Biomass Composition of Microalgae Local Mixed Culture using POME (Palm Oil Mill Effluent) Medium

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Abstract
Palm Oil Mill Effluent (POME) as a microalgae local mixed culture medium is useful for recycling waste water as well as a source of nutrients by utilizing inorganic materials to synthesize lipids, carbohydrates, and proteins for microalgae local mixed culture. POME has fundamental nutrients and organic materials COD and BOD. This research was conducted for 7 days of cultivation of local mixed culture grown in Bold's Basal Medium (BBM) as a control medium and medium contained POME with concentrations of 2.5%, 5%, 7.5%, 10%, 12.5%, and 15% as treatments. The parameters observed included cell count, dry weight, carbohydrates, proteins and microalgae lipids content and reduction of BOD and COD.

The results showed that POME increased the growth of local mixed culture by 1.80 x 10^6 cells/ml and biomass by 0.33 mg/ml at 10% concentration respectively. Optimum lipid content was 99.27 mg/ml at 10% concentration, optimum carbohydrate was 30.32 mg/ml at 12.5% concentration, and optimum protein was 2906 µg/ml at 10% concentration. BOD and COD on POME medium decreased after cultivation with local mixed culture.

Keywords: POME, microalgae, growth, biomass.

Introduction

New renewable energy is an important aspect in line with the decline in fossil fuel production (petroleum) in the world. Moreover, petroleum fuel subsidies continue to increase in various countries because they use them as fuel for transportation and industrialization. The use of fossil fuels continuously is not environmentally friendly, has no aspects of sustainability and increases climate change. Plus the loss of fossil fuels is air pollution due to the incomplete combustion process[17].

Many researchers have turned to developing renewable and environmentally friendly fuels, one of which uses microalgae. Microalgae is a resource for third generation biodiesel energy. The advantage of microalgae is that it produces high biomass without the need for large tracts of land capable of utilizing inorganic materials to synthesize lipids, the raw material for biodiesel production. Microalgae are able to solve two problems namely air pollution due to carbon dioxide and a crisis of energy needs. In addition, microalgae are able to grow in wastewater under high salinity and alkalinity, tolerant of temperature fluctuations, high carbon conditions, and variations in light intensity[9].

Handling of liquid waste by using it as a microalgae culture medium has benefits because it is useful in recycling liquid waste as well as a source of nutrients for microalgae. One local mixed cuture of microalgae developed by the Faculty of Biology, Universitas Gadjah Mada, is the Glagah isolate. Glagah isolate is a consortium of microalgae isolated from Glagah beach, Yogyakarta. Based on Suyono et al[33,34] research percentage of Glagah isolate lipids in open ponds (raceway pond) was around 2.9% and lipid content reached 4.5 mg/L with lipid cell quota reaching 2.64 n/g cells and productivity of 0.71 mg/L/day. Increasing lipid content in Glagah isolate can use liquid waste in addition to using standard medium because they contain carbon residues and inorganic materials that can be utilized. This can reduce the cost of microalgae cultivation.

Several studies have reported the use of POME (Palm Oil Mill Effluent), which is liquid waste from palm oil processing as a medium for cultivating microalgae, and its potential to increase the content of metabolites such as lipids and bioremediation[9]. Moreover, Indonesia is a major producer of palm oil since 2008 with a productivity of palm oil in 2015 amounted to 31,070,015 tons and estimated productivity in 2016 amounted to 33,229,381 tons and in 2017 amounted to 35,359,384 tons[15].

Palm oil production certainly produces liquid waste in the form of POME. In 1 ton of oil palm fresh fruit bunches can produce 0.6 tons of POME. The content of pollutants in POME is quite high seen from Chemical Oxygen Demand (COD) of 15,103-65,100 mg / L and Biological Oxygen Demand (BOD) of 8,200-35,000 mg/L. Total Suspended Solids (TSS) are around 1,330-50,700 mg/L.

POME still has a high nutrient for microalgae growth with ammonia content of 12-126 mg/L. Liquid waste such as POME must be treated before being discharged into the environment. The palm oil industry uses facultative anaerobic ponds to reduce COD and BOD content, but has a negative impact on the environment because it produces CH_4 and CO_2 into the atmosphere and requires extensive land and long hydraulic resident times (HRT)[30].

In addition, other minerals such as Fe, Zn, P, Mg, Ca, and K needed by microalgae are found in POME[22]. So treatment using microalgae is one solution to overcome these problems.

From the facts above, the researchers tried to find out the optimization of growth, biomass, and lipid content, carbohydrates, and protein culture consortium of microalgae Glagah isolates in POME medium. Glagah isolates are expected to be cultivated in POME medium capable of producing growth (cell/ml), biomass (mg/ml). Lipid percentage (% dry weight), lipid content (mg/L), lipid cell quota (ng/cell) and lipid productivity (mg/L/day) are higher than the control medium (BBM) and total nitrogen (mg/ L) and phosphorus (mg/L) in POME before and after cultivation using Glagah isolates.

Material and Methods

Making Bold's Basal Medium (BBM): Medium BBM was made by dissolving nutrient stock according to the recipe with distilled water. Nutrient stock consists of macronutrient ($NaNO_3$, $MgSO_4.7H_2O$, NaCl, K_2HPO_4, KH_2PO_4, $CaCl_2.2H_2O$, H_3BO_3, EDTA), micronutrient and iron ($FeSO_4.7H_2O$).

Making POME Medium: POME medium was sterilized with autoclave and then diluted by distilled water with the composition as in table 1.

Microalgae treatment and cultivation: Cultivation was carried out in 500 mL culture vial by mixing 450 mL BBM medium and tofu wastewater medium (5%, 7.5% and 10%) each with 50 mL of Glagah isolate mixed culture stock (initial cell density ± 2.9 x 10^2 cells/mL).

Furthermore, cultivation was carried out for 7 days with aeration and irradiation for 24 hours (light intensity 2000-2500 lux). POME was filtered with an 80-mesh filter to reduce solid suspension. POME was stored in a refrigerator at 4° C. Some essential parameters of POME are calculated such as COD and BOD which are calculated before and after cultivation.

Cell Count: Calculation of the number of cells was carried out every day for 7 days of observation with 1 mL of culture taken and put in a 2 mL tube. Furthermore, the number of cells was calculated using a haemocytometer under a microscope equipped with optilab with a magnification of 100x. The cell count is calculated based on the following formula[27]:

$$number\ of\ cells\ (\frac{cell}{mL}) = \frac{cell\ counts}{5}\ x\ 10^4$$

Calculation of doubling time (td) is based on a formula:

$$t_d = \frac{ln2\ t}{ln.N_t\ /N_0}$$

where Nt = number of cells at the end of the exponential phase, N_0 = number of cells at the beginning of the exponential phase and t = time interval.

Meanwhile, the specific growth rate (μ) is based on a formula[32]:

$$\mu = \frac{0.693}{t_d}$$

Dry Weight: Total biomass was measured by the gravimetric method which was modified. Microalgae culture biomass was measured by measuring dry weight, previously weighed by empty tube, dry weight measurement was carried out by taking 15 ml of sample and then put in a tube. Next, it was centrifuged at 8000 rpm for 15 minutes. Then, the supernatant part is removed and left for the pellet. After that, put in the oven at a temperature of 30-34°C overnight (overnight) until a constant weight is reached. Dry weight calculation is done based on the following formula[32]:

$$Dry\ weight\ (\frac{mg}{mL}) = \frac{(final\ weight\ of\ sample\ -\ empty\ tube\ weight)}{sample\ volume}$$

Biomass productivity is calculated by the formula[23]:

$$B = \frac{\Delta X}{t}$$

where ΔX is the difference between biomass concentration on 4 and 0 days and t is time.

Table 1
Composition of POME medium

POME Medium	Composition in 1000 mL medium	
	Aquades (mL)	POME (mL)
2.5 %	975	25
5 %	950	50
7.5 %	925	75
10 %	900	100
12.5 %	875	125
15%	850	150

Lipid content: Lipid content was measured using the Bligh and Dryer method[5]. 10 ml microalgae samples were centrifuged for 15 minutes at 4000 rpm, the pellets formed were separated and the supernatant was then counted in volume. Every 1 ml of pellet is added with 2 ml of methanol and 1 ml of chloroform and then vortexed. After mixing, the pellet was added with 1 ml of chloroform and 1 ml of distilled water and then divortexed again. Then the mixture was centrifuged for 15 minutes. After the separation occurs, the yellow underside is lipid and it taken with a dropper. Chloroform was evaporated in an ESCO isotherm oven at 40°C so that only lipids remain. Lipid weight was obtained from the difference between the weight of the empty cup and the weight of the cup with lipids.

Lipid productivity (L mg L-1 day-1) is calculated by the following formula[23]:

$L = \Delta X/t$

where Δx is the difference in lipid concentration between 4 and 0 day and t is time.

Carbohydrate Content: 5 ml of sample was taken and then centrifuged at 3300 rpm for 10 minutes and the supernatant removed. Then the pellet was added with 5% phenol as much as 500 µL, then divortexed and allowed to stand for 10 minutes. After that 1 ml concentrated sulfuric acid was added through the wall, then divortexed and allowed to stand for 20 minutes. Making a standard curve from standard glucose with a concentration of 0.025 g/L; 0.05 g/L, 0.1 g/l; 0.25 g/L and 0.5 g/L, absorbance was measured using a spectrometer with a wavelength of 490 nm[11].

Protein content: Calculation of protein content is done by Bradford method. The standard protein used is Bovine Serum Albumin (BSA). The concentrations used are 1, 2, 3, 4, 5, 6, 7 and 8 ppm. Absorbance was measured using BioTek ELISA Reader at a wavelength of 595 nm. Protein content testing is done by the following method. Samples were taken as much as 2 mL and put in a 2 ml tube. The sample was centrifuged at 3000 rpm for 10 minutes. The pellet were then sampled. The pellets were added with 1 ml of SDS 10% solution. Then the sample is heated at 95° C for 5 minutes.

Samples were put in the refrigerator at 4°C for 5 minutes. Samples were taken as much as 8 µl with a P20 scale micropipette and put into a 500 µl microplate. Samples were added with 200 µl Bradford reagent. After that the data was read by the BioTek ELISA Reader at a wavelength of 595 nm. The protein content is determined using a standard curve.

Data Analysis: Data taken were cell count, dry weight, chlorophyll, carbohydrate, protein, and lipids analyzed using Microsoft Excel and continued with statistical analysis using ANOVA and DMRT to determine whether there were any real differences between treatments. Then the data was presented in the form of graphs and histograms.

Results and Discussion

Microalgae growth is influenced by nutrient content both macro and micro. The need for absorption of these nutrients is influenced by temperature, light, and pH[7]. POME is known to have high nutrient content such as nitrogen and phosphorus, and other nutrients as well as BOD and COD[18]. Some previous studies have shown that POME can be used as a microalgae growth medium and its nutrient content can affect the growth rate and biochemical content in microalgae. This shows that the composition of nutrients in the medium can affect the metabolism of microalgae.

This research was conducted with the aim to determine the effect of using POME on the growth of Glagah isolate mixed culture including cell density, biomass, lipids, carbohydrates, proteins, BOD and COD. The medium used is POME with concentrations of 2.5%, 5%, 7.5%, 10%, 12.5% and 15%.

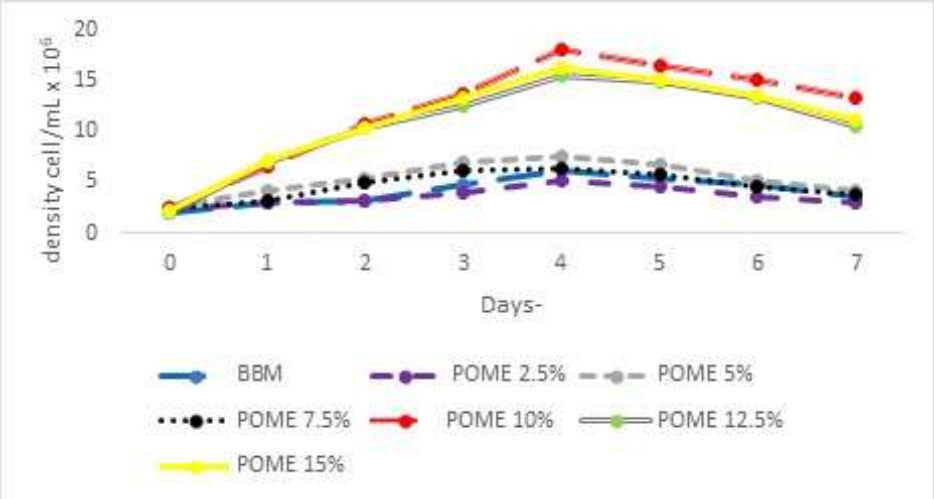

Figure 1: Number of cells (mL-1 cells) of Glagah isolate culture in cultivation using BBM medium and medium containing POME

The control treatment used as a comparison is the Bold Basal Medium (BBM). The cell count is done periodically every 24 hours starting from day 0 to day 7.

The growth phase includes the lag phase which is the initial period of slow growth, the second exponential or log phase is where growth and division are fast, the third phase is of relative growth reduction which occurs when cells experience limited growth, the fourth is the stationary phase where there is a balance of cell growth with cell death. The death phase is the phase where cell death is greater than cell growth[26]. All treatments both control and POME experienced a peak logarithmic phase on day 4 (Figure 1). On that day also the Glagah isolate mixed culture biomass reached the highest value (Figure 2).

Increased growth of Glagah isolate mixed culture cells is caused by positive interactions between Glagah isolate mixed cultures and POME medium. The logarithmic phase occurs because the mixed culture of Glagah isolate is able to absorb nutrients in POME waste optimally.

DMRT test results showed that the use of POME as a cultivation medium at various concentrations was significantly different from the control treatment. However, based on the graph in the POME treatment, the concentration of 10% has the highest average value than other concentrations of 1.80 x 10^6 cells / ml (Figure 1). Nutrients that play a role in the growth of Glagah isolate mixed cultures are nitrogen in the form of ammonium and nitrate[6].

Glagah isolate mixed culture utilizes phosphates and nitrates found in POME as a source of nutrients to help cell division. POME has rich BOD, COD, nitrate, and phosphate contents. Growth in the number of cells is positively related to an increase in the amount of POME medium concentration.

Based on the six POME treatments, the highest cell count was shown at a 10% concentration treatment that was 1.80x10^6 cells/ml on the 4th day where a logarithmic or exponential phase occurred. Figure also shows that at 10% the concentration has the highest biomass. This is because in addition to nutrients, Glagah isolate mixed cultures need light to carry out photosynthesis and metabolize. A nutrient-rich medium and organic matter in addition to making high cell count growth can also inhibit because it inhibits the penetration of light used by mixed Glagah isolate cultures to photosynthesize and metabolize[15,31].

The combination of nutrients and organic material that is too rich causes turbidity so that the color of the medium becomes black[14]. This makes the culture of Glagah isolate mixed cultures cultivated on 15% POME medium which do not produce large cell growth numbers like the 10% POME medium.

Figure 2 shows the change in biomass content during 7 days of cultivation which overall showed fluctuation. The highest biomass was shown at a concentration of 10% on the 4th day of 0.33 mg/ml. However, in the DMRT analysis both the control treatment and the POME medium did not show significant difference. The content of mixed biomass culture of Glagah isolate cultivated in POME medium showed a correlation with the results of cell number growth (Figures 1 and 2).

The increase in the amount of biomass is directly proportional to the increase in cell density. Therefore, the yield of dry biomass from the dashing mixed culture will also increase with the concentration of POME media in accordance with the results of cell density.

Microalgae can grow well in conditions where there is a source of nitrogen. The increase in microalgae biomass increases with the addition of POME concentrations. According to Viena[36], the addition of different POME to the conditioned microalgae culture will show an increase in the amount of biomass. This is due to microalgae using nitrates for cell growth.

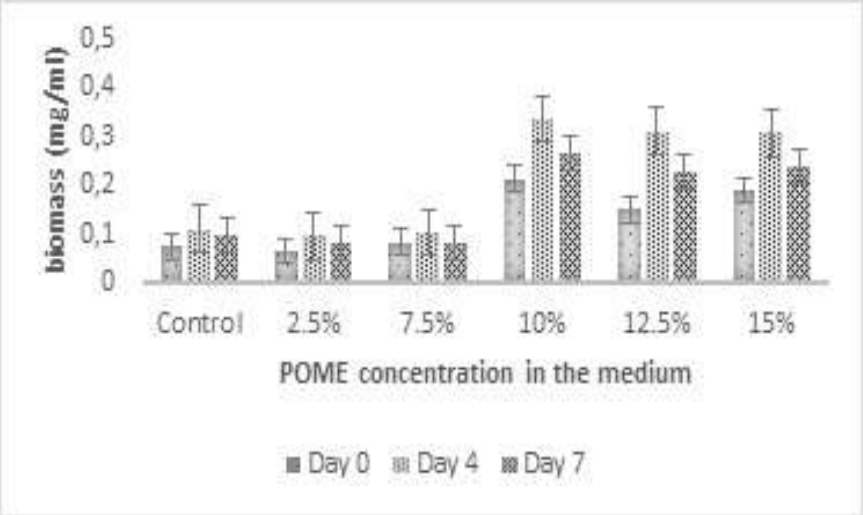

Figure 2: Dry weight (mgml) of Glagah isolate culture on cultivation using POME

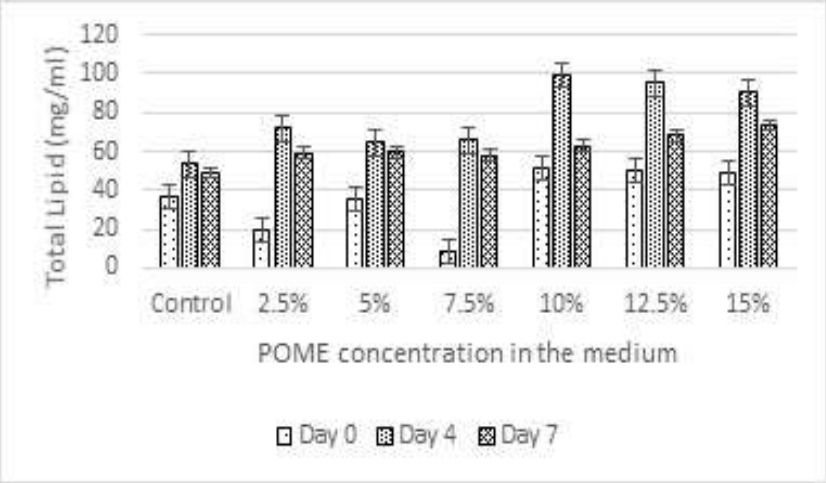

Figure 3: Lipid content (mg/ml) of Glagah isolate culture on cultivation using POME

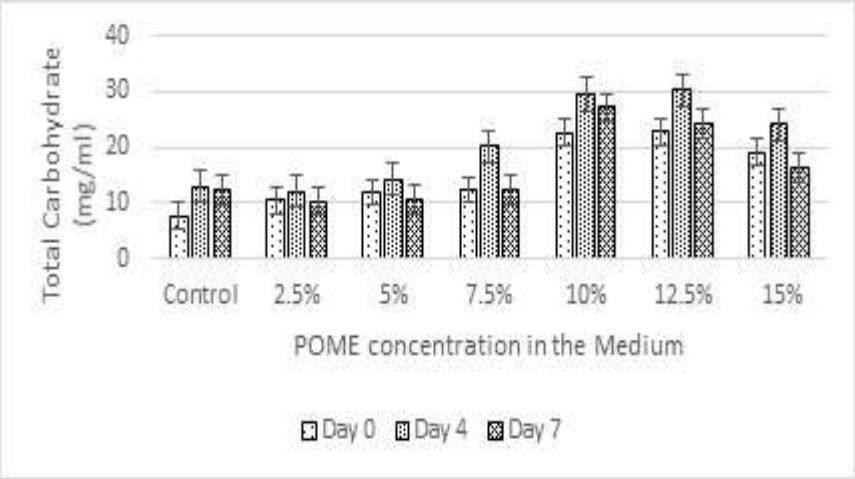

Figure 4: Carbohydrate content (mg/ml) of Glagah isolate culture in cultivation using POME

From figure 3 we get the fact that the highest total lipid content was produced by the Glagah isolate mixed culture on the 10% POME medium on the 4th day with a value of 99.27 mg / ml. However, based on Anova and DMRT test at 5% level, it showed that the lipid content of Glagah isolate mixed culture in the control treatment was not significantly different from all POME medium treatments.

Microalgae store lipids in a variety of ways and depend on species, growth conditions, growth phase, and harvest time. When microalgae cells grow actively, the main lipids found in cells are in the form of glycolipids and phospholipids. The overall lipid profile changes when microalgae enter an inactive growth phase or face environmental stress. Microalgae will slow the rate of proliferation and begin to store triacylglycerides (TAGs) as the dominant lipids in cell vacuoles[6].

Biomass and lipids are used to find the strains with the best absorption of nutrients to optimize lipid production. In other words, high biomass yield is positively correlated with high lipid production[19]. Figure explains that a 10% POME concentration on the 4th day produces the highest biomass and lipid content. These results indicate that the production of lipids is directly proportional to the biomass produced.

Manipulating environmental stress and stress tolerance of microalgae are widely used to increase lipid production. Nutrients that lack mainly nitrogen are one of the most effective induction factors for triggering and increasing lipid accumulation in microalgae. Lipid content can be increased by reducing nitrogen nutrient sources[38]. However, in this study the POME medium has high nitrogen and organic matter content so that the lipid content obtained is not significant.

The culture of Glagah isolate mixture on the medium 12.5% on the 4th day when the exponential phase occurred resulted in a total carbohydrate value of 30.32 mg/ml. Carbohydrates are formed in the chloroplast but also in the cytosol (in the case of prokaryotes carbohydrates synthesized in the cytosol). Carbohydrate production in microalgae has two main objectives, namely as a structural component in cell walls and as a component of storage in cells. Carbohydrates as storage compounds provide energy needed for the

organism's metabolic processes and allow temporary survival in dark conditions[12,29].

In general, storage of compounds such as proteins, lipids, and carbohydrates allows microalgae to adjust their growth to changing environmental conditions[21]. Carbohydrates include broad categories of both monosaccharides and their polymers (di-, oligo-, and polysaccharides).

Nitrogen is an essential nutrient for microalgae growth. It plays a role in the formation of vital components such as DNA, proteins, pigments[24]. As a photosynthetic organism, Glagah isolate mixed cultures have pigments that are used to harvest light energy. Chlorophyll is the main pigment of photosynthesis. Chlorophyll consists of tetraphyrrole rings containing magnesium, nitrogen, and long chain terpenoid atoms. This makes the provision of nitrogen can increase the formation of chlorophyll. Increased chlorophyll formation can increase the rate of photosynthesis and more energy production. Nitrogen is an important element in the growth of microalgae.

Nitrogen is an important nutrient after carbon formation in biomass. Nitrogen is generally available in the form of nitrate (NO_3^-) and ammonia (NH_4^+)[33]. The source of nitrogen in this experiment is POME. High nitrogen content in the medium increases the synthesis of chlorophyll pigments in cells. As the chlorophyll content increases, the rate of photosynthesis will also increase. If the photosynthesis process increases, carbohydrates produced from the photosynthesis process will also increase. Carbohydrates are formed through the process of photosynthesis, most carbohydrates are used as an energy reaction to respiration.

Carbohydrates are used for energy sources. Energy produced through photosynthesis is used to increase the number of cells stored in the form of carbohydrates, especially starch and cellulose. In this study, an increase in carbohydrate production in photosynthesis is an energy source. Microalgae have the ability to change their biomass composition under stress conditions to accumulate more carbohydrates[34].

Phosphorus is also an important nutrient that plays a vital role in the formation of organic molecules such as RNA, DNA, nucleic acids, phospholipids, and ATP[12]. Low concentrations of phosphorus are stored as polyphosphates. The polyphosphate functions are stored as regulators of intracellular orthophosphate concentrations and they are depleted under phosphorus starvation. Carbohydrates, as mentioned above, are synthesized in chloroplasts and in the cytosol. When the cytosolic concentration of phosphorus is low, triose phosphate is stored in chloroplasts and then carbohydrates are synthesized[34].

Moreover, it is known that carbohydrate synthesis is not a process of consuming inorganic phosphorus and that ADP-glucose pyrophosphorylase, an enzyme that controls carbohydrate synthesis is activated by 3-phosphoglycerate, and is inhibited by inorganic phosphorus.

Therefore, the level of carbohydrate accumulation, in eukaryotes and prokaryotes is determined by the ratio of 3-phosphoglycerate to inorganic phosphorus[13]. POME has a high organic phosphorus content which is then used by microalgae for photosynthesis[14].

Based on figures 5, the results showed that the highest protein content of Glagah isolate mixed culture at a concentration of 10% POME medium was 2906 µg/ml on the 4th day. Anova and DMRT test at 5% level showed that the total protein content and protein productivity of microalgae Glagah isolate mixed cultures in control treatments were significantly different from the treatment of POME medium concentration of 10%, 12.5%, and 15%. In addition, the nutrient content in POME has a positive correlation with protein production.

Nitrogen is used as a source of nitrogen in the formation of proteins in the form of ammonium and nitrate. Phosphorus is used as a universal energy source in photosynthesis, respiration, and the process of protein formation. One of the nutrient content in POME that causes an increase in the number of Glagah isolate mixed cultures is nitrate.

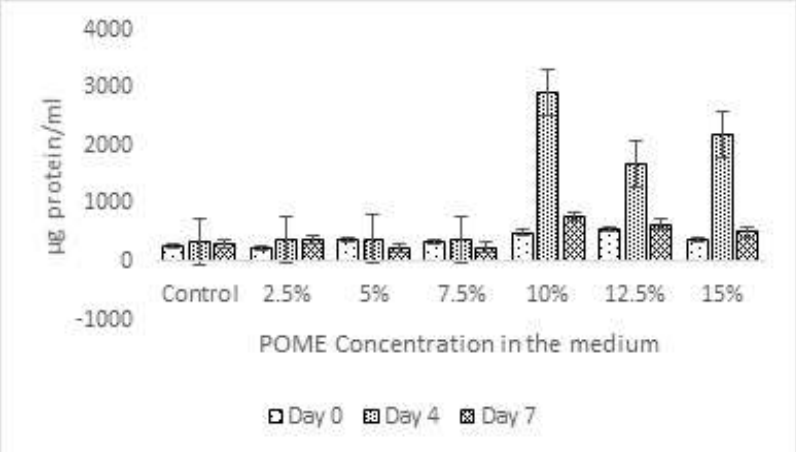

Figure 5: Protein content (µg/ml) of Glagah isolate culture in cultivation using POME

However, increasing the concentration of POME medium inhibits protein production. It is assumed that the high nitrogen content in the form of nitrate in POME has a contribution in inhibition. Glucose is a useful carbon source for microalgae. Increased growth and respiration rate can be influenced by carbon or glucose sources compared to other carbon sources, where glucose is used by microalgae to form organic compounds such as lipids and proteins[28].

If the nitrogen and phosphorus content are balanced, then biomass will have a high protein content. The N: P ratio of microalgae is not only influenced by the N: P ratio of culture media, but also differs between various species or groups of microalgae. P is mainly used in microalgae cells to produce rRNA, which is part of the ribosome. Fast-growing microalgae species have high rRNA content to produce new proteins and tend to have high P content[20].

Free amino acids, nucleotides, and urea are forms of organic N which are highly bioavailability for microalgae. Peptides or proteins have slightly lower bioavailability[8]. Usually part of the organic N present in wastewater is slowly provided by bacteria that live in symbiosis with microalgae[25]. This causes the concentration of 10% POME medium to have a high protein content, because it provides sufficient nutrients and is not inhibited, besides the turbidity level that inhibits photosynthesis is not as much as a 15% concentration. It also correlates with cell density in Glagah isolate mixed culture. The higher are the number of cells, the higher is the protein content produced.

BOD explains the ability of microalgae to oxidize organic matter into CO_2 and water using oxygen molecules as oxidizing agents. Therefore, BOD can deplete dissolved oxygen[10]. Therefore, BOD is a measure of the demand for microorganism respiration in metabolizing organic material in wastewater. A decrease in BOD indicates the use of nutrients[1].

In this study there was a decrease in BOD number in each treatment of POME concentration for 7 days which proves the use of organic material for metabolism. In addition, the decrease in BOD figures is evident that Glagah isolate mixed culture is capable of being a bioremediation agent.

POME with a high content of organic substances begins to degrade well by microalgae. In the adaptation phase, microalgae can survive until the lag phase so that nutrients are gradually reduced. The reduction of nutrients in POME is in line with the decrease in organic matter. Yunardi[39] reported that the performance of microalgae is better than bacteria in reducing the COD concentration because microalgae can grow in water containing low carbon.

This is because of the ability to utilize dissolved carbon from the air. Figure 7 shows the ability of Glagah isolate mixture culture to remove organic substances and nutrients in POME for 7 days. The analysis shows that microalgae have the ability to reduce organic substances and nutrients in POME. Cultivation of Glagah isolate mixed culture in POME for 7 days shows its ability to reduce organic, nutritional, and produce biomass production, carbohydrates, proteins, and lipids.

Microalgae converts waste into carbon (zero waste) and produces high density liquid energy[37]. The efficient growth of microalgae in wastewater depends on critical variables, including pH, temperature, and nutrient availability. POME contains nitrogen mostly in the form of ammonia. However, the balance between ammonia (NH_3) and ammonium (NH_4^+) depends on pH and temperature. The ratio of ammonia to ammonium increases 10-fold for each unit of pH increase. Some researchers report the negative effects of ammonia on the growth of microalgae associated with pH.

Ammonia at concentrations of over 2 mM and pH of more than 8 in high-level oxidation ponds inhibited the growth of microalgae[2]. pH in the growth medium is important because it can affect the binding of metals to the cell wall. This process involves the transfer of protons which depends on the level of protonation which is determined by the pH of the surrounding media. On the other hand, pH parameters can affect growth through increased absorption of inorganic nutrients.

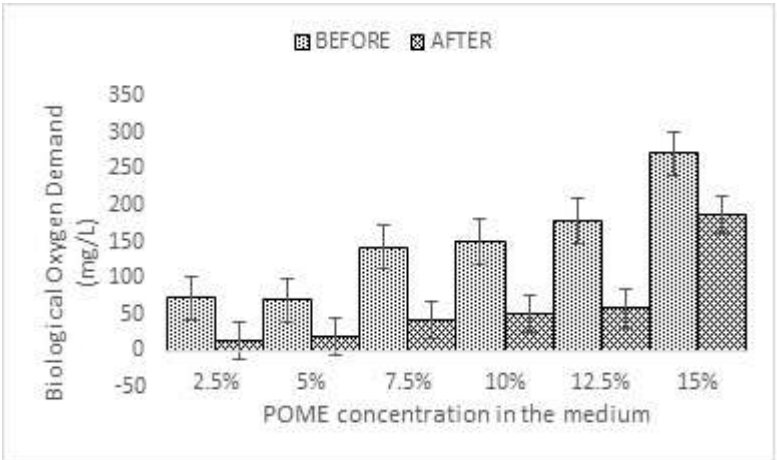

Figure 6: BOD (mg/L) Glagah isolate culture before and after cultivation using POME

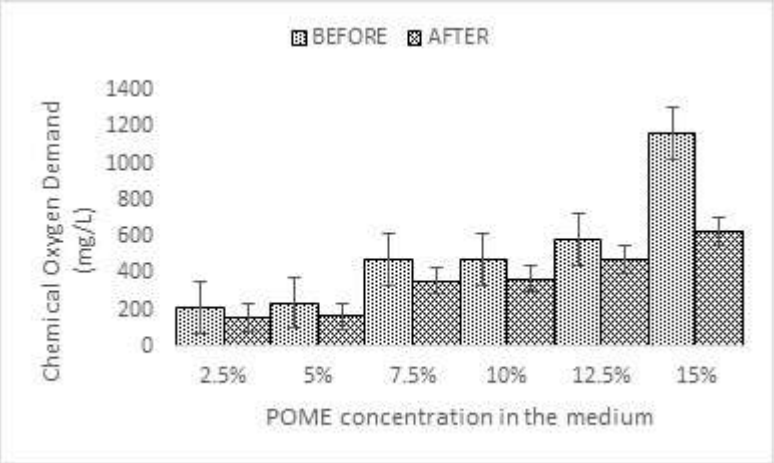

Figure 7: COD (mg/L) of Glagah isolate culture before and after cultivation using POME

Table 2
Glagah culture pH indicator on cultivation using POME

Days	0	1	2	3	4	5	6	7
Control	7	7	7	7	7	7	7	7
2.5%	5	5	5	6	6	6	6	6
5%	5	5	5	6	6	6	6	6
7.5%	5	5	5	6	6	6	6	6
10%	5	5	5	5	5	5	5	6
12.%	5	5	5	5	5	5	5	6
15%	5	5	5	5	5	5	5	6

In this study the control concentration had a normal pH of 7 whereas the concentration of 2.5%, 5%, 7.5% had an initial pH of 5, then on the 4th day it rose to 6. Concentrations of 10%, 12.5%, and 15% had an initial pH of 5 and only rose to 6 on day 7.

The results obtained in this study are consistent with other studies where most species of microalgae grow optimally around neutral pH (7.0-7.6), although the optimal pH is the initial culture pH where microalgae adapts to grow[4], but these microalgae cannot grow at pH 10. Therefore, changing the pH in the media can limit algal growth through inhibition of metabolism. Under acidic conditions, nutrients can be absorbed to be changed and thus affect the growth of microalgae[16]. In addition, pH levels at concentrations of 10%, 12.5%, and 15% were more acidic and positively correlated with cell density, biomass, carbohydrate, lipid, and protein contents higher than concentrations of 2.5%, 5%, and 7.5%.

Conclusion

The use of POME waste increased the growth of Glagah isolate mixed cultures by 1.80x 10^6 cells/ml and biomass by 0.33 mg/ml on the 4th day at a concentration of 10%. Optimum lipid content of 99.27 mg/ ml at a concentration of 10%, optimum carbohydrate content of 30.32 mg/ml at a concentration of 12.5%, optimum protein content of 2906 µg/ml at a concentration of 10%, BOD and COD on POME medium decreased after cultivation using Glagah isolate mixed culture.

Acknowledgement

This work was supported financially by Ministry of Research, Technology and Higher Education, Republic of Indonesia through Directorate of Research, Universitas Gadjah Mada, Indonesia.

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(Recceived 22nd November 2019, accepted 01st February 2020)