



CONFERENCE PROGRAMS AND ABSTRACT

**The 3rd FIRST 2019 INTERNATIONAL CONFERENCE
FORUM IN RESEARCH, SCIENCE, AND TECHNOLOGY
OCTOBER 09-10, 2019
*Palembang, Province of South Sumatera
Indonesia***

Organized by :



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PREFACE

The 3rd FIRST 2019 (Forum in Research, Science, and Technology) International Conference was initiated and organized by State Polytechnic of Sriwijaya in collaboration with Management and Science, University (MSU), Malaysia and National Chin-Yi University of Technology, Taiwan. The theme of the conference was “Integration of Advanced Technology to Enhance Social Welfare”. The 3rd FIRST 2019 International Conference facilitated the participants from all over the world to meet face to face to open chances in establishing connection and collaboration among them. It was not only for the researchers in academics, but also in industries and governments. This conference became an effective media to link the researchers from many parts of the world conference, for exchanging, sharing, following up and discussing the results of the latest research, industry’s needs, and government regulatory policies. The 3rd FIRST 2019 International Conference became worthwhile platform for researchers to present their finding in the areas on multidisciplinary of Engineering and Science (Track 1), Computer Science and ICT (Track 2), and Social Science (Track 3). It has also provided an opportunity for the professionals and researchers to learn and share about the latest development and research in those 3 tracks.

The 3rd FIRST 2019 International Conference attracted so many authors not only from Indonesia but also from other countries, such as Japan, Taiwan, and Malaysia. There were 180 papers were accepted in the 3rd FIRST 2019 International Conference, including 89 papers for Track 1 (Engineering and Science), 46 papers for Track 2 (Computer Science and ICT), and 45 papers for Track 3 (Social Science). In The 3rd FIRST 2019 International Conference, there were 4 keynote speakers and 2 invited speakers. As the keynote speakers, there were Prof. Yasushi Kiyoki, Ph. D from KEIO University, Japan, Prof. Nurul Taufiqu Rochman, M. Eng, Ph. D, From LIPI, Indonesia, Prof. Tjiptohadi Sawarjuwono, M. Ec., Ph. D., Ak, from Universitas Airlangga, Indonesia, and Prof. Win-Jet Luo, from National Chin-Yi University of Technology, Taiwan. As the invited speakers, there were Dr. R. Wisnu Nurcahyo, DVM from Universitas Gadjah Mada, Indonesia, and Assoc. Prof. Dr. Intan Zaurah binti Mat Darus from Universiti Teknologi, Malaysia. The 3rd FIRST 2019 International Conference committee would like to say thank you very much for all the participants and their respected institutions that have supported for the success of the 3rd FIRST 2019 International Conference, and also for all of the guess and sponsors of the 3rd FIRST 2019 International Conference.

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Lipid Extraction from Microalgae *Spirulina Platensis* for Raw Materials of Biodiesel

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Abstract. Biodiesel derived from microalgae has been considered as one of the substitutes for fossil fuels. There are many microalgae with high lipid content that can be used for the raw material of biodiesel. Microalgae *Spirulina platensis* in the past few years has been developed to become biodiesel feedstock, previously this microalga was widely used in food supplements. The purpose of this research is to study the growth of microalgae in lab scale cultivation, to extract lipids by maceration, Soxhlet, and osmotic methods and analyze the biodiesel obtained from the transesterification process. Cultivation was carried out in laboratory scale with bright dark lighting conditions (12:12 hours), using Walne medium, pH of 7-10 and salinity of 25-30 ppt. Cell density was observed using a microscope and was calculated using a hemacytometer. From the results of the study at the cultivation stage, it was found that the optimum pH for the growth of microalgae *Spirulina Platensis* was at pH of 9 and lipids obtained from maceration, osmotic and Soxhlet extraction methods are 5.5%, 0.6%, and 9%. Biodiesel which had the highest calorific value was obtained through extraction using the maceration method with a calorific value of 11200 cal/gram and density 0,875 gram/cc.

1. Introduction

Energy has become one of the primary needs of human. However, the energy sources are getting limited. The limitation of energy resources become a serious global issue caused by using common fuel derived from petroleum that cannot be renewed. To reduce the dependence on petroleum fuels, a solution that appears to meet futuristic needs on fuels is the use of renewable energy and environmentally friendly alternative fuels such as biodiesel. Plant oil extractions and animal fats are currently being developed into an environmentally friendly energy source. Traditionally, plant oil extractions have long been used for raw materials biodiesel like rapeseed in Europe, soybean in America and palm oil in Asia [1]

In accordance with the development of raw material sources and the technology used in producing biodiesel, until now it has reached the third generation. The first generation of biodiesel was produced from refined edible vegetable oil and animal fats with alkaline catalysts through the



transesterification process. In the second generation, biodiesel was produced from materials other than edible oil with various alternative technologies. Now it has entered the third generation, where biodiesel or known as fatty acid methyl esters (FAME) is produced from microalgae. Oil from microalgae according to [1] has far higher productivity than oil produced from oil crops. Biodiesel from microalgae through three stages of the process, namely cultivation, extraction and transesterification [2]

It is predicted that in the future, algal biomass will be an attractive source for biodiesel feedstock, especially because its oil production per hectare can reach 10 times higher than other biodiesel-oil plants [3]. Microalgae growth like plants, they need sunlight, carbon dioxide, water, and inorganic salts to live. One of the microalgae that can be developed into biodiesel is *Spirulina platensis* [4,5]. According to [6] stated that *Spirulina platensis* is not easily affected by fluctuating environmental conditions, especially physical and chemical environmental conditions such as light intensity, water temperature, salinity, and nutrient limitations. *Spirulina strains* are commonly found naturally in waters with high alkalinity that contains carbonate or bicarbonate. Until now, 35 species of *Spirulina* have been found. The most well-known species include *S. platensis*, *S. major*, *S. princeps*, *S. laxissima*, *S. subtilissima*, *S. caldaria*, *S. curta*, *S. subsalsa*, and *S. spirulinoides*. The characteristics of *Spirulina* cells are as follows: cylindrical cells, greenish blue, 6–8 μm in diameter (*S. platensis*), 4–6 μm (*S. maxima*), gas-filled cytoplasmic granules, helical branching filament, 3–5 mm in size [7]. The lipid contains microalgae *Spirulina platensis* according to [8] is quite high at 4-16%.

One of the challenges in using microalgae as a raw material for making biodiesel is the process of extracting the oil which is difficult and expensive, so this study continues to be developed. There are several methods in extracting microalgae, including the Folch method, Bligh and Dryer method and Superior solvent extraction method. These methods were modified by the writer to get better results. The solvent extraction method was modified by mechanical treatment of algae cells or disruption cells to break the cell wall which aimed to push oil out of the algae cells. Disruption cells extraction can be conducted by osmotic shock [9]. Several methods of microalgae extraction had been studied by [10] on microalgae *Botryococcus brauni* obtained that the extraction method with high lipid yield was using the soxhlet method. In this study, the Maseration and Osmotic methods were used in lipid extraction from *Spirulina platensis* microalgae and compared with the soxhlet method. The maceration method according to [11] is simple with the principle of extraction through soaking dry algae with a solvent for a certain time. While the osmotic method is an extraction method that utilizes the osmotic pressure of a solvent to damage the semipermeable blocking of microalgae cells so that it is expected to accelerate lipids out of microalgae cells. The results of this study were expected to obtain an optimal extraction method in obtaining lipids from microalgae *Spirulina platensis*. The results of the lipids obtained then be processed into biodiesel with the transesterification process. In this study also carried out cultivation of microalgae *Spirulina platensis* under bright dark lighting conditions (12:12) hours, using Zarouk medium, pH of 7-10 and salinity of sea water media 35 ppt.

2. Research Method

This study began with microalgae cultivation which was previously obtained from the Institute for Brackish Water Aquaculture Center of Life Feed Laboratory at Jepara. In order to prevent contamination by bacteria and fungi in the cultivation process, all nutrient stock, sea water media and equipment used must be sterilized first. In this microalgae cultivation used ratio 80:20 between algae seeds and sea water media. Sea water media with 35 ppt salinity and microalgae seeds were mixed with the addition of 1 ml of Zarouk medium as nutrition and 1 ml of the vitamin. In maintaining microalgae stock culture, optimum growth conditions (especially light) need to be maintained. In this study, microalgae were cultivated with a 20 watt lamp with 12:12 hours of lighting (bright: dark) [12]. The gas exchange process was carried out using a hose made with two branches, one part of the hose was directed to the inside of the erlenmeyer and the other part was transferred to the outside of the erlenmeyer. During the 11-day cultivation process, an analysis of the density of *Spirulina platensis* microalgae cells was carried out. The cultivation using condition of pH 7, 8, 9 and 10.

In extraction studies using dried microalgae obtained from the Center for Brackish Water Aquaculture Jepara. Lipid extraction was carried out by using maceration and osmotic extraction methods. The solvent used to extract in this study was n-hexane as a nonpolar solvent and based on [13] would be easily extracted by using n-hexane. The maceration extraction process was carried out by weighing 10 grams of dried algae and was placed in a three-neck flask. Then 75 ml of n-hexane [13] was added to the three-neck flask with a reflux condenser and magnetic stirrer. Agitation was turned on at a speed of 650 rpm for 24 hours. Then waiting for the mixture formed two layers of lipids and solvents. The final step was a distillation, to separate the solvent with lipids. The lipid yields obtained was calculated by yield percent. The extraction method with osmotic shock was carried out by weighing 10 grams of dried algae at three neck flask. Add 75 ml HCL 5 M into a three-neck flask with a reflux condenser and magnetic stirrer. Agitation was turned on at a speed of 650 rpm for 24 hours. Let the mixture form two layers of lipids and solvents. Washing the filtrate with 200 ml of distilled water and 200 ml of hexane. Then distillation was carried out to separate solvents and lipids. The final step was to analyze the % of lipid yields obtained. After the extraction process, it was continued with the formation of fatty acid methyl ester (FAME) using the transesterification process. The transesterification process was conducted by preparing equipment water batch, two neck flask, condenser, stative, and a thermometer. Heating algae lipids in a two-neck flask with a temperature of 55⁰ C. Dissolving KOH into methanol at a ratio of 1:6. Mixing the solution with algae lipids when the temperature had reached 55⁰ C. This transesterification reaction lasted for two hours and formed two layers, the lower layer was a mixture of triglycerides and the upper layer was FAME (Fatty Acid Methyl Ester). The analyses conducted were to find out the calorie value with bomb calorimeter and density measured by picnometer.

3. Results and Discussion

3.1 *Cultivation of Spirulina platensis Microalgae*

The results of cell density calculation on the growth are shown in Table 1.

Table 1. *Spirulina platensis* microalgae cells with pH variations.

Observation time (Days)	Cells Number (cell/mL)			
	pH 7 (x 10 ⁴)	pH 8 (x 10 ⁴)	pH 9 (x 10 ⁴)	pH 10 (x 10 ⁴)
0	0,520	0,520	0,520	0,520
1	0,694	0,867	1,215	0,867
2	0,868	1,215	1,562	1,215
3	1,041	1,562	1,909	1,736
4	1,215	1,736	2,256	1,909
5	1,562	2,083	2,604	2,256
6	2,083	2,430	2,951	2,604
7	2,604	3,125	3,472	3,298
8	2,777	2,951	3,298	3,125
9	2,430	2,604	3,125	2,777
10	2,256	2,256	2,777	2,430
11	1,736	1,909	2,430	2,256

The following Figure 1 shows a graphical picture of the *Spirulina plantesis* microalgae growth during the 11-day cultivation period at pH of 7,8,9 and 10.

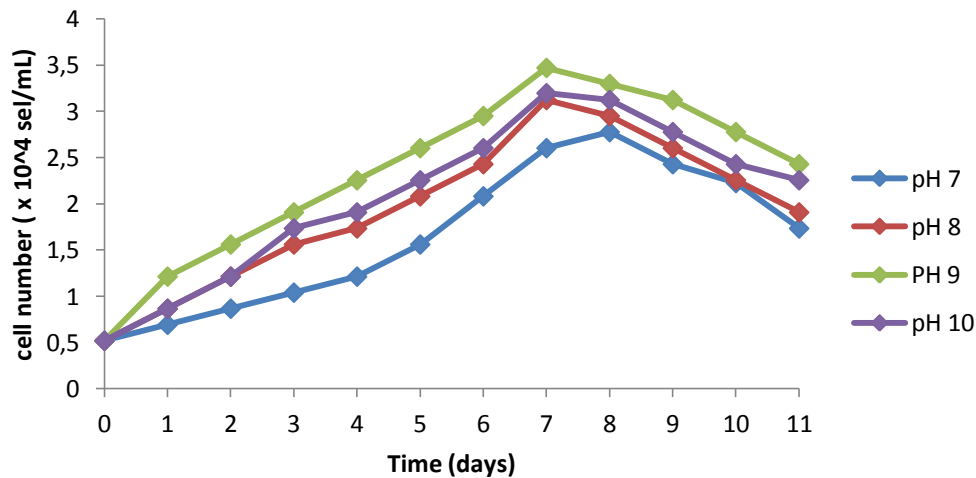


Figure 1. *Spirulina platensis* microalgae cells during cultivation

Based on Figure 1 it can be seen that the optimum pH for the growth of microalgae *Spirulina platensis* is at pH 9. At this pH 9, microalgae cells have the highest density compared to another pH. This is in accordance with [11] stated that the pH value in media the growth of *Spirulina* microalgae ranges from 8-11. pH of 9, it is considered work optimally by activating several enzymes that are not active at another pH. Most algae grow under normal pH conditions between 6 and 8, but some *Cyanobacteria* algae such as *Spirulina platensis* grow in alkaline/alkaline conditions [14]. In Figure 1 shows the development of microalgae population during 11 days of culture. In general, it can be seen that the growth of spirulina in this study showed a growth pattern which is divided into lag phase, exponential phase, decreased phase of growth rate, stationary phase, and death phase.

The lag phase treatment at pH of 7 clearly shown compared to other. In this lag phase, the number of microalgae cells was 0.694×10^4 cells/ml. The lag phase with pH of 7 was due to the low level of acidity in spirulina growing media, it made algae cells unable to adapt well. The lag phase that occurs at pH of 8, 9 and 10 in this study was assumed to occur in a short time so that it was not clearly seen in the observations within 24 hours. Other researcher states that on growth curves sometimes show incomplete growth patterns, it is not due to the absence of one of the phases, but the phase takes place so fast that it is difficult to describe.

The highest microalgae growth phase took place on the 7th day with a number of cells $3,125 \times 10^4$ cells/ml at pH of 8; $3,472 \times 10^4$ cells / ml at pH of 9 and $3,298 \times 10^4$ cells / ml while the highest growth phase of microalgae at pH of 7 took place on 8th day with a number of cells $2,7778 \times 10^4$ cells/ml. It was because of the slow phase of microalgae cell adaptation at pH of 7 called the exponential growth phase. In this phase, exponential growth, microalgae cells divided rapidly and experienced an increasing cell number. It was because of microalgae cells balanced or in a stable culture.

The decline phase growth rate occurred with the exponential phase ending. In this phase occurred between the 8th day to the 11th day. Decreasing in growth rate occurred because cells begin to nutritional deficiencies and due to the shadow of the cell itself (self-shading). According to [15], the increasing of cells concentration in culture will increase self-shading which further decreases the rate of growth.

The next phase was the stationary phase. The stationary growth phase is characterized by a balanced growth rate with the rate of death because the increase in population density is balanced with the rate of death, so there is no more population growth. In this study, the stationary phase in each treatment was not clearly visible. It was likely because of the stationary phase took place quickly so that it was not observed at an interval of 24 hours.

The lowest number of cells phase, in this study, occurred on the 11th day. The increasing algae population that occurred will cause nutrients to decrease very quickly and affect the decline in growth rate, and continue into the stationary phase and death phase. The number of cells in this death phase was 1.736×10^4 cells / ml at pH of 7; $1,909 \times 10^4$ cells / ml at pH of 8; $2,430 \times 10^4$ cells / ml at pH of 9 and $2,256 \times 10^4$ cells / ml at pH of 10.

3.2 Lipid Extraction Process

The following Table 2 is presented for the data of lipid extraction results using maceration, osmotic and soxhlet methods

Table 2. Lipid Extraction Results

Extraction Method	% Yield
Maseration	5,5
Osmotic	0,6
Soxhlet	9

Based on the data above it can be analyzed that the maceration method produces higher % yield than the osmotic method which is 5.5% for the maceration process and 0.6% for the osmotic method. However, when compared with the soxhlet method, the maceration method produces lower lipid yield. According [10], that the Soxhlet method is the best method for obtaining a lot of lipids from microalgae *Botryococcus brauni*, the sample is extracted perfectly because it is conducted repeatedly. The solvent used in this method was n-hexane. It is based on the nature of fat which is not soluble in water or polar solvents but can only be dissolved in non-polar solvents. Lipid extraction using soxhlet method with n-hexane solvent is also influenced by the type of microalgae used. In this study, the microalgae used were dry so that there was no water content in microalgae. As [15] states, it is possible to extract oil from a microorganism using n-hexane solvents by minimizing the water content in the microorganism. Conducting microalgae extraction using the soxhlet method even though it produces high yield %, some researchers claim that the soxhlet method is quite expensive and requires higher energy. Therefore, it is necessary to conduct a deeper further research to get other methods at a lower cost. The lowest lipid extraction results found in the osmotic method was only 0.6%. The osmotic method extracts lipid compounded by suddenly decreasing osmotic pressure on a microorganism so that it caused damage to the cells. However, this osmotic method was less optimal for extracting lipids from dried microalgae. Because of the dried material of the semipermeable membrane had been damaged due to the drying process, so there was no more osmotic pressure [16]. It was assumed as the cause of the low yield of microalgae lipids.

3.3 Analysis of Density and Caloric Value of FAME

Fatty acid methyl ester (FAME) obtained was then analyzed for its density and caloric value. But before the analysis is conducted, it is necessary to carry out a purification step which aims to eliminate some of the impurity parameters in a mixture of methyl esters, namely: free fatty acids, soap, glycerol, water, alcohol, and residual catalysts. The following are the results of the analysis of density and caloric value.

Table 3. Analysis of Density and Caloric values of FAME

Extraction Method	Density (g/cm ³)	Caloric Value (cal/g)
Maseration	0,875	11200
Osmotic	0,855	9800
Soxhlet	0,860	10400

The density of all FAME has met the Indonesian Standard (SNI 7182:2015: biodiesel density ranging from 0.850 - 0.890 g / cm³). Caloric value is the amount of energy produced by complete combustion of material or fuel. The test results obtained the caloric value for biodiesel produced by extraction using the maceration process was 11200 cal / g, while the caloric value for biodiesel from the osmotic method was 9800 cal / g and caloric value from the soxhlet method was 10400 cal / g. The caloric values minimal for Biodiesel according to EN 14214 is 10.898 cal / g and when compared to the caloric value of biodiesel produced only biodiesel with lipid derived from maceration methods that met the standard.

4. Conclusion

Based on the results of the research, it can be concluded that in cultivation studies, the optimum pH in microalgae cell growth of *Spirulina platensis*. is 9, while the growth peak of *Spirulina platensis* microalgae occurs on the 7th day for pH of 8.9 and 10 with the number of cells at each pH of 3.125×10^4 cells/ml; $3,472 \times 10^4$ cells/ml and $3,298 \times 10^4$ cells/ml. The result of dry extraction lipid, the maceration extraction method produces higher % lipid yield of 5.5% while the osmotic extraction method only produces % lipid of 0.6%. However, compared to the Soxhlet method, the result is higher, which is 9% lipid. The biodiesel produced in terms of density of has met the standards SNI 7182:2015 and for the caloric value of FAME using maceration extraction method has met EN 14214 standard.

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