Growth of Microalgae *Spirulina platensis* on Media Containing Palm Oil Mill Effluent.

I. Effendi*, I. Nurrachmi, A. N. F. Tarigan
Universitas Riau, Pekanbaru, Riau, Indonesia
*Corresponding author: helpingirwan@gmail.com

Abstract. *Spirulina platensis*, a Cyanobacteria, is used as a natural food in fish hatcheries and a food supplement for humans. These microalgae are very nutritious, containing 63-68% protein, 18-20% carbohydrates, and 2-3% fat. Increased production of palm oil has serious side effects on the aquatic environment due to the disposal of palm oil mill effluent (POME). POME are thought to be suitable as nutrients and media to microalgae. This study aimed to determine the effect of POME with different nutrient variations on the growth of these microalgae. This study used a randomized block design (RBD) with 2 factors, namely factor A (POME concentration) which consisted of 3 levels, namely 20 ppm (A1), 40 ppm (A2), and 60 ppm (A3) and factor B (media) namely Walne (B1), and Guillard (B2) with 3 repetitions. The results showed that the addition of POME had positive effect on the growth of *S. platensis*. The higher the POME concentration the better the growth rates obtained for both types of media. For Walne medium, the best growth was obtained on day 10 of A3B1 (69,447.98 sins/ ml). Meanwhile, for Guillard medium, the highest growth was achieved on day 8 of A3B2 (82,027.6 sins/ ml).

1. Introduction

*Spirulina platensis* is a very potential microalgae as a food source because 1 acre *Spirulina* farm can produce 20 times better protein than 1 acre soybeans or corn and 200 times better than beef [1]. Apart from the high protein content, *Spirulina* has several advantages compared to other types of microalgae, namely relatively fast production [2] and the resulting biomass is easy to harvest. This is due to the large size of the cells so that they can be separated from the media through filtration using a 20 µm filter. *Spirulina* cells are easily digested because the layer is a thin membrane, not like cellulose which is difficult to digest, but the membrane is a sugar group that is easily digested and absorbed [3, 4].

*S. platensis* is a class of algae from Cyanobacteria that can be used as natural food in fish and shrimp hatchery businesses because it has high enough nutrients, including 63-68% protein, 18-20% carbohydrates, and 2-3% fat [5]. Currently, microalgae cultivation technology can be applied in a wider field, namely in liquid media. In this case, there are two advantages, namely producing microalgae biomass which can be used as a food source and reducing the cost of synthetic nutrient needs by utilizing nutrients contained in waste [6].

Indonesia is the largest producer of palm oil in the world since displacing Malaysia from the throne of the largest producer in 2006 [7]. The production of crude palm oil (CPO) has serious side effects on the environment, mainly due to disposal of palm oil processing waste (POME) which has not been processed optimally so that it pollutes the environment [8]. In terms of quantity, it is known that from 1 ton of CPO produced, it takes no less than 5 tons of palm oil (fresh fruit bunch) and produces 5-7.5 tons of process wastewater, more than 50% will be POME. POME that has not been processed has a content consisting of 95% water, 0.6-0.7% oil and 4-5% solids including 2-4% suspended solids [9].

Cultivation of algae using waste is an effective and efficient alternative. POME which is rich in minerals such as N, P, K and various other minerals are very suitable for use as nutrients and media in plants. Nutrients possessed by POME are required by plants, POME is rich in organic compounds and carbon dioxide. POME contains large amounts of nitrogen, phosphate, calcium, magnesium and potassium so it can be used as fertilizer [8]. This study aims to determine the effect of adding POME to cultivation media on the growth of *S. platensis*. 

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

Published under licence by IOP Publishing Ltd
2. Methodology

This research is an experimental study using a randomized block design (RBD) of two factors, namely factor A (POME concentration) and factor B (type of medium) with three replications. Factor A consists of three levels; 20 ppm (treatment A1), 40 ppm (treatment A2), and 60 ppm (treatment A3). Meanwhile, factor B consists of two levels, namely medium Walne [9] and Guillard medium [10]. Walne medium (treatment B1), and Guillard medium (treatment B2), each with a concentration of 1 ml/liter [11]. The experimental unions were A1B1, A1B2, A2B1, A2B2, A3B1, A3B2 and experimental control (seawater without media). The POME used is obtained from PT. Wira Karya Pramitra, Kampar, Riau Province, Indonesia. Meanwhile, cultured *S. platensis* microalgae were obtained from stocks of the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Affairs, University of Riau.

A plastic bottle measuring 1 liter is first cleaned and rinsed with clean water and sterilized using 70% alcohol and then air dried and labeled. This plastic bottle is then filled with 625 ml of sea water (25 ppt salinity) which is filtered using a water filter. Furthermore, the cultivation medium was spiked with chlorine powder at a dose of 1 mg/liter, which is for the purpose of sterilizing the cultivation medium from microbes and other bacteria and left for 24 hours. The next step is the addition of Na-thiosulfate crystals (0.5 grams/liter) which aims to neutralize water and eliminate chlorine odors. Next is the addition of POME media according to the required concentrations, namely 20, 40, 60 and 0 ppm. The microalgae seeds are then stocked and the initial density calculated, the results obtained are used as the initial density of stocking. This cultivation medium was aerated continuously until the end of the research.

Calculation of the number and growth of *S. platensis* (relative growth rate) was carried out on days 0, 2, 4, 8, 10, 12 and 14 after inoculation. The count of cells was carried out under a microscope using the Sedgewick Rafter Counting Chambers. In addition, the water quality (pH, temperature, salinity) was also measured for each experimental unit during the study.

3. Result and Discussion

The results showed that the addition of POME to the cultivation medium had a positive effect on the growth of *S. platensis*. The higher the concentration added, the higher the growth of these microalgae. In Walne’s medium, for all treatment units, the number of microalgae cells decreased until the second day after inoculation. However, this number started to multiply on the 4th day. This number continued to increase and reached the 10th day. Then it began to decrease on the 12th day and so on until the end of this experiment. While in the control treatment, the number of microalgae cells continued to decline until the end of the experiment (Table 1). Among all the treatments, the best growth was obtained on the 10th day of the A3B1 treatment (Figure 1), namely the use of Walne cultivation medium added with 60 ppm POME (69,447.98 sin/ml).

Table 1. Growth of *S. platensis* (sin/ml) on Walne medium containing POME.

| Unit    | Day 0  | Day 2  | Day 4  | Day 6  | Day 8  | Day 10 | Day 12 | Day 14 |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|
| A1B1    | 15.063 | 13.566 | 35.286 | 38.736 | 50.785 | 57.983 | 50.180 | 44.840 |
| A2B1    | 15.063 | 13.959 | 38.768 | 43.131 | 54.564 | 57.484 | 53.408 | 43.004 |
| A3B1    | 15.063 | 18.662 | 45.329 | 50.552 | 65.276 | 69.447 | 62.569 | 52.823 |
| Control | 15.063 | 5.010  | 3.630  | 2.898  | 2.314  | 1.804  | 1.443  | 1.050  |
Figure 1. Growth of *S. platensis* (sin/ml) on Walne medium with different POME concentrations.

On guillard cultivation medium, the highest growth was achieved on day 8 of A3B2 treatment (82.027.6 sin/ml). For all treatment units, the number of microalgae cells decreased until the second day after inoculation. However, this number started to multiply on the 4th day. This number continued to increase and reached the 8th day. Then it began to decline on the 12th day and so on until the end of this experiment (Table 2). While in the control treatment, it can be seen that the microalgae population has decreased drastically from day 1 to the end of this experiment (Figure 2).

Table 2. Growth of *S. platensis* (sin/ml) on Guillard medium containing POME.

| Unit   | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 |
|--------|-------|-------|-------|-------|-------|--------|--------|--------|
| A1B2   | 15.063,69 | 14.501,06 | 29.978,77 | 32.558,39 | 41.804,67 | 39.490,45 | 35.074,31 | 30.244,16 |
| A2B2   | 15.063,69 | 18.110,40 | 38.301,49 | 43.142,25 | 59.033,97 | 60.084,93 | 52.473,46 | 44.108,28 |
| A3B2   | 15.063,69 | 18.535,03 | 51.475,58 | 65.753,72 | 82.027,60 | 76.539,28 | 69.585,99 | 65.286,62 |
| Control| 15.063,69 | 5.010,62 | 3.630,57 | 2.898,09 | 2.314,23 | 1.804,67 | 1.443,74 | 1.050,96 |

The use of POME as a growing medium for *S. platensis* has been reported by several researchers previously. Two workers [12] cultivated the algae in POME containing media with a concentration of 20%, 40% and 60% V. Urea, NaHCO3 and TSP were given once every 2 days as a supply of additional nutrients. The results obtained showed that the best cultivation medium was POME with a concentration of 20%. The best nutrient supply composition is Urea 25 mg/l, TSP 50 mg/l and NaHCO3 200 mg/l. At the same treatment for each cultivation medium, the maximum growth rate obtained was μ = 0.128/day. It was reported [13] a successful effort on clarifying the palm oil mill effluent (POME) using commercial polymer and then treating it by growing Spirulina. The experiment proved that Spirulina can be cultivated by using POME as partial media treating the POME to qualify the standards before discharge.

Other researchers [14] reported nutrient removal from anaerobically treated palm oil mill effluent by *S. platensis* and *Scenedesmus dimorphus*. An experiment was conducted phycoremediation in anaerobically digested palm oil mill effluent using Cyanobacterium, *S. platensis* [15]. Another group of researcher [16] reported the utilization of microalgae cultivated in palm oil mill wastewater to produce lipid and carbohydrate by employing microwave-assisted irradiation. Preliminary research was conducted by growing *S. platensis* on POME medium with various concentrations, namely 25%, 50%, 75%, and 90% POME on batch system. Dilution rate of *S. platensis* and the reduction rate of pollution level of POME on continuous photobioreactor was also successfully conducted [17].
Figure 2. Growth of *S. platensis* (sin/ml) on Guillard medium with different POME concentrations.

Media, fertilizers or nutrients are the most important elements for the growth of phytoplankton including *S. platensis*. Optimum nutrient conditions are needed to obtain high productivity values for microalgae culture along with good biomass quality. Giving the right dose is critically required to support the effectiveness of the growth of phytoplankton. The successful use of POME as a nutrient for *S. platensis* growth is understandable. POME as a waste still has high nutritional value. Nutritional value can be utilized directly as a source of energy and carbon source for these microalgae. POME is palm oil liquid waste which still contains a lot of dissolved solids. Most of these dissolved solids come from lignocellulosic material containing oil which comes from palm fruit [18].

Lignocellulose in POME is the largest constituent of woody plants. Lignocellulose consists of lignin, hemicellulose, and cellulose material. The chemical properties of these lignocelluloses make them of high biotechnological value. POME is a colloid suspension containing 95-96% water, 0.6-0.7% oil and 4-5% total fat and solids. POME is released from the industry as a brown liquid with temperatures between 80°C and 90°C and moderately acidic with a pH value of 4.0-5.0. Typically POME contains an average value of 6000 mg/l of oil and fat. The average POME contains BOD (biological oxygen demand) ranging from 8,200-35,000 mg/l and COD (chemical oxygen demand) ranging from 15,103-65,100 mg/l which will become pollutants when discharged directly into free waters [19, 20, 21, 22, 23, 24].

4. Conclusion
The addition of POME had positive effect on the growth of *S. platensis*. The higher the POME concentration the better the growth rates obtained for both types of media. For Walne medium, the best growth was obtained on day 10 of A3B1 treatment (69,447.98 sins/ ml). Meanwhile, for Guillard medium, the highest growth was achieved on day 8 of A3B2 treatment (82,027,6 sins/ ml).

Acknowledgment
We would like to express our gratitude to PT. Wira Karya Pramitra, Kampar, Riau Province, Indonesia, which has provided POME waste in this research. Our gratitude also goes to all parties who have participated.

References
[1] Spolaore P, C Joannis-Cassan, E Duran, A Isambet 2006 Commercial applications of microalgae. J. Biosci. Bioeng. 101(2): 87–96.
[2] Natalia N, B Amin, I Effendi 2019 Effect of addition of different nitrate concentration on *spirulina platensis* biomass with semi outdoor system. *Asian J. of Aq. Sci.* 2 (1) 127-131.
[3] Soni R, A K Sudhakar and R S Rana 2017 *Spirulina* – From growth to nutritional product: A review. Trends in Food Sci. and Tech. 69 A 157-171.
[4] Hanryani P, E Efriyeldi and I Effendi 2019 The effect of different light colors on the biomass...
growth of *Spirulina platensis*. *Asian J. of Aq. Sci.* 2(2) 132-137.

[5] Teimouri M, A K Amirkolaie and S Yeganeh 2013 The effects of *spirulina platensis* meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 396–399 14-19.

[6] Budiyono I, Syaichurozi, S Sumardiono and S B Sasonoko 2014 Production of *Spirulina platensis* biomass using digested vinasse as cultivation medium. *Trends in Appl. Sci. Res.* 9(2) 93-102.

[7] Jayed M H, H H Masjuki, M A Kalam, T M I Mahlia, M Husnawan and A M Liaquat Prospects of dedicated biodiesel engine vehicles in Malaysia and Indonesia. *Ren. and Sust. En. Reviews* 15(1) 220-235.

[8] Singh R P, M H Ibrahim, N Esa and M S Iliyana 2010 Composting of waste from palm oil mill: a sustainable waste management practice. *Rev. in Env. Sci. and Biotech.* 9 331–344.

[9] Phang. 2002. *Spirulina culture in digested sago starch factory waste water.* *J. of Appl. Phycology* 12(3–5) 395-400.

[10] Ahmad A L, S Ismail and S Bhatia 2003 Water recycling from palm oil mill effluent using membrane technology. *Desalination* 157 87-95.

[11] Salihu A and Z Alam 2012 Palm oil mill effluent: a waste or a raw material? *J. of Appl. Sci. Res.* 8(1) 466-473.

[12] Walne P R (1970) Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria, and Mytilis. *Fish. Invest.* 26 1-62.

[13] Kang K H, Z J Qian, B M Ryu and S K Kim 2011 Characterization of growth and protein contents from microalgae *Navicula incerta* with the investigation of antioxidant activity of enzymatic hydrolysates. *Food Sci. Biotech.* 20(1) 183-191.

[15] Nainggolan J G M, A Tanjung and I Effendi 2018 Growth of *Spirulina platensis* in indoor and semi outdoor culturing systems. *Asian J. of Aq. Sci.* 2(1) 22-28.

[16] Kumar P, S Kuppusamy, H M Yusop, S Isa and S Alwi 2011. POME treatment using *Spirulina platensis* Geitler. *Int. J. of Curr. Sci.* 1 11-13.

[17] Rajkumar R and M S Takriff 2015 Nutrient removal from anaerobically treated palm oil mill effluent by *Spirulina platensis* and *Scenedesmus dimorphus*. *Der Pharmacia Lettre*, 7(7):416-421.

[18] Zainal A, Z Yaakob, M S Takriff, R Rajkumar and J A Ghani 2012 Phycoremediation in anaerobically digested palm oil mill effluent using *Cyanobacterium, Spirulina Platensis*. *J. of Biobased Mat. and Bioenergy*. 6(6) 704-709.

[19] Nur M M A, D Kristanto, T M Setyoningrum and I G S Budiaman 2017. Utilization of microalgae cultured in palm oil mill wastewater to produce lipid and carbohydrate by employing microwave-assisted irradiation. *Recent Innov. in Chem. Eng.* 9(2) 107-116.

[20] Suharyanto T, S Permatasari and K Syamsu 2012 Production of *Spirulina platensis* in continuous photobioreactor using palm oil mill effluent media. *E-Journal Menara Perkebunan*, 82(1), 1-9.

[21] Abdullah N and F Sulaim 2013 The oil palm wastes in Malaysia. In Biomass Now - Sustainable Growth and Use. *IntechOpen*. doi.org/10.5772/55302.

[22] Kamyab H, S Chelliapan, M F M Din, S Rezania, T Khademi and A Kumar 2018. Palm oil mill effluent as an environmental pollutant. *Intechopen*. doi.org/10.5772/intechopen.75811.

[23] Madaki Y S and L Seng 2013 Palm oil mill effluent (Pome) from Malaysia palm oil mills: waste or resource. *Int. J. of Sci. Env. and Tech.* 2(6) 1138-1155.

[24] Marrez D A, M N Mohamed, Y S Yousef, Y D Zakaria and M H Aziz 2014 Evaluation of chemical composition for *Spirulina platensis* in Different Culture Media. *Res. J. of Pharma. Biol. and Chem. Sci.* 5(4) 1161-1171.