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Research Article

Pretreatment of palm oil mill effluent (POME) for *Spirulina* cultivation

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Abstract. Palm Oil Mill Effluent (POME) is liquid waste produced from palm oil processing. The quantity of POME in Indonesia has increased from year by year. POME is not a toxic material, but it will be very dangerous if it is thrown directly without pre-processing, because it will harm the aquatic ecosystem due to its high content of COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand). POME has also a high content of nitrogen (N) and phosphor (P), which make POME can be used as medium for microalgae growth. Therefore, this research is aimed to study pretreatment and to utilize its nutrient content (N, P) for medium of spirulina growth. Pretreatment of POME research was conducted by using two stage of cultivation (*Chlamydomonas* and *Chlorella*) and dilution factor (0-4x). The result of this pretreatment was then used for *Spirulina* growth. *Spirulina* cultivation was conducted by nutrient addition and without nutrient addition. This research showed that the best performing variation of dilution rate to cultivate *Spirulina* is 4x dilution. It showed that 4x dilution could reduce COD content until 128,33 mg/L. Besides that, in 4x dilution, microalgae as *Chlamydomonas*, *Chlorella*, and *Spirulina* had the highest growth rate as compared to 2x dilution and without dilution. The best wild algae for pretreatment of POME is *Chlorella*. Because by using wild algae *Chlorella*, COD content could be reduced until 128,33 mg/L and achieve the lowest COD content compared with *Chlamydomonas*. For microalgae growth, addition of nutrient is better than without nutrient, because nutrient could enhance *Spirulina* photosynthesis.

Keywords: *Chlamydomonas*, *Chlorella*, POME pretreatment, *Spirulina* cultivation

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1. Introduction

Over the last ten years, palm oil mill effluent (POME) has undergone treatment in a sequence of ponds, including anaerobic, aerobic, or facultative ponds, to decrease the concentration of pollutants in the wastewater prior to its discharge into the river. Nevertheless, these traditional approaches are deemed unsustainable and environmentally unfriendly since they result in the release of a significant quantity of trash containing high levels of nutrients into water bodies, which in turn contributes to the production of greenhouse gases and air pollution (Campos et al., 2016). As a result of the deteriorating impact on environmental health, stricter regulations have been put in place regarding industrial discharge (Stavropoulos et al., 2017). Every palm oil mill must actively engage in research and development for improved waste management, in addition to implementing zero-waste management technologies (Abdullah & Sulaiman, 2013).

Researchers have recently shown significant interest in obtaining algal biomass cultivated in wastewater. This approach aims to reduce production costs while simultaneously providing benefits such as nutrient removal from wastewater, carbon capture, and the production of valuable biomass. This biomass can be used for various commercial applications, including animal feed, biofertilizers, and biopharmaceutical products (Bansal et al., 2018). A recent study, conducted by Nur et al. (2019), has demonstrated that POME can be used as a substrate for cultivating the marine diatom *Phaeodactylum tricornutum*, resulting in the production of sulfated exopolysaccharide and the removal of nutrients. Research indicates that the ideal concentration of POME for cultivating microalgae is 30%. Beyond this concentration, the growth rate may be hindered (Nur et al., 2019). Phycoremediation has been employed for more than 70 years in wastewater treatment to improve treatment processes by utilizing the efficient photosynthetic metabolism of organisms and effectively capturing CO2 (Delrue et al., 2016). Nevertheless, the concentration of organic and inorganic substances in the wastewater may occasionally above the optimal threshold for microalgae's adaptability. Hence, understanding the toxicological impacts and identifying the optimal concentration of non-inhibitory levels of wastewaters is crucial in order to maintain a high rate of biomass growth and achieve a high treatment capacity.

POME is distinguished by its high concentration of organic materials and nutrients, which makes it a promising and cost-effective substrate for culturing microorganisms, particularly microalgae (Kamarudin, et al., 2015). Nevertheless, like other wastewaters, POME is also recognized to include a specific spectrum of inhibitors and hazardous substances. Specifically, it comprises around 0.6–0.7%

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palm oil, 95–96% water, 4–5% total solid, and 2–4% suspended solids (Irvan, 2018). As stated in (Hariz et al., 2019), POME contains the following nutrient concentrations: 2726 mg/L of COD, 1270 mg/L of TOC, 316.7 mg/L of TN, and 257.6 mg/L of phosphate. Within this set of components, there exist chemicals that can impede the proliferation of microalgae by diminishing their growth, photosynthetic activity, enzymatic activity, and respiration (Yan & Pan, 2002). Cultivating microalgae in POME is difficult due to the light-blocking effect of the dark brown POME. Nevertheless, prior to microalgae growing, it is possible to do pre-treatment of POME (i.e. chemical, biological, and mechanical pre-treatment) in order to allow light to pass through.

Pretreatment is done with and without dilution. In this research, several things will be obtained including the optimum dilution and type of wild algae to reduce BOD and COD content with mineral content (N, P, K) that is still high enough to utilize POME as a medium for microalgae growth and development. The main objectives of this research are to treat POME to reduce COD and BOD levels so that it can be used as a medium for *Spirulina* growth, to obtain optimum conditions for POME pretreatment by dilution, and to evaluate wild algae species of *Chlamydomonas* and *Chlorella* in POME pretreatment to reduce COD and BOD levels.

2. Materials and Methods

2.1 Cultivation of wild microalgae on POME

POME waste to be used as growth media was first filtered to remove impurity solids. Furthermore, the POME used as media was varied without dilution, with 2x dilution, and with 4x dilution. The resulting media was then added with *Chlorella* and *Chlamydomonas* starters as a pretreatment process of POME waste before being used as a growth medium for *Spirulina*.

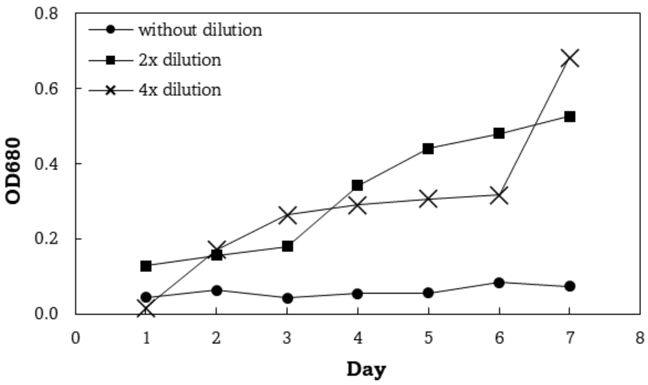
2.2 Cultivation of Spirulina on POME

Spirulina cultivation was carried out by adding microalgae stock to POME media that was varied without dilution, with 2x dilution, and with 4x dilution. Cultivation was carried out in a photobioreactor. The photobioreactor used was equipped with air aeration and lighting using lamps (12 hours light, 12 hours dark). Meanwhile, the cultivation temperature followed the room temperature (25 ± 2°C). Spirulina cultivation was also observed with and without the addition of nutrients (urea and KH_2PO_4).

3. Result and Discussion

3.1 Wild microalgae growth on POME medium

In this study, POME used as a growth medium was varied without dilution, with 2x dilution, and with 4x dilution. The POME pretreatment process was carried out using wild microalgae (*Chlorella* and *Chlamydomonas*).



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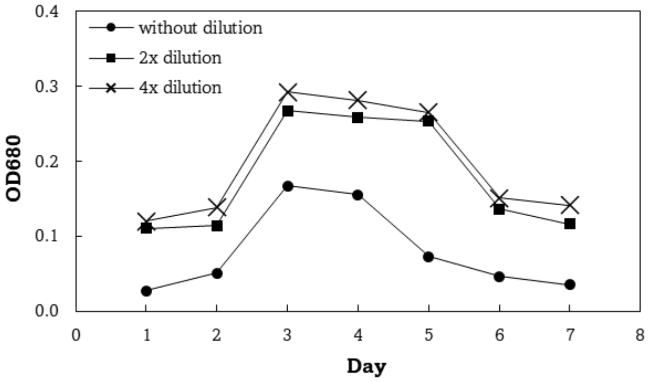

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Figure 1. Wild microalgae growth rate curve. (a) *Chlamydomonas*, (b) *Chlorella*

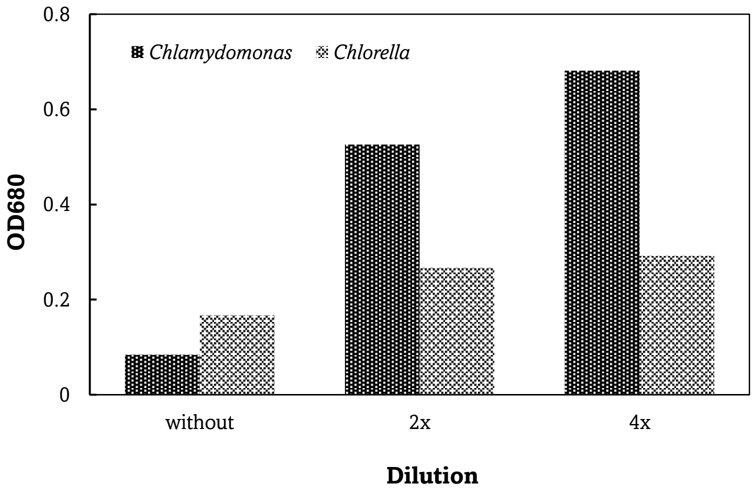


Figure 2. Maximum optical density of wild microalgae

Based on **Figure 1** and **2**, it can be seen that in the variable without dilution, *Chlorella* has better growth than *Chlamydomonas*. This is indicated by the maximum optical density of *Chlorella* which is 0.167 while *Chlamydomonas* is 0.084. However, in the 2x and 4x dilution variables, *Chlamydomonas* has a greater maximum optical density than *Chlorella*. This is indicated by the 2x dilution variable maximum optical density of *Chlamydomonas* is 0.526 while *Chlorella* is 0.267. At 4x dilution the maximum optical density of *Chlamydomonas* is 0.681 and *Chlorella* is 0.292. It can be seen that the growth rate of wild algae on POME media 4x dilution is the highest growth rate compared to media without dilution and 2x dilution. This phenomenon can occur because in media without dilution and 2x dilution the light used by wild algae to carry out photosynthesis is blocked by media that are too dark. The lack of incoming light intensity results in inhibited growth of wild algae cells in media without dilution and 2x dilution (Jasni et al., 2020).

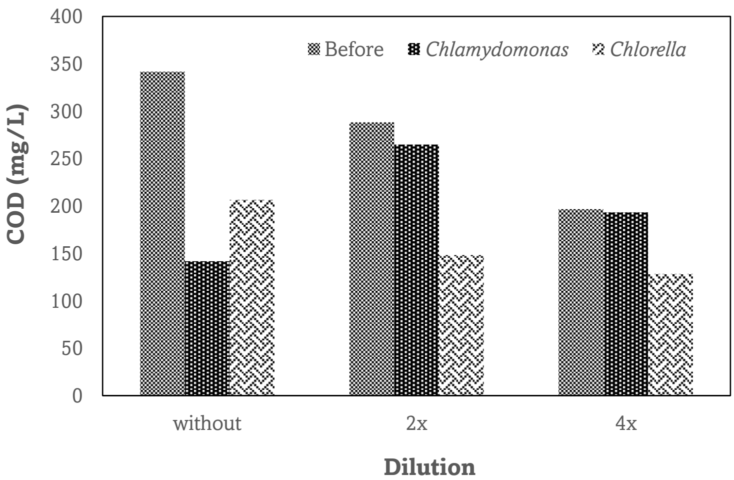
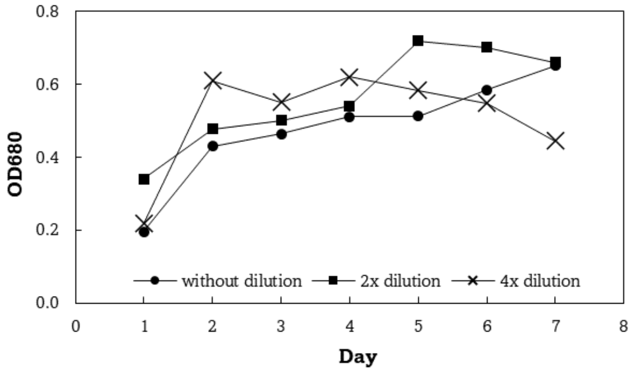


Figure 3. COD content on POME medium before and after treatment

Figure 3 show that the higher the dilution level, the COD content in POME will decrease. The COD content in POME also decreased significantly by using wild algae cultivation such as *Chlamydomonas* and *Chlorella* for 7 days. This is because the longer the residence time will provide many opportunities for microorganisms to break down the organic materials contained in the waste. This high COD value indicates that the organic matter content before wastewater treatment is very high. In the process of overhauling organic matter, microbes use organic matter as a food source. So that at the end of the treatment process using chlorella the organic matter content is getting less which results in a decrease in COD levels.

Figure 4 show that the maximum optical density of *Spirulina* is in cultivation using POME media without dilution with the addition of nutrients and using *Chlamydomonas* as wild algae to reduce the COD content in POME, which reaches 0.860. This is because *Spirulina* requires nutrients C, H, O, N, P and K to photosynthesis. It can be seen that the growth rate of spirulina on POME media 4x dilution is the highest growth rate compared to media without dilution and 2x dilution. This phenomenon can occur because in media without dilution and 2x dilution the light used by spirulina to carry out photosynthesis is blocked by media that are too dark. The lack of light intensity resulted in inhibited growth of spirulina cells in media without dilution and 2x dilution (Jasni et al., 2020).



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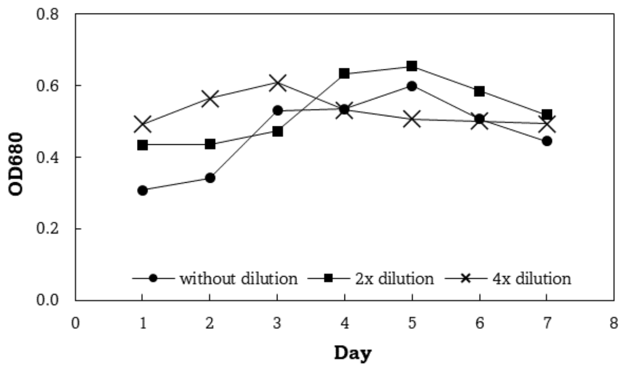

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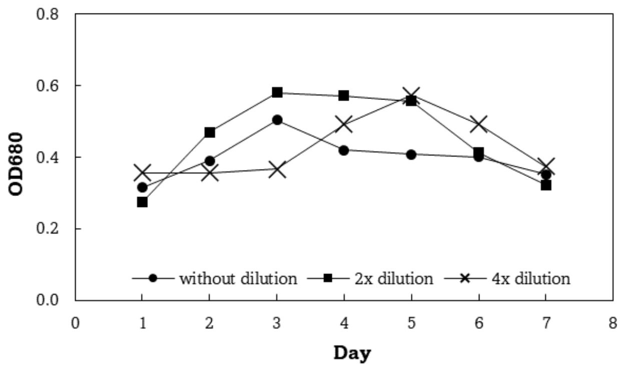

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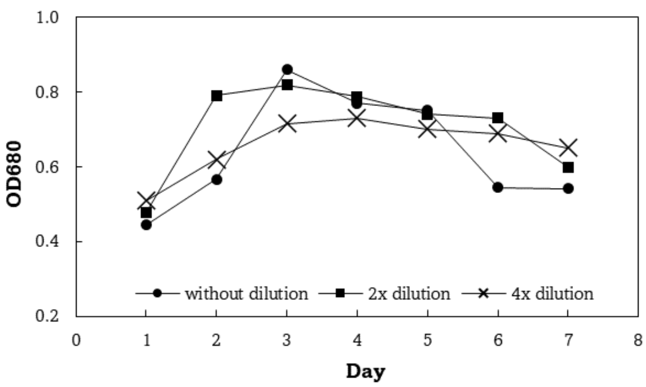

(d)

Figure 4. *Spirulina* growth rate curve. (a) POME-*Chlamydomonas* without nutrient, (b) POME-*Chlorella* without nutrient, (c) POME-*Chlorella* with nutrient, (d) POME-*Chlamydomonas* with nutrient

Table 1. Growth rate microalgae in different growth medium

Microalgae	Growth medium	1/day	Reference
Chlamydomonas	Bean sprouts extract	0.91	Prihantini (2000)
	Wellwater	0.93	
	Beneck medium	0.79	
Chlorella	Walne medium	0.584	Suminto (2008)
	Guillard's F/2 medium	0.655	
	Erdschreiber medium	0.385	
Spirulina	Freshwater	0.365	Handajani (2006)
	Saltwater	0.271	
Chlamydomonas	Freshwater	0.348	This work
Chlorella	Freshwater with urea and KH_2PO_4 addition	0.175	This work

From the **Table 1** above, it can be seen that each microalgae have a different growth rate in different mediums. This is because the growth rate of microalgae photosynthesis is influenced by several factors, including temperature, light intensity, and nutrients. Treatment using several culture media has a different effect on lag phase time. In addition, the degree of acidity (pH) also greatly affects the growth of microalgae with different tolerances. However, among the factors described above, the most influential factor on microalgae growth is nutrient composition. Complete nutrients in microalgae culture media will allow microalgae to grow well even though the medium is acidic or alkaline. If the composition of nutrients in the microalgae culture medium is poor, excessive or deficient, microalgae will tend to experience relatively constant growth in alkaline culture media (Prihantini, 2000).

4. Conclusion

The best POME pretreatment to be able to cultivate *Spirulina* is by doing 4x dilution compared to POME without dilution and 2x dilution. The best wild algae used for POME pretreatment is *Chlorella* wild algae. By using *Chlorella* wild algae, the COD content can decrease to 128.33 mg/L compared to using *Chlamydomonas* species. As a nutrient for microalgae growth, the variable with the addition of nutrients is better than without the addition of nutrients, because good nutrition will increase the growth of *Spirulina*.

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