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Utilization of Palm Oil Liquid Waste for Media Culture and Analysis of Chemical Composition of Microalga *Skeletonema costatum*

Pemanfaatan Limbah Cair Kelapa Sawit untuk Media Kultur dan Analisis Komposisi Kimia Mikroalga Skeletonema costatum

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Abstract

This research was conducted in October 2022 at the SUPM Natural Feed Labor/Maritime and Fisheries Polytechnic Pariaman. The purpose of this study was to obtain a suitable dose of palm oil wastewater for culturing *Skeletonema costatum* microalgae, to analyze the chemical composition of *Skeletonema costatum* microalgae, to identify the ability of *Skeletonema costatum* to utilize nitrate and phosphate content in palm oil wastewater, to see the effect of *Skeletonema costatum* on water quality which has been contaminated with palm oil liquid waste. The method used was an experimental method with a one-factor Completely Randomized Design (CRD) consisting of 6 (six) treatment levels and 3 (three) replications. The treatment given was palm oil wastewater with different concentration treatment levels, namely 0%, 10%, 15%, 20%, 25%, 30% with 3 (three) repetitions so that a total of 18 experimental units, culture carried out for 6 days. The results showed that palm oil wastewater had an effect on the abundance of *Skeletonema costatum*. The best treatment for *Skeletonema costatum* culture media was P5 (300 ml/L), cell abundance 329,260 cells/mL, biomass (dry weight) 7.27 g, chlorophyll-a 0.84, nitrate 0.759 mg/L, phosphate 0.301 mg/L, protein 30.70%, fat 0.291% and carbohydrates 21.96%. Meanwhile, a good CO_2 is P0 (control), the best dissolved oxygen level is P5 (300 ml/L). Water quality parameters for a temperature of 16^0C, pH 8.2.

Keywords: Palm Liquid waste, Abundance, Nitrate, Phosphate

Abstrak

Penelitian ini dilaksanakan pada bulan Oktober 2022 di SUPM Pakan Alami Tenaga Kerja/Politeknik Kelautan dan Perikanan Pariaman. Tujuan dari penelitian ini adalah mendapatkan takaran air limbah kelapa sawit yang sesuai untuk budidaya mikroalga *Skeletonema costatum*, menganalisis komposisi kimia mikroalga *Skeletonema costatum*, mengetahui kemampuan *Skeletonema costatum* dalam memanfaatkan kandungan nitrat dan fosfat pada air limbah kelapa sawit, melihat pengaruh Skeletonema costatum terhadap kualitas air yang telah terkontaminasi limbah cair kelapa sawit. Metode yang digunakan adalah metode eksperimen dengan Rancangan Acak Lengkap (RAL) satu faktor yang terdiri dari 6 (enam) taraf perlakuan dan 3 (tiga) ulangan. Perlakuan yang diberikan adalah air limbah kelapa sawit dengan taraf perlakuan konsentrasi berbeda yaitu 0%, 10%, 15%, 20%, 25%, 30% dengan 3 (tiga) kali pengulangan sehingga total terdapat 18 satuan percobaan, budidaya dilakukan selama 6 hari. Hasil penelitian menunjukkan bahwa air limbah kelapa sawit berpengaruh terhadap kelimpahan

Skeletonema costatum. Perlakuan terbaik pada media kultur *Skeletonema costatum* adalah P5 (300 ml/L), kelimpahan sel 329.260 sel/mL, biomassa (berat kering) 7,27 g, klorofil-a 0,84, nitrat 0,759 mg/L, fosfat 0,301 mg/L , protein 30,70%, lemak 0,291% dan karbohidrat 21,96%. Sedangkan CO_2 yang baik adalah P0 (kontrol), kadar oksigen terlarut yang terbaik adalah P5 (300 ml/L). Parameter kualitas air untuk suhu 16^0C, pH 8,2.

Kata kunci: Limbah Cair Kelapa Sawit, Kelimpahan, Nitrat, Fosfat

1. Introduction

The development of oil palm plantations in Indonesia has increased very significantly. Riau Province has the largest oil palm plantation in Indonesia, with an area of 2,399,173 hectares in 2014 (Mahajoeno et al., 2008). Biogas Plant Tandun (2015) reported that PKS biogas effluent contained macronutrients, namely N = 675 mg/L, P = 90-100 mg/L, K = 2,400 mg/L, Ca = 860 mg/L, and Mg = 800 mg/L, as well as micronutrients, namely Fe= 126 mg/L, Mn= 9.22 mg/L, Zn= 1.1 mg/L, and B= 5.18 mg/L. Actions to prevent and mitigate the negative impacts of the palm oil industry continue to be carried out, one of which is using phytoplankton microalgae. Phytoplankton microalgae are autotrophic organisms that use sunlight, carbon dioxide, nitrates, and phosphates for growth (CO_2+H_2O light/ $C_6H_{12}O_6$+O_2). In addition, microalgae also have an essential role in human life. This is due to the high nutritional value contained in microalgae (Hashanah, 2011).

The use of phytoplankton microalgae in wastewater treatment has several advantages compared to using chemicals. Some of the advantages of using phytoplankton microalgae in wastewater treatment include: the principle of the processing process runs naturally like natural ecosystems so it is very environmentally friendly, does not produce secondary waste, can reduce carbon emissions, and high biomass production (Kawaroe, 2010). In this study, the phytoplankton microalgae used was the diatom Skeletonema costatum, which is a tropical marine microalgae. Skeletonema costatum is a natural food that is small in size, contains good nutrients, and is food for larvae or zooplankton. The nutritional quality of macroalgae depends on the content of proteins, carbohydrates, lipids and fatty acids. Skeletonema costatum has several advantages over artificial feed, because it has its own autolytic enzymes so it is easily digested by the larvae and does not contaminate the culture media (Ryther & Goldman *in* Sutomo, 2005). Information regarding the culture and chemical composition of Skeletonema costatum using palm oil wastewater is still very rare, so researchers are interested in conducting research on the chemical composition of Skeletonema costatum cultured at different concentrations of palm oil wastewater which refers to the research of Dahril et al. (2016) to see the best concentration for the growth of Skeletonema costatum and the ability of Skeletonema costatum to utilize palm oil waste as a living medium.

2. Material and Method

2.1. Time and Place

This research was carried out in October-December 2022 at the SUPM Natural Feed Laboratory/Maritime and Fisheries Polytechnic Pariaman

2.2. Methods

The method used in this study was an experimental method with a one-factor Completely Randomized Design (CRD) consisting of 6 (six) treatment levels and 3 (three) repetitions. The treatment to be given in this study was palm oil wastewater with different levels of concentration treatment, namely 0%, 10%, 15%, 20%, 25%, and 30%, with 3 (three) repetitions, so that a total of 18 trial units.

2.3. Procedure

One of the essential factors in the culture of Skeletonema costatum is the sterile condition of both the equipment and the room where the culture is. Then, filtering the waste is carried out. The filter media used to filter the waste is the Dahril filter. This filter is made of a plastic drum with four layers of filters. The first layer is palm fiber, then a layer of charcoal, then gravel, and the last layer of sand, which neutralizes all suspended organic matter in the liquid waste.

Pure culture of *Skeletonema costatum* is carried out in the natural feed laboratory of the Pariaman Marine and Fisheries Polytechnic. It must be carried out aseptically to avoid all kinds of pathogens. The culture room is equipped with an Air Conditioner (AC), which regulates the room temperature so that the room temperature is 16 0C. Lamps are used in culture racks as a source of lighting for the photosynthesis process. For the source of air, they were using a mini blower. 20,000 ml of culture was used as the initial seed for mass culture using palm oil mill wastewater as a growth medium. *Skeletonema costatum*, which was inoculated, was 20,000 mL.

3. Result and Discussion

3.1. Nitrate and Phosphate Concentration

The results of nitrate measurements during *Skeletonema costatum* culture are in Table 1.

Table 1. Nitrate measurements during *Skeletonema costatum* culture

Treatment	Day 1		Day 3	
	Nitrate	Abundance	Nitrate	Abundance
T0	0.664±0.009[a]	21.48±3.90[a]	0.482±0.011[a]	31.85±4.21[a]
T1	0.690±0.012[b]	77.41±2.80[b]	0.586±0.013[b]	85.56±4.01[b]
T2	0.705±0.005[c]	95.56±4.01[c]	0.634±0.031[c]	131.67±4.49[c]
T3	0.709±0.005[c]	120.00±1.11[d]	0.657±0.039[c]	267.41±4.49[d]
T4	0.831±0.009[d]	138.89±9.49[e]	0.730±0.004[d]	285.93±14.33[d]
T5	0.888±0.002[e]	182.22±5.09[f]	0.759±0.015[d]	329.26±18.90[e]

Based on the results of the analysis of nitrate content in Table 1, it shows that the highest average nitrate content was at P5 (300 ml/L), namely on the first day 0.888 mg/L, on day 3 (three) it was 0.759 mg/L and the average The lowest nitrate content was at P0 (control), namely on the first day 0.664 mg/L, on day 3 (three) 0.482 mg/L. The higher the dose of palm oil waste given to the *Skeletonema costatum* culture media, the higher its nitrate content. According to Mackentum (1969), phytoplankton can grow optimally at nitrate concentrations ranging from 0.9 to 3.5 mg/L. At concentrations below 0.01 mg/L or above 4.5 mg/L, nitrate can be a limiting factor for the growth of phytoplankton. Based on the level of water fertility on nitrate content, oligotrophic waters have nitrate levels of 0-1 mg/L, mesotrophic with nitrate levels of 1-5 mg/L, and eutrophic with nitrate levels of 5-50 mg/L (Wetzel, 1975).

Table 2. Phosphate measurement during *Skeletonema costatum* culture

Treatment	Day 1		Day 3	
	Phosphate	Abundance	Posphate	Abundance
T0	0.895±0.004[a]	21.48±3.90[a]	0.210±0,003[a]	31.85±4.21[a]
T1	0.706±0.003[b]	77.41±2.80[b]	0.244±0,006[b]	85.56±4.01[b]
T2	0.745±0.001[c]	95.56±4.01[c]	0.250±0,004[b]	131.67±4.49[c]
T3	0.789±0.008[d]	120.00±1.11[d]	0.281±0,003[c]	267.41±4.49[d]
T4	0.893±0.003[e]	138.89±9.49[e]	0.289±0,003[d]	285.93±14.33[d]
T5	0.964±0.005[f]	182.22±5.09[f]	0.301±0,002[e]	329.26±18.90[e]

Based on the results of the analysis of nitrate content in Table 1, it shows that the highest average nitrate content was at P5 (300 mL/L), namely on the first day 0.888 mg/L, on day 3 (three) it was 0.759 mg/L and the average The lowest nitrate content was at P0 (control), namely on the first day 0.664 mg/L, on day 3 (three) 0.482 mg/l. The higher the dose of palm oil waste given to the *Skeletonema costatum* culture media, the higher its nitrate content. According to Mackentum (1969), phytoplankton can grow optimally at nitrate concentrations ranging from 0.9 to 3.5 mg/l. At concentrations below 0.01 mg/L or above 4.5 mg/L, nitrate can be a limiting factor for the growth of phytoplankton. Based on the level of water fertility on nitrate content, oligotrophic waters have nitrate levels of 0-1 mg/L, mesotrophic with nitrate levels of 1-5 mg/L, and eutrophic with nitrate levels of 5-50 mg/L (Wetzel, 1975).

The dissolved oxygen content during this study was good enough for cherax growth, namely the lowest was 4.2 mg/L and the highest was 5.8 mg/L or an average of 5 mg/L. In accordance with the opinion of Boyd (1982) in Faiz et al. (2021) the optimum range of dissolved oxygen values for crustacean growth is above 5 mg/L, thereby providing good growth and survival.

3.2. Skeletonema costatum Cell Abundance and Biomass

Based on the results of calculating the abundance of *Skeletonema costatum* cells contained in Table 3, which was carried out for five days, the abundance of *Skeletonema costatum* in Table 3, shows that the highest average abundance was at P5 (300 mL/L), namely on day 3 namely 329.26 cells/mL. The lowest average abundance was at P0 (control) on day 5, 16.30 cells/mL. The total density of Skeletonema costatum increased in each treatment. The large number of *Skeletonema costatum* cells at P5 (300 mL/L) was due to the given palm oil wastewater containing high nitrate and phosphate content. The density of Skeletonema costatum cells was determined by the nutrients present in the culture media. The high density of Skeletonema costatum cells is due to nutrients containing sufficient amounts of nitrate elements so that the needs of microalgae for nutrition are met, and growth is maximized, while the lowest average number is at P0 (control) because the culture container does not meet the nutrients needed by Skeletonema costatum. Inoculation of the first stocking seeds in each treatment was the same, namely 20,000 cells/mL. This proves that Skeletonema costatum can grow well. The results of the analysis of variance (ANOVA) showed that differences in the concentration of palm oil wastewater given in

Skeletonema costatum culture media had a significant effect on the abundance of *Skeletonema costatum* cells ($p<0.05$).

Table 3. Calculation of *Skeletonema costatum* cell abundance

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
T0	21.48±3.90[a]	23.33±5.09[a]	31.85±4.21[a]	27.41±5.58[a]	16.30±1.70[a]
T1	77.41±2.80[b]	80.37±2.31[b]	85.56±4.01[b]	55.93±3.57[b]	45.59±14.20[b]
T2	95.56±4.01[c]	115.93±5.59[c]	131.67±4.49[c]	85.85±4.01[c]	71.48±1.70[c]
T3	120.00±1.11[d]	132.59±6.51[d]	267.41±4.49[d]	92.52±2.81[c]	76.37±12.30[c]
T4	138.89±9.49[e]	183.70±7.40[e]	285.93±14.33[d]	115.56±5.09[d]	96.33±6.11[d]
T5	182.22±5.09[f]	224.07±4.50[f]	329.26±18.90[e]	205.184±7.56[e]	112.22±10.18[d]

The primary role of this element is to stimulate vegetative growth and increase the number of cells/ind. The difference in cell density of each medium is caused by differences in the nutrient content contained in each medium. The suitability of the media type for microalgae cultivation will produce optimum growth (Andersen, 2005).

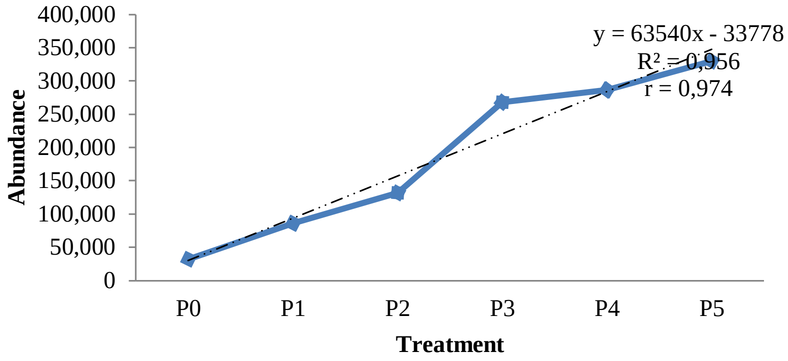

Figure 1. Regression graph of *Skeletonema costatum* cell abundance

Figure 1 shows that the administration of palm oil wastewater to *Skeletonema costatum* culture has a robust positive correlation (r = 0.974). Administration of palm oil wastewater affects the abundance of *Skeletonema costatum* cells by 97%. The low abundance at P0 (control) was due to the untreated treatment or not being given palm oil effluent, so more nutrients were needed to grow *Skeletonema costatum*. The abundance of P5 increases because the number of doses is large enough to fulfill the nutrients *Skeletonema costatum* needs. The difference in growth rate in each treatment was caused by the ability of cells to absorb nutrients contained in the culture media (Aulia, 2017).

Table 4. Biomass of *Skeletonema costatum* during culture

Treatment	Day 3	Day 5
T0	2.23±0.01[a]	1.07±0.04[a]
T1	3.04±0.04[b]	1.55±0.02[b]
T2	4.17±0.03[c]	2.43±0.04[c]
T3	5.79±0.03[d]	2.57±0.02[d]
T4	6.38±0.03[e]	3.54±0.02[e]
T5	7.27±0.04[f]	4.02±0.02[f]

Based on the calculation results of *Skeletonema costatum* biomass in Table 4, it shows that the highest average biomass is at P5 (300 ml/l), namely on day 3 is 7.27 g, on day 5 is 4.02 g and the lowest average biomass was at P0 (control), namely on day 3 is 2.23 g, on day 5 is 1.07 g. The low biomass at P0 is because the containers are not treated, so the nutrients in the containers cannot meet the needs of *Skeletonema costatum* to grow. The decrease in biomass on the fifth day is because the third day is the peak of *Skeletonema costatum* growth, and the fourth and fifth day is the death phase. On the fifth day, many *Skeletonema costatum* died, so the biomass of the *Skeletonema costatum* decreased. The high biomass of *Skeletonema costatum* at P5 is because the growth of *Skeletonema costatum* is going well. After all, the concentration of palm oil waste given is relatively high, so the macro elements needed by *Skeletonema costatum*, such as nitrate, phosphate which are in palm oil wastewater, can already meet the nutrient needs of Skeletonema costatum so that it can develop properly.

3.3. Chlorophyll-a

Based on the calculation results of Chlorophyll-a in Skeletonema costatum in Table 5, the highest average chlorophyll-a is at P5 (300 mL/L), namely 0.84. Based on the results of the Analysis of Variance (ANOVA), the difference in the concentration of palm oil liquid waste given in the culture media significantly affects the amount of chlorophyll-a present in *Skeletonema costatum*. Assessment of the level of water fertility through the number of individual types of phytoplankton may give different results for each kind of phytoplankton due to differences in the volume size of each type of phytoplankton (Sulastri, 2002).

Table 5. Results of calculation of chlorophyll-a in *Skeletonema costatum*

Treatment	Day 3
T0	$0.46{\pm}0.07^{a}$
T1	$0.56{\pm}0.02^{b}$
T2	$0.64{\pm}0.01^{c}$
T3	$0.70{\pm}0.01^{d}$
T4	$0.76{\pm}0.02^{e}$
T5	$0.84{\pm}0.04^{f}$

3.4. Chemical Composition of Skeletonema costatum

Based on the results of the analysis of the protein content of *Skeletonema costatum* in Table 6, it shows that the highest average protein content is at P5 (300 ml/l), namely on the first day 26.91%, on the third day is 30.70%. The lowest moderate protein content was at P0 (control) on the first day at 18.36% and on day three at 20.31%. The high protein content in *Skeletonema costate* at P5 is because the macro elements needed by algae, such as nitrogen, phosphate, and iron present in palm oil wastewater, can already meet the need for nutrients for *Skeletonema costatum*. *Skeletonema costatum* is a type of natural feed that is often used in aquaculture. Which is rich in nutrients such as protein, fat, crude Omega3 (ƆƆ3), and HUFA (Highly Unsaturated Fatty Acid), which is relatively high (Widianingsih et al., 2010). This opinion is reinforced by Supriyantini et al. (2013), who stated that *Skeletonema costatum* is a quality feed with a relatively high nutritional content. The nutritional value of Skeletonema costatum has a protein content ranging from 21.63-32.05% (Herawati & Hutabarat, 2015). Protein functions as a building substance and maintains cells and tissues of the body, regulating water balance and energy sources (Almatsier, 2003).

Table 6. Protein content in *Skeletonema costatum*

Treatment	Day 1	Day 3
T0	$18.36{\pm}0.01^{a}$	$20.31{\pm}0.02^{a}$
T1	$24.31{\pm}0.05^{b}$	$26.25{\pm}0.05^{b}$
T2	$24.33{\pm}0.01^{b}$	$26.90{\pm}0.03^{c}$
T3	$25.11{\pm}0.03^{c}$	$28.88{\pm}0.03^{d}$
T4	$26.85{\pm}0.03^{d}$	$29.04{\pm}0.07^{e}$
T5	$26.91{\pm}0.01^{e}$	$30.70{\pm}0.11^{f}$

Based on the results of the analysis of the fat content of *Skeletonema costatum* in Table 7 it shows that the highest average fat content is at P5 (300 mL/L), namely on the first day 0.291%%, on day 3 (three) the amount of fat present on *Skeletonema costatum* was 1.566%% and the lowest average fat content was at P0 (control) namely on the first day 0.035%, on day 3 (three) 0.162%. The high-fat content in *Skeletonema costatum* at P5 is because the macro elements needed by algae, such as nitrates and phosphates present in palm oil wastewater, can fulfill the need for nutrients for *Skeletonema costatum*. Fat is a feed compound that will be deposited as an energy reserve and to support growth. At high salinity, nutrients are used for growth but not optimum; this is because, at high salinity, *Skeletonema costatum* adapts by carrying out the process of osmosis (Erlina et al., 2004).

Table 7. Fat content in *Skeletonema Costatum*

Treatment	Day 1	Day 3
T0	$0.035{\pm}0.000^{a}$	$0.162{\pm}0.004^{a}$
T1	$0.140{\pm}0.001^{b}$	$0.263{\pm}0.006^{b}$
T2	$0.142{\pm}0.001^{c}$	$0.296{\pm}0.002^{c}$
T3	$0.144{\pm}0.001^{d}$	$1.104{\pm}0.004^{d}$
T4	$0.209{\pm}0.001^{e}$	$1.556{\pm}0.005^{e}$
T5	$0.291{\pm}0.001^{f}$	$1.566{\pm}0.008^{e}$

Based on the results of the analysis of the carbohydrate content of *Skeletonema costatum* in Table 8, it shows that the highest average carbohydrate content is at P5 (300 mL/L), namely on the first day 20.01%, on the third day 21.95% and the average content the lowest carbohydrate was at P0 (control), namely on the first day 13.20%, on the third day 15.18% the high carbohydrate content of *Skeletonema costatum* was at P5 because of the macro elements needed by algae such as nitrogen, phosphate and iron present in waste Liquid palm oil can already meet the need for nutrients for Skeletonema costatum. In addition to its nutritional content, *Skeletonema costatum* contains fatty acids, free amino acids, ß-1,3 glucan, and polysaccharide cell walls (Granum et al., 2002). Chu et al. (1982) stated that carbohydrates increased according to the age of the microalgae in line with the reduced nutrients in the culture medium. The primary function of carbohydrates in metabolism is as a fuel for oxidation and providing energy for metabolic processes (Martin et al., 1987)

Table 8. Carbohydrate content in *Skeletonema costatum*

Treatment	Day 1	Day 3
T0	13.20±0.02[a]	15.18±0.02[a]
T1	15.13±0.04[b]	16.20±0.01[b]
T2	17.32±0.03[c]	19.20±0.03[c]
T3	19.59±0.03[d]	20.26±0.03[d]
T4	19.91±0.02[e]	20.68±0.03[e]
T5	20.01±0.02[f]	21.95±0.03[f]

4. Conclusions

Based on the results of the study, it showed that palm oil wastewater affected the abundance of *Skeletonema costatum*. The best treatment for *Skeletonema costatum* culture media was P5 (300 mL/L), *Skeletonema costatum* cell abundance 329,260 cells/mL, biomass (dry weight) 7.27 g, chlorophyll-a 0.84. Then, based on the results of the analysis of nitrate and phosphate also provided good content for *Skeletonema costatum* culture media, namely in treatment P5 (300 mL/L) in which the nitrate content was 0.759 mg/L, phosphate 0.301 mg/L. In contrast, the best CO_2 was P0 (control), and the best-dissolved oxygen level the best is P5 (300 mL/L). Conditions for water quality parameters for temperature 16^0C, pH 8.2, while for good protein, fat, and carbohydrate content, namely treatment P5 (300 mL/L) with a protein content of 30.70%, 0.291% fat, and 21 carbohydrates 96%.

5. Suggestion

Based on the research that has been done, it is suggested that natural feed cultivators culture *Skeletonema costatum* using palm oil waste at a dose of 300 mL/L. Future researchers should use palm oil wastewater at a dose of 300 mL/L using other types of microalgae such as *Thalassiosira* sp.

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