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Microalgae as A Bioremediation Agent for Palm Oil Mill Effluent: Production of Biomass and High-Added Value Compounds

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Abstract

Palm oil mill effluent (POME) is high strength wastewater obtained from palm fruit processing, which contains high chemical oxygen demand (COD), biological oxygen demand (BOD), and other contaminants. The pollutant load in POME can serve as a source of nutrients for microalgae growth. As a result, the goal of this work was to utilize *Spirulina sp.* and *Nannochloropsis oculata* to reduce the nutritional content of POME while producing biomass rich in high-value chemicals. The cultivation was conducted in a batch reactor using various POME fractions (0-20%) under 5,000 lux light intensity and continuous aeration at a temperature of 22-28°C and a salinity of 30 ppt for 14 consecutive-days. The results demonstrate that *Spirulina sp.* produced the most biomass at 15% POME, accounting for 4.67±0.95 g/L with 0.57±0.11 1/day of growth rate and 3.33% of COD reduction efficiency. Meanwhile, *Nannochloropsis oculata* thrived in 20% POME, producing 4.43±0.36 g/L biomass, 1.18±0.31 1/day growth rate, and 14.43% COD reduction efficiency. In the proximate analysis. *Spirulina sp.* and *Nannochlorpsis oculata* provided 0.87%; 1.11% lipid and 1.03%; 0.86% protein, respectively.

Keywords: biomass, cultivation, microalgae, *Nannochloropsis oculata*, palm oil mill effluent, *Spirulina sp.,* wastewater

1. Introduction

Palm oil is an agricultural crop that rapidly grows in Indonesia and produces more than 18 million tons of oil per year, as a feedstock for vegetable oils. The demand for palm oil in Indonesia is estimated at approximately 20 million tonnes by 2025 to meet food and industry needs (Khatiwada et al., 2021). As a result, the production is also followed by the rise of palm oil mill effluent (POME).

To avoid environmental pollution, further processing is needed in this circumstance. POME contains a large amount of organic matter, in particular carbohydrates, amino acids, free organic acids, organic pollutants, fiber, and other organic substances (Abdulsalam et al., 2018). POME also has inorganic compounds needed to grow plants like nitrogen, phosphorus, calcium, sodium, and potassium (Alam et al., 2022). Susilo et al (Susilo et al., 2016) reported that the microorganism activity can be used in the biotechnological treatment processes to remove nutrients or pollutant load in POME such as microalgae.

Microalgae are microscopic single-celled organisms of various sizes, shapes, and types, with photosynthetic pigments, and photoautotrophs, and found in aquatic environments (Dayana et al., 2022). Microalgae can be found in a type of photosynthetic microorganisms that is capable of providing valuable compound such as lipids which may have the potential to make biofuels through transesterification (Chowdury et al., 2020; Randrianarison and Ashraf, 2017).

Currently, various studies are continuously carried out to obtain microalgae strain that can grow and well develop in the Indonesian region for further use such as bioremediation. The utilization of microalgae cultivated in POME is one of the efforts to degrade and convert almost all organic materials in the effluent. Microalgae that can be used in POME treatment are *Spirulina sp.* and *Nannochloropsis oculata*.

Spirulina sp. is a species of blue green algae with high protein content, containing amino acids, lipids, fatty acids, carbohydrates, vitamins and minerals (Sofiyah & Suryawan,

2021). Meanwhile, *Nannochloropsis oculata* has a fairly high fat content with a total amount ranging from 37% to 60% dw (Putra et al., 2022). The growth of *Spirulina sp.* and *Nannochloropsis oculata* is strongly influenced by the availability of nutrients and environmental conditions (Buwono and Nurhasanah, 2018; Sukmawan, Antara and Arnata, 2014).

Based on the influence of microalgae growth and waste characteristics, POME is deemed to be one of the possible materials that can be employed as microalgae growth medium to produce biomass (A, Khaswar and D, 2023; Mahdi *et al.*, 2022). Cheah et al. (2018) found out that *Chlorella sorokiniana* grew in 30% of acid heat pretreated POME resulting 2.12 g/L of biomass and 11.21% of lipid. Emparan et al. (2019) also reported that *Nannochloropsis sp.* produced 1.27 g/L of biomass at 10% of POME. Meanwhile, Erlangga et al. (2019) obtained 184.1 mg/L of *Chlorella pyrenoidosa* biomass while cultivated in 25% of POME. In this study, the effect of various POME concentrations (0-20%) on biomass production, growth rate, and COD removal efficiency was investigated by employing two different local marine microalgae, namely *Spirulina sp.* and *Nannochloropsis oculata* grown in Kw 21 fertilizer with no further nutrient addition. Aside from it, the COD removal efficiency was also measured along with the value-added compounds contained in biomass using proximate analysis.

2. Methodology

2.1. Materials

The materials used in this study were *Spirulina sp.*, *Nannochloropsis oculata*, POME waste, tofu waste, NaCl solution (SAP, Indonesia), H_2SO_4 (SAP, Indonesia), $KMnO_4$ (SAP, Indonesia), $C_2H_2O_4$ (SAP, Indonesia), and NaOH (SMARTLAB, Indonesia). *Spirulina sp.* and *Nannochloropsis oculate* were purchased from Jembrana Marine and Fisheries Polytechnic.

2.2. Cultivation of Microalgae

Microalgae cultivation equipment, including containers (Batch Reactor) with a volume of 1 liter, an aerator, aquarium hose, TL lamp, oven, petri dish, pH meter, refrigerator, plastic tube and buch funnel are the tools employed in this research. Cultivation of *Spirulina sp.* and *Nannochloropsis oculata* was performed by using a flask which acted as a bioreactor with a total working volume of 1 liter (Figure S2). As much as 20% (v/v) of culture was mixed with POME and saline water with a ratio of 0%:80%, 5%:75%, 10%:70%, 15%:65% and 20%:60% (v/v).

In this research, the cultivation was conducted at a temperature of 22-28°C and a salinity of 30 ppt. The reactor (Figure 1) was operated in a laboratory with a light intensity of 5000 lux referring to Palanisamy et. al., Hamidi et.al., and Widihastuti et. al. (Palanisamy et al., 2021; Hamidi et al., 2023; Widihastuti et al., 2022). In this study, the cultivation process was carried out for 14 days with a batch system. On the final cultivation day, harvesting was carried out using filter paper connected to a vacuum pump. The analysis of optical density (OD) and COD was carried out every day towards the medium. Whereas, the final suspended biomass was analyzed using the proximate method.

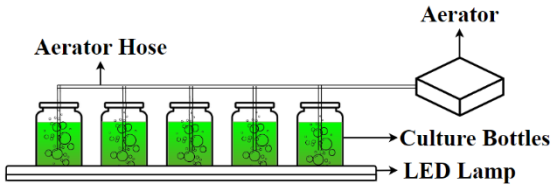

Figure 1. Installation batch reactor for cultivation of microalgae

2.3. Optical Density (OD)

To analyze the OD, a spectrophotometer was used with the type of Ori. The spectrophotometer was used to measure the absorbance of each sample as a function of wavelength (λ) which was 750 nm. Before the sample analysis, it was necessary to calibrate the spectrophotometer by inserting a blank solution (aquades) into the cuvette and then placing it in the spectrophotometer to observe the absorbance. The scale shown must be 0 because the blank solution is a pure solvent. In the next step, cultivation samples were taken sufficiently using a measuring pipette, then put into a cuvette and placed into the cuvette holder. Growth of *Spirulina sp.* and *Nannochloropsis oculata* was determined by OD at a wavelength of 750 nm. There is a direct relationship between optical density and dry biomass. This relationship is obtained by testing carried out on the control medium which is then connected to the graph. With linearization, standard curves for the following variables can be obtained (Figure S3) and (Figure S4).

2.4. Measurement Of Specific Growth Rate

The growth rate of POME content on the growth of *Spirulina sp.* and *Nannochloropsis*

oculata was analyzed daily after 14 days. The specific growth rate (μ) is calculated by Eq. (1) as follows:

$$\mu = ln\frac{(OD_1/OD_0)}{(t_1/t_0)} \quad (1)$$

Remark:
μ (1/day) : specific growth rate
OD : optical density on day
t (day) : time

2.5. Chemical Oxygen Demand Analysis

The COD analysis was undertaken based on Dewi et al method (Dewi et al., 2022). To analyze the COD content in the sample, 10 ml of 0.01 N $C_2H_2O_4$, 5 ml of 4 N H_2SO_4, and $KMnO_4$ solution were needed. It is necessary to standardize the KMnO4 solution by mixing 10 ml $C_2H_2O_4$ 0.01 N, 5 ml $H2SO_4$ 4 N into an Erlenmeyer, then heating it to a temperature of 70-80°C. Then the solution was titrated with $KMnO_4$ until the color changed to red wine (b ml). The normality of KMnO4 was calculated using Eq. (2) below:

$$N_{KMnO_4} = \frac{N_{C_2H_2O_4} \times V_{C_2H_2O_4}}{V_{KMnO_4}} \quad (2)$$

Remark:
N : normality
V (ml) : Volume

Analysis of the COD content was conducted by mixing 10 ml of sample, 5 ml of 4 N H_2SO_4, and 3.2 ml of $KMnO_4$ solution into an Erlenmeyer. Then the mixture was heated up to a temperature of 70-80°C. Afterwards, 10 ml of 0.01 N $C_2H_2O_4$ was added. The solution was titrated with standardized $KMnO_4$ and recorded the required volume of $KMnO_4$ (a ml). COD was calculated using Eq. (3) below:

$$COD = \left[(a \times b)N_{KMnO_4} - (V \times N)_{C_2H_2O_4}\right] \times 8000 \quad (3)$$

Remark:
COD (ppm) : chemical oxygen demand
N : normality
V (ml) : volume

2.6. Proximate Analysis

The proximate analysis of microalgae biomass consists of water and ash content by a gravimetric method in accordance with SNI 2354.2:2015, and SNI 2354.1: 2010. Whilst the lipid content was conducted by solid-liquid extraction based on SNI 2354.3- 2017 using a Soxhlet, and protein content was determined by calculating the total nitrogen according to SNI 01-2354.4:2006.

2.7. Data Analysis

Data obtained were collected in triplicate. One-way analysis of variance (ANOVA) was performed using IBM SPSS version 26 software with a confidence level of 95%. Further analysis (Tukey test) was applied to determine which concentration factor had a significant effect ($p < 0.05$).

3. Results and Discussion

3.1. Effect of POME Fractions

As demonstrated in Figure 2, *Spirulina sp.* and *Nannochloropsis oculata* successfully grew for 14 days in a batch mode on all POME fractions. The nitrogen, phosphorus, and carbon sources possessed essential roles in the growth rate and biomass of both microalgae, which led to a higher outcome when compared to control due to the nutritional content of POME (Table 1).

Table 1. Characteristics of POME

Parameter	Unit	Result
Physical Test		
pH	-	8.36
Temperature	°C	24.4
TDS	mg/L	7,800
TSS	mg/L	1,115
Turbidity	NTU	925
Salinity	%	3.2
Chemical Test		
Ammonia as N	mg/L	1.43
BOD5	mg/L	578.45
COD	mg/L	1,652.7
Nitrate as N	mg/L	11.5
Oil & Grease	mg/L	0.78
Total Phosphate	mg/L	15.01
Total N	mg/L	215

Based on Figure 2 the addition of POME had a positive impact since the biomass kept increasing especially when it reached the exponential phase. The amount of 215 mg/L of N, 15.01 mg/L of P, and 619.76 mg/L of C boosted up the growth resulting in the high biomass production. Albeit the optimal C:N:P ratio for microalgae growth is 56:9:1 (Nur et al., 2019; Sari et al., 2022) where the ratio in this study was around 14.283:14.324:1, both microalgae were still able to grow in POME with no extra nutrients. According to Samanta et al. and Lie et al. (Samanta et al., 2013; Li et al., 2019), microalgae can adjust the C, N, and P concentrations in their biomass to the C, N, and P supply in the wastewater for effective C, N, and P removal. As a result, the nutrients in POME were successfully utilized during cultivation.

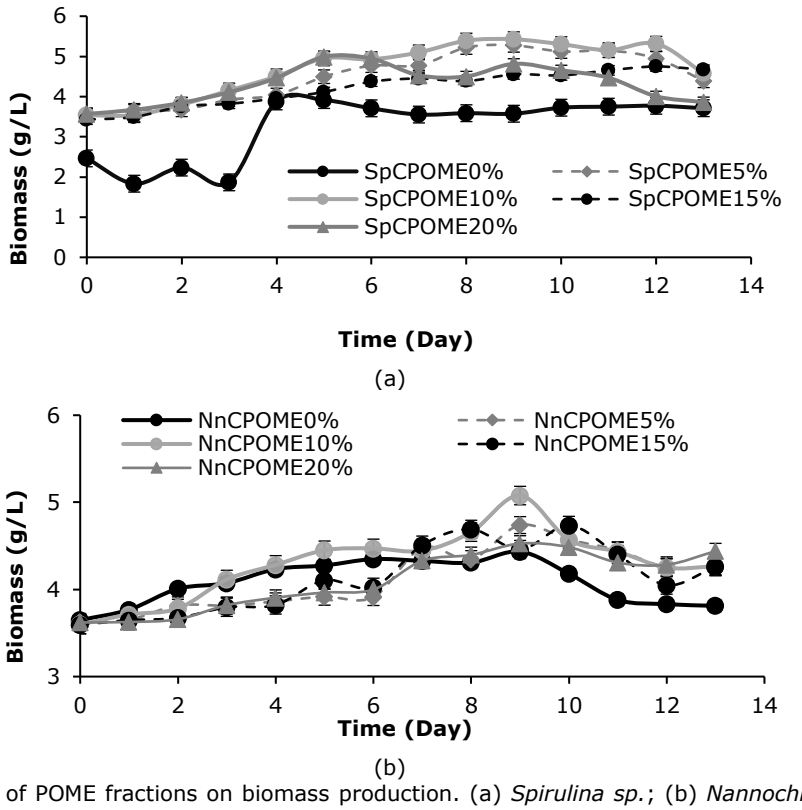

Figure 2. Effect of POME fractions on biomass production. (a) *Spirulina sp.*; (b) *Nannochloropsis oculata*. Average values are shown (n = 3).

There are several stages to the growth of microalgae, encompassing (1) the lag phase, during which microorganisms adapt to their medium; (2) the exponential phase, which is characterized by rapid growth and visible active cells; (3) the stationary phase; (4) the phase of decreased growth; and (5) the death phase (Chowdury, Nahar and Deb, 2020; Istirokhatun, Aulia and Utomo, 2017; Lee, Jalalizadeh and Zhang, 2015; Pradana et al., 2020). The lag phase in this study was very short, which was less than 48 hours. This phenomenon occurred because the strain of both microalgae was added when it was at the exponential phase (Istirokhatun et al., 2017).

Cell division initiates the exponential/log phase, which is characterized by an increase in growth rate that raises cell density (Lee et al., 2015). According to Figure 2, the lowest exponential phase occurred on days 4-5 with the lowest biomass produced at 0% (control). Control merely consisted of distilled water which had fewer nutrient sources than POME. Hence, it inhibited the growth of *Spirulina sp.* and *Nannochloropsis oculata*. Meanwhile, the highest exponential phase varied from one to another for *Spirulina sp.* There was an obvious difference between 0%, 20% (on days 5-6) and 5-15% (on days 10-11) of POME fractions. However, *Nannochloropsis oculata* managed to achieve the highest biomass yield on the same days (day 10-11).

The stationary phase could not be monitored in this study since the growth of cell density was calculated once every 24 hours (Istirokhatun et al., 2017). The distance between the decreasing phase and the stationary phase is generally relatively short, so that calculations are needed with intensities more than once in 24 hours. The following phase is the decreasing phase, which is distinguished by a reduction in cell density. *Spirulina sp.* and *Nannochloropsis oculata* both experienced varied reductions in cell density depending on the POME concentrations. The quickest decline in *Spirulina sp.* occurred on days 6-8 for 0%, 5%, and 20% of POME fractions. While at 10-15%, the decreasing phase was attained on day 11-12. As opposed to this, *Nannochloropsis oculata* reached the decline phase on days 9-10 for 0–10% and on day 10-11 for 15-20%, respectively. Overall, both microalgae experienced a declining phase between days 10-11. This stage took place as a result of the waste's nutrients starting to deplete over time. Correspondingly, a cell's rate of growth was lower than its rate of death (Chowdury, Nahar and Deb, 2020; Istirokhatun, Aulia and Utomo, 2017).

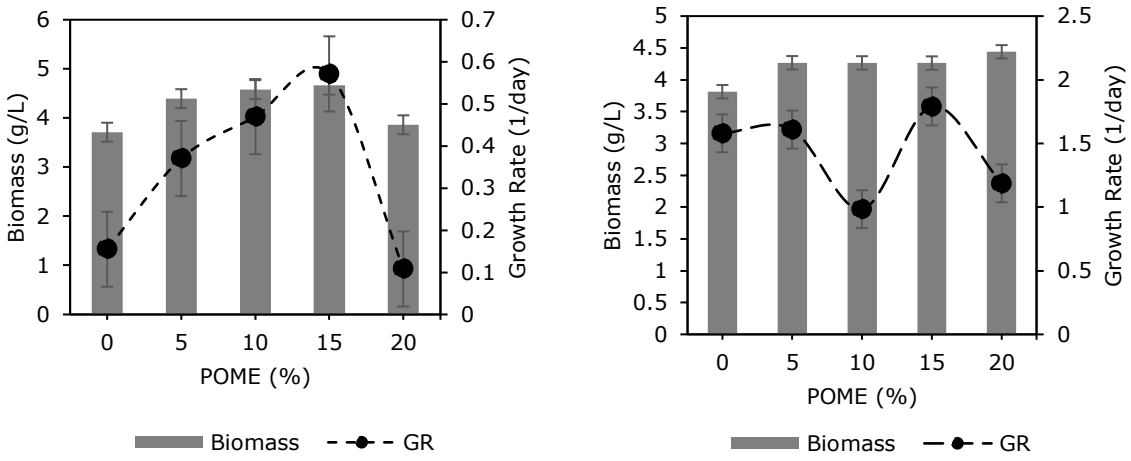

Figure 3. Effect of POME fractions on biomass and growth rate on 14th Day. (a) *Spirulina sp.;* (b) *Nannochloropsis oculata*. Average values are shown (n = 3). Sharing letters indicate insignificant value (P > 0.05) at confidence levels of 95%.

The death phase is the subsequent stage. On days 11 and 12, *Nannochloropsis oculata* entered the death phase, where the concentration of POME was proportional to the time of cell death which was getting longer. This was so that cells' long-term nutritional needs could still be met by POME's nutrient content. In *Spirulina sp.*, the death phase occurred on day 13. *Spirulina sp.* had low resistance to the high POME fractions so nutrients could not be completely consumed by algal cells which led to the inhibitory effect. Whereas, *Nannochloropsis oculata* was able to survive at 20% of POME implying that this strain was more resistant to high pollutants load. This research supports earlier research, which showed that *Spirulina plantesis* (Christwardana and Hadiyanto, 2022*;* Effendi, Nurrachmi and Tarigan, 2021*;* Palanisamy *et al.*, 2023) and *Nannochloropsis sp.* in POME continued to grow after 4 days (Emparan, Harun and Jye, 2019; Palanisamy *et al.*, 2023). However, the majority of the exponential phase reached on day 10 resulting in the highest biomass production.

Figure 3 shows that adding 5-20% POME had a notable effect in comparison to the control. Indicating that microalgae in this fraction were able to utilize the waste to its fullest extent. The maximum growth rate and biomass for *Spirulina sp.* was at 15% and for *Nannochloropsis oculata* was at 20%, which amounts to approximately 0.57±0.11 1/day; 4.67±0.95 g/L and 1.18±0.31 1/day; 4.43± 0.36 (P > 0.05). However, the growth rate and biomass of *Spirulina sp.* were reduced by 20% of POME, which resulted in lower biomass but did not generate a significant difference (P > 0.05); these values were 0.10±0.03; 3.86±0.18 g/L, respectively. According to Dewi et al. and Gramegna et al. (Dewi *et al.*, 2022; Gramegna *et al.*, 2020), an abundance of nutrients can limit the amount of biomass that can be generated for certain microalgae.

Proportionately, microalgae took more time to acclimate to the media. *Spirulina sp.* cells' inability to react as a result of the 20% of POME components limited or even completely stopped their growth. The overbalance of wastewater was arduous to digest causing toxic metabolites to appear (Wang *et al.*, 2010; Wang *et al.*, 2018). Interestingly, *Spirulina sp.* produced the most biomass out of the two studied strains. *Nannochloropsis oculata* was found to be more resistant in POME, even though the change was quite marginal. This finding was in line with Hadiyanto et al and Shah et al research (Hadiyanto *et al.*, 2017; Shah *et al.*, 2014) that *Nannochloropsis oculata* can survive at 10-30% POME fraction obtaining 0.04-0.091 g/L. *Spirulina sp.* produced 267 mg/L/day biomass in digested POME utilizing a continuous photobioreactor (Suharyanto et al., 2014).

In this study, the turbidity of POME could prevent light from penetrating the medium, resulting in a reduction in growth (Nur et al., 2022). To lessen the POME's color intensity, the pre-treatment process must be carried out physically, biologically, and chemically before further step (Low et al., 2021). Some pre-treatment methods that can be performed to decrease the turbidity of POME are shown in Table 2.

Table 2. Pre-treatment methods in wastewater for POME

Input Media	Pre-treatment Method	Microalga	Yield of Productivity	Final Effluent (% COD)	References
Digested POME	Dilution in water lily (8 days)	*Spirulina platensis*	0.945 g/L biomass	96.9	(Hadiyanto et al., 2014)
Digested POME	Addition of activated carbon	*Spirulina dimorphus*	0.17 g/L biomass	77.4	(Takriff et al., 2016)
Raw POME	Filtration, centrifugation and autoclave	*Isochrysis galbana*	0.142 mg/L/day biomass	80.1	(Shah et al., 2014)
		Pavlova lutheri	0.130 mg/L/day	63	
Digested POME	Filtration	*C. sorokiniana*	n.a	90.3	(Nwuche, 2014)

3.2. Effect of Microalgae on COD Decreased Level in POME

The COD was a crucial indicator selected to evaluate the POME's quality since, as organic matter, it was closely related to the degree of overall pollution. The POME initial COD (1,652 mg/L) needs to be reduced to a manageable level because it was too high. The total COD significantly dropped to 700-750 mg/L between 0-14 days (Table 3). The time of COD breakdown may be attributed to the microalgal metabolism, which is active in absorbing the organic fraction for their growth and biomass synthesis. On day 14, the POME showed COD reductions that allowed for the increase of biomass ($P > 0.05$). During a 14-day cultivation period, the maximum COD removal efficiencies were 12.67% for *Spirulina sp.* and 14.43% for *Nannochloropsis oculata*. The final COD ($P > 0.05$) was reported lower than the initial one ($P < 0.05$). It is interesting to observe that the relationship between the COD reduction and POME concentrations was linear where the highest reduction for both microalgae was achieved at 20% POME fraction. Due to the contaminants load at greater concentrations increased as well.

The microalgae used organic matter as a carbon source for growth, resulting in a decrease in COD over the treatment period. Organic carbon is a crucial source for the development of microalgal cells. Carbon makes up the majority of the content of microalgal cells (Emparan et al., 2019). Microalgae culture is a bioprocess that may remove 80-90% of inorganic nutrients from wastewater while simultaneously providing biomass with a high added value at a reasonable cost (Salama *et al.*, 2017; Tawfik *et al.*, 2022). Nonetheless, the removal efficiency in this study did not surpass previous findings because there was no pre-treatment step conducted as shown in Table 1. Discart et al (Discart et al. 2014) added that at the same time microalgae produced organic matter that may be responsible for the increase in COD.

The death cell of microalgae could also take a role in this phenomenon creating a higher final COD result on the final cultivation period. Likewise, the microalgae cells were in the heterotrophic growth metabolism. Arroyo et al reported that mixotrophic cultivation can produce 3-4 times higher biomass than heterotrophic did, respectively (Heredia-Arroyo et al., 2011).

Mixotrophic is a condition where additional nutrients are added in the medium so microalgae can survive in a more sufficient condition. This circumstance usually occurs while cultivating microalgae in the wastewater. Abdel et al (Abdel-Raouf et al., 2012) and Nur et al (Nur et al., 2023) found that wastewater medium still consists of low nutrient sources for microalgae. Hence, the addition of nutrients is required to generate its energy or cell metabolism called a mixotrophic cultivation condition.

Table 3. COD results at various POME concentrations

POME (%)	Initial COD Concentration (ppm)		Final COD Concentration (ppm)		COD Removal Efficiency (%)	
	SP	**NO**	**SP**	**NO**	**SP**	**NO**
5	748.38^{ab}	705.37^{a}	730.96^{a}	670.96^{a}	2.33	4.88
10	774.19^{ab}	748.38^{b}	748.38^{a}	713.97^{a}	3.33	4.60
15	774.19^{ab}	756.98^{b}	748.38^{a}	722.58^{a}	3.33	4.54
20	756.98^{b}	834.40^{b}	748.38^{a}	713.97^{a}	12.67	14.43

SP (*Spirulina sp.)* and NO (*Nannochloropsis oculata);* All values are an average of means (n = 3)
Sharing letters indicates significant value ($P < 0.05$) at a 95% confidence level

Many investigations on various species of microalgae grown in POME media for COD removal have been published. Hadiyanto et al (Hadiyanto Hadiyanto et al., 2017) removed COD from 10%, 30%, and 50% POME samples using suspended free cells of *Nannochloropsis oculata*, resulting in a 34% COD reduction. Emparan et al (Emparan et al., 2019) investigated *Nannochloropsis oculata* cells immobilized in biological sodium alginate at 10% POME produced COD removal of 55%. Suharya et al (Suharyanto et al., 2014) used *Spirulina platensis* in a continuous photobioreactor with a 90% POME fraction resulting in a 5% COD reduction. Hadiyanto et al (H Hadiyanto et al., 2014) obtained a 50% COD decrease when cultivating *Spirulina sp.* in 35%, 50%, and 65% with a pre-treatment process using the aquatic plant.

In this regard, different species of robust microalgae can be blended in the future to achieve improved COD removal effectiveness accompanied by the pretreatment process of POME. Several researchers have documented the successful usage of *Nannochloropsis oculata*, *Spirulina sp.* cells and other microalgal species to remove COD from POME (Ahmad *et al.*, 2015; Hadiyanto, Soetrisnanto and Christwardhana, 2014; Rajkumar and Takriff, 2015). According to earlier research, biomass concentration and COD removal effectiveness were impacted by POME concentration and the kind of microalgae species employed for POME treatment.

3.3. Proximate Analysis of Microalgae Biomass

The high added value of proximate content was observed in microalgae biomass as shown in Table 4. The analysis was performed at the highest biomass produced in *Spirulina sp.* and *Nannochloropsis oculata.*

Table 4. Result of proximate analysis of microalgae biomass

Parameter	% Content	
	Spirulina sp.	*Nannochloropsis oculata*
Water	91.05	75.65
Ash	4.27	7.49
Lipid	0.87	1.11
Protein	1.03	0.86

Proximate composition showed that the water content ranked as the highest composition in the biomass since the analysis was performed using wet biomass. As previously mentioned by (Baumgardt *et al.*, 2016; Cecchin *et al.*, 2020; Ma *et al.*, 2016), *Nannochloropsis oculata* is considered a marine microalga for food and fuel purposes. *Nannochloropsis oculata* is regarded as a possible oleaginous model microalga due to its high photosynthetic efficiency, high lipid yield (37-60% of dry weight), well-established genetic toolbox, and relatively mature technology for large-scale outdoor growing systems. Meanwhile, *Spirulina sp.* is reported to consist of a high amount of protein. Therefore, this alga is potentially used as a functional food and supplement with protein content ranging from 65-70% of dry weight (Liestianty *et al.*, 2019; Suherman *et al.*, 2022).

The concentration of lipids and protein in microalgae remained low in this investigation. Aside from the wet biomass analysis, the culture factor in POME could be the cause of the lower recovery of certain chemical components. Chemicals such as nitrate and $FeCl_3$ added to the wastewater medium can help enhance the recovery of lipid and protein proteins in microalgae (Nur et al., 2022).

4. Conclusion

Microalgae were cultivated in a variety of POME concentrations (%) for research purposes. POME has the potential to be employed as a medium for *Spirulina sp.* growth at a 15% fraction, yielding 4.67±0.95 g/L biomass, 0.57±0.11 1/day, and 3.33% COD reduction efficiency. Meanwhile, *Nannochloropsis oculata* produced 4.43±0.36 g/L biomass, 1.18±0.31 1/day, and 14.43% COD reduction efficiency at 20% POME. *Nannochloropsis oculata* was shown to be more resistant in POME because it can survive in larger concentrations of wastewater. In this study, the lipid and protein compounds remained low, indicating that cultivation in POME should be altered by adding chemical compounds to increase the contents.

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Supplementary Materials

Figure S1. Cultivation of Microalgae

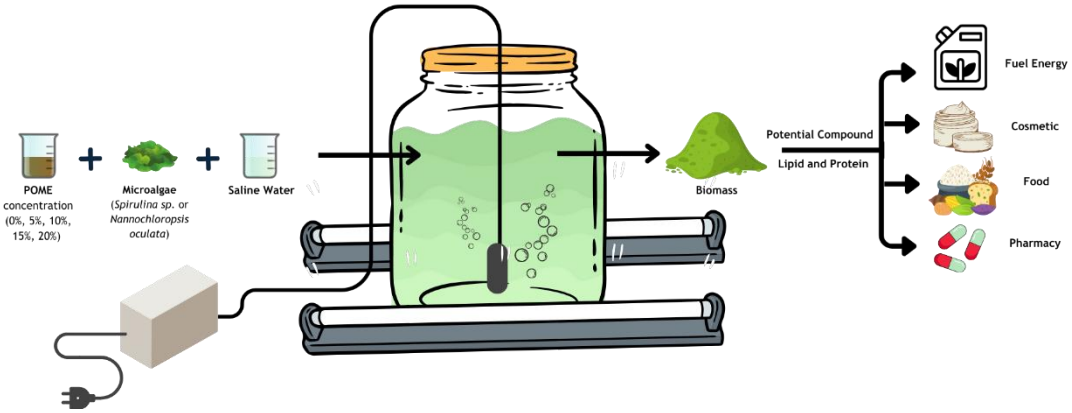

Figure S2. Cultivation of Microalgae Setup

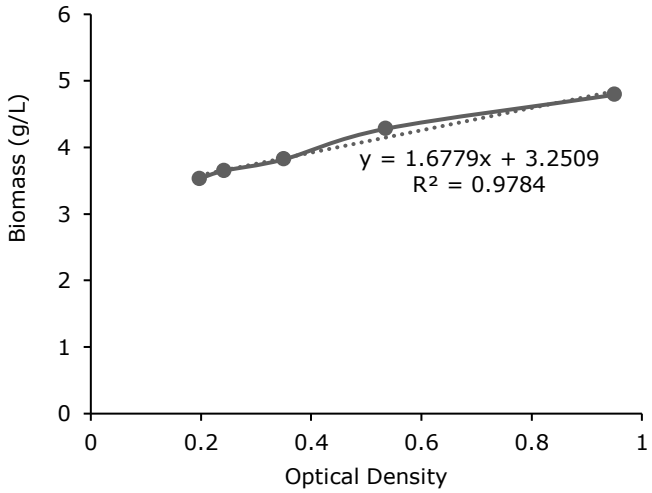

Figure S3. Standard Curve of *Spirulina sp.*

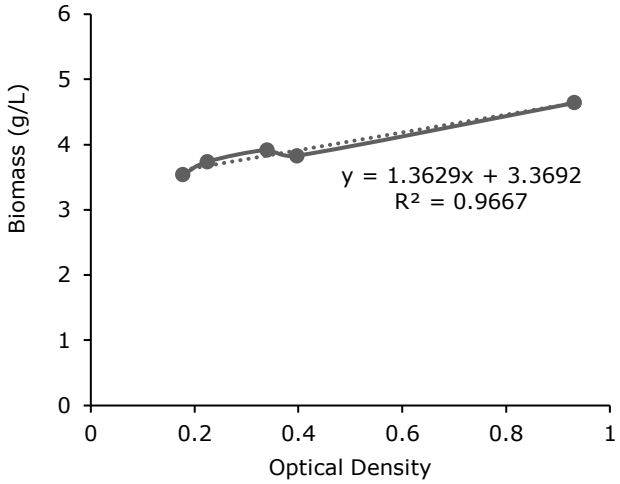

Figure S4. Standard Curve of *Nannochloropsis oculata*