac.*, *211*(1–4), 195–214.

Samsudin, A., Azmi, A. S., Nawi, M. N. M., & Halim, A. A., 2018. "Wastewater treatment by microalgae-bacteria co-culture system", *Malays. J. Microbiol.*, *14*(3), 131–136.

Shen, X., Chu, F., Lam, P. K. S., & Zeng, R. J., 2015. "Biosynthesis of high yield fatty acids from Chlorella vulgaris NIES-227 under nitrogen starvation stress during heterotrophic cultivation", *Water Res.*, *81*, 294–300.

Shen, Y., Gao, J., & Li, L., 2017. "Municipal wastewater treatment via co-immobilized microalgal-bacterial symbiosis: Microorganism growth and nutrients removal", *Bioresour. Technol.*, *243*, 905–913.

Syafiqah Hazman, N. A., Mohd Yasin, N. H., Takriff, M. S., Hasan, H. A., Kamarudin, K. F., & Mohd Hakimi, N. I. N., 2018. "Integrated palm oil mill effluent treatment and CO_2 sequestration by

microalgae. *Sains Malays.*, *47*(7), 1455–1464.

Tan, K. A., Morad, N., Norli, I., Lalung, J., & Omar, W. M. W., 2018. "Post-treatment of palm oil mill effluent (POME) using freshwater green microalgae", *Malays. J. Microbiol.*, *14*(3), 145–151.

Taufikurahman, T., & Shafira, H., 2019. "Comparison between Chlorella vulgaris and Chlorella pyrenoidosa in biomass and protein content , cultivated in bioslurry and grown under various LED comparison between Chlorella vulgaris and Chlorella pyrenoidosa in biomass and protein content , cultivate." Joint Symposium on Plant Scienes and Products, Bandung, Indonesia.

Toh, P. Y., Tai, W. Y., Ahmad, A. L., Lim, J. K., & Chan, D. J. C., 2016. "Toxicity of bare and surfaced functionalized iron oxide nanoparticles towards microalgae", *Int. J. Phytoremediation*, *18*(6), 643–650.

Tuser, C. (2020, January). *What is Wastewater?* Water & Waste Digest.

Wang, Y., He, B., Sun, Z., & Chen, Y. F., 2016. "Chemically enhanced lipid production from microalgae under low sub-optimal temperature", *Algal Res.*, *16*, 20–27.

Wu, T. Y., Mohammad, A. W., Jahim, J. M., & Anuar, N. (2010). Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes", *J. Environ. Manage.* 91(7), 1467–1490.

Yoo, C., Jun, S. Y., Lee, J. Y., Ahn, C. Y., & Oh, H. M., 2010. "Selection of microalgae for lipid production under high levels carbon dioxide", *Bioresour. Technol.*, *101*(1 SUPPL.), S71–S74.

Yu, H., Kim, J., & Lee, C., 2019. "Nutrient removal and microalgal biomass production from different anaerobic digestion effluents with Chlorella species", *Sci. Rep.*, *9*(1), 1–13.

Zhu, S., Huang, W., Xu, J., Wang, Z., Xu, J., & Yuan, Z., 2014. "Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga Chlorella zofingiensis", *Bioresour. Technol.*, *152*, 292–298.