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THE POTENTIAL OF TROPICAL MICROALGAE AS FLOCCULANT IN HARVESTING PROCESS

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ABSTRACT: This environmental biotechnology research aims to obtain a diversity of freshwater tropical microalgae which has the ability to form a floc naturally to be utilized as bioflocculants in the process of harvesting other tropical microalgae biomass that does not have such ability. The microalgae *Chlorella vulgaris, Chlorella sorokiniana, Chlorococcum* sp., *Closterium* sp., *Oscilatoria* sp., *Monorapidhium* sp, *Ankistrodesmus* sp. and *Scenedesmus obliquus* are cultivated in batch culture and their environmental condition is controlled at 25°C, fed with 5% pure CO_2 with a flow rate of 5 L/min. To determine the ability of microalgae strain as microalgae flocculant or not, it is necessary to calculate recovery efficiency. The addition of *Scenedesmus obliquus* and *Ankistrodesmus* sp. as flocculant microalgae followed by slow mixing allows better interaction between flocculant microalgae and non-flocculant microalgae, so the floc size increases. *Scenedesmus obliquus* and *Ankistrodesmus* sp. can be used as flocculant species because the value of settling for both types of microalgae is more than 50% for 60 minutes while other microalgae species can be categorized as non-flocculant microalgae since within 60 minutes settles less than 50%. The study also proved that no greenhouse gas was formed during the bioflocculation process. Thus the method of bioflocculation with *Scenedesmus obliquus* and *Ankistrodesmus* sp. is feasible to be applied to harvest microalgae biomass on an industrial scale.

Keywords: Bioflocculation, Tropical microalgae, Flocs, Harvesting, Diversity

1. INTRODUCTION

In a series of microalgae biomass production systems, the harvesting stage must be through first before entering any processing stage of the microalgae, such as to be bioenergy raw materials, foodstuffs, raw materials of drugs and cosmetics. In order to obtain high biomass production, it is necessary to do research on harvesting with the right method without causing new problems. Since microalgae biomass concentrations are often very low with size only a few micrometers (1 to 30 μm), then harvesting of microalgae becomes difficult [1] and expensive [2]. The cost of harvesting can be reduced significantly by choosing a more efficient and economical harvesting technique. Although some harvesting techniques have currently known, the main disadvantage of such of them is requiring expensive operational costs due to high energy dependence and less environmentally friendly.

Centrifugation is one of the most commonly used harvesting techniques because its ability to produce 80-90% harvested biomass from cultivation results in only one stage thus increasing efficiency in the harvesting stage [3], [4]. Harvested microalgae can reach up to 20% of total solids when using centrifugation [1]. However, centrifugation is a technique with high capital, energy and operational costs [5].

In addition, this process allows the components in the cell to be damaged [3], [6]. Another technique that is commonly used is sedimentation, but this technique is inefficient with time and requires space to build a storage pond. Harvesting of microalgae using filtration techniques can only be done to harvest microalgae which larger than 100 μm and have filamentous or colonized cell shape, such as *Spirulina* sp. and *Micractinium* sp. [7].

Harvesting of microalgae biomass using chemical flocculants and biofilms has been done by previous researchers. They mention that flocculation is ideal for harvesting microalgae smaller than 100 μm and not colonizing. Flocculation using chemicals can also lead to nutrient degradation, changes in temperature and dissolved O_2 [8]. Chemical flocculants may contaminate the water used as microalgae culture medium. Water remaining after harvesting when disposed directly into the environment must be processed first to meet the required quality standards. The process requires a lot of money and energy. The addition of chemical compounds can also cause

changes in the composition of microalgae cells [9], [10].

Bioflocculation is a technique of harvesting microalgae whose principle is similar to flocculation, which is the difference is the flocculant material used. Bioflocculation uses living things such as bacteria, fungi, or microalgae as flocculants. Flocculation using bacteria [11] and fungi [12] are alternatives to replace chemical flocculants, so the effects of chemical pollution on culture media can be reduced. However, the use of fungi and bacteria as flocculants requires additional special media as a source of energy for its growth. In addition, bacteria and fungi can contaminate microalgae [11], [13].

The use of microalgae as flocculants does not require different growth media so it reduces additional costs. In addition, it will prevent bacterial contamination of the microalgae to be harvested [13]. Most importantly, bioflocculation by utilizing microalgae is more environmentally friendly because residual water harvesting that not contain chemicals can be reused or can be disposed of without requiring special treats with a high cost.

In addition, microalgae flocculants can reduce dependence on energy used in the harvesting process until microalgae processing becomes a useful product. Thus, the problems to be studied include the fastest and most stable microalgae floc formulation test by taking into account the gradient criteria of floc-forming speed. In addition, optimal flocculant microalgae ratios of non-flocculant microalgae need to be determined to obtain maximum yield. To find out how far the bioflocculation technique gives an effect of efficiency in harvesting, it will be extracted to obtain by-products, such as lipids and starch.

By studying the floc-forming speed gradient, it is particularly expected to provide information on the diversity of microalgae as the flocculant agent that gives the highest biomass yield. In general, this research is expected to provide a scientific contribution to the development of microalgae biomass production technology that is more economical, efficient and environmentally friendly.

2. RESEARCH METHODOLOGY

2.1 Cultivation of Flocculant and Non Flocculant Microalgae

Microalgae were cultivated using an artificial growth medium of Provasoli Haematococcus Media (PHM). Microalgae that grow in light and climate are controlled 25°C in temperature, fed with 5% pure CO_2 with a flow rate of 5 L · min^{-1}, illuminated by fluorescent lamps (4000 lux) with 16/8 hour light/dark cycle [14]. All microalgae were tested on its ability to improve the recovery efficiency.

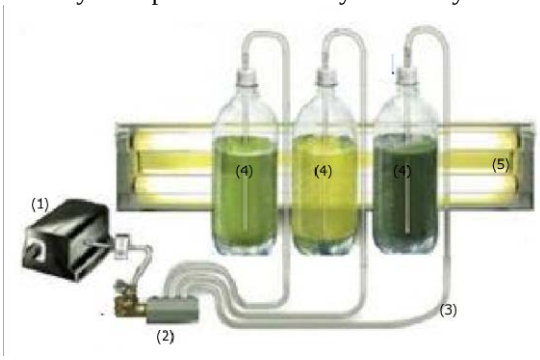

Fig. 1 Scheme of Microalgae Cultivation in Batch Culture, not to scale
(1) aerator, (2) regulator to regulate aeration rate, (3) silicone hose for aeration channel, (4) bottle of culture, (5) fluorescent lamps

2.2 The Study of Flocculation (%) of Microalgae by Flocculants and Bioflocculants

The precipitation of non flocculating microalgae with bioflocculant is obtained from the calculation of OD_{750} (Optical Density) data using equation 1 [13].

$$recovery\ (\%) = \frac{OD_{750}(t_0) - OD_{750}(t)}{OD_{750}(t_0)} . 100 \quad (1)$$

$OD_{750}(t_0)$ is the turbidity of the sample taken at time zero and OD_{a750} (t) is the turbidity of the sample taken at time t.

To determine the ability of microalgae strain as microalgae flocculant or non-flocculant microalgae, it is necessary to calculate recovery efficiency, i.e. recovery by non-flocculant microalgae to flocculating microalgae divided by non-flocculant microalgae recovery without the presence of flocculant microalgae [13].

$$recovery\ efficiency\ (\%) = \left[1 - \frac{\frac{OD_{a750}(t)}{OD_{a750}(t_0)}}{\frac{OD_{b750}(t)}{OD_{b750}(t_0)}}\right] . 100 \quad (2)$$

$OD_{a750}(t_0)$ and $OD_{a750}(t)$ are the turbidities of samples of non-flocculating microalga with flocculating microalga taken at time zero and at time t, respectively. $OD_{b750}(t_0)$is the turbidity of sample of non-flocculating microalga taken at time zero and OD_{b750} (t) is the turbidity of the same sample taken at time t (Fig. 2)

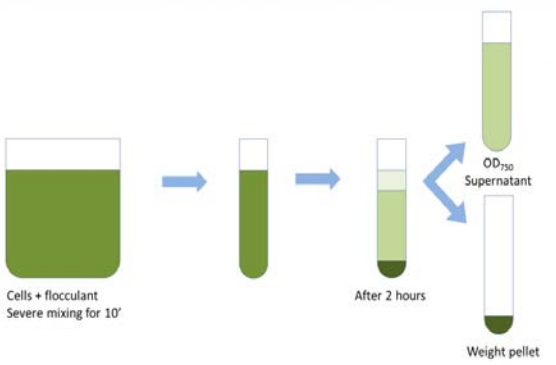

Fig. 2 Determination of Recovery Efficiency

3. RESULT AND DISCUSSION

Flocculation tests have been performed on 8 types of microalgae identified morphologically, as shown in Figure 3. The 6 of 8 microalgae were *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlorococcum* sp., *Closterium* sp., *Oscillatoria* sp., and *Monorapidhium* sp. (Fig. 3 a, b, c, d, e, f) in the first 1 to 6 hours visual observation remained unflocculated and sedimentation, while the other 2 species were *Scenedesmus obliquus*, *Ankistrodesmus* sp. (Fig. 3g, h) experienced floc formation followed by a settling time of fewer than 60 minutes of observation.

The experimental results have shown that the addition of *Scenedesmus obliquus* and *Ankistrodesmus* sp. as flocculant microalgae followed by slow mixing allows better interaction between flocculant microalgae and non-flocculant microalgae, so the floc size increases.

Settling time depends greatly on flock size. The larger the size of the floc that is formed, the faster settling time. Flocculation efficiencies is influenced by several factors, i.e pH [14], dosage of biofloccurrent [11], [15] and proper mixing [12] . Initial pH values of culture can be adjusted to improve flocculation efficiency [14]. Incorrect doses may decrease flocculation efficiency. Mixing is necessary to increase the contact between microalgae cells so as to facilitate the formation of large flocs in a shorter time than without adequate mixing [12].

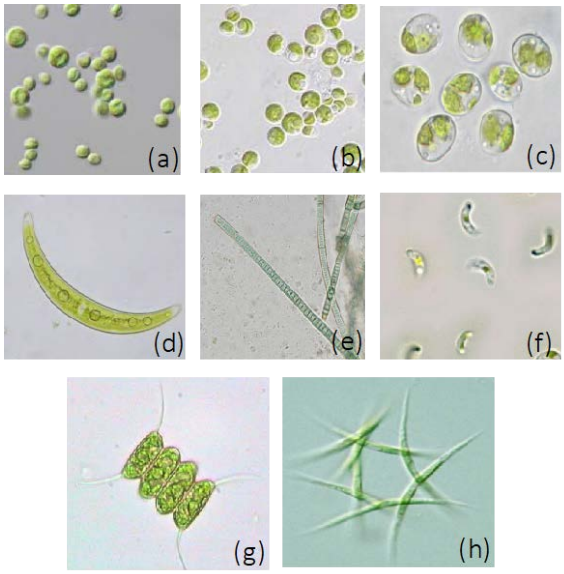

Fig. 3 Non flocculant microalgae: a) *Chlorella vulgaris*, b) *Chlorella sorokiniana*, c) *Chlorococcum* sp., d) *Closterium* sp., e) *Oscilatoria* sp., f) *Monorapidhium sp.* Flocculant microalgae: g) *Scenedesmus obliquus*, h) *Ankistrodesmus* sp.

Flocculant formation occurring in the flocculant microalgae can be seen in Fig. 4, while the microalgae flocculation capability and ability within 60 minutes of observation can be seen in Fig. 5. Figure 5 shows that the addition of *Scenedesmus obliquus* into culture followed by slow mixing allows increasing interaction between the flocculant microalgae cells and the non-flocculant microalgae so that the flocculation performance is efficient.

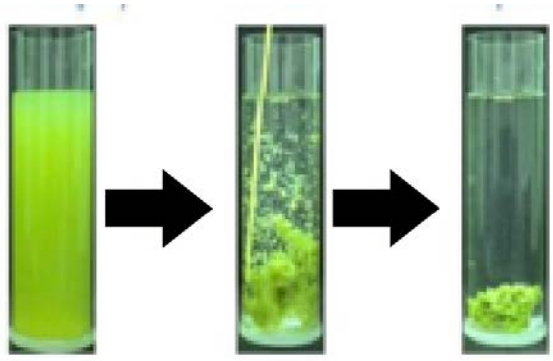

Fig. 4 The formation of floc on *Scenedesmus obliquus* within 60 minutes of observation

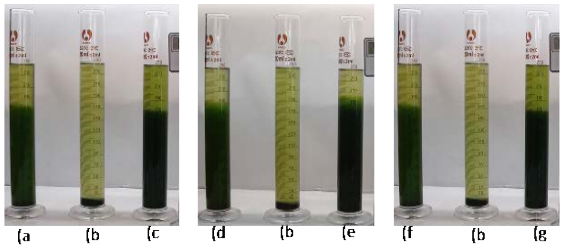

Fig. 5. The ability of microalgae flocculation within 60 minutes of observation: a) *Chlorella vulgaris,* b) *Scenedesmus obliquus* c) *Chlorella sorokiniana,* d) *Chlorococcum* sp., e) *Closterium* sp., f) *Oscilatoria* sp., g) *Monorapidhium* sp.

On a laboratory scale, the effectiveness of dewatering technology in relation to the process of harvesting of many microalgae biomass has been reported to have achieved significant efficiencies, but increasing to a larger scale still make problems, i.e less economically viable if harvesting implemented to produce products such as biofuel [5], [16]. In contrary, bioflocculation harvesting is an environmentally friendly method, consuming less energy, can take place relatively quickly (within minutes).

The flocculant and non-flocculant microalgae species were determined from the settling percentage (%) calculated on the basis of the OD_{750nm} value of each microalgae (Eq. 1). The value of OD_{750nm} for *S. obliquus* and *Ankistrodesmus* sp. initially 0.20 ± $7.16x10^{-3}$ and 0.18 ± $9.17x10^{-3}$ respectively and subsequently decreased rapidly after the first hour to 0.09 ± $4.13x10^{-3}$ and 0.095 ± $3.89x10^{-3}$, then decreased slowly until the 12^{th} hour became 0.04 ± $6.16x10^{-3}$ and 0.05 ± $3.38x10^{-3}$. Salim et al. [13] state that species potentially made as microalgae flocculants are microalgae with a settling value (%) of 50 or more within 60 minutes.

Table 1. Percentage of the settling value in the first 60 minutes

Tropical microalgae	Settling value
Flocculant microalgae	
4. *Scenedesmus obliquus*	55.1% ± 1.24
5. *Ankistrodesmus* sp	52.3% ± 3.71
Non flocculant microalgae	
1. *Chlorella vulgaris*	17.3% ± 0.88
2. *Chlorella sorokiniana*	16.7% ± 1.13
3. *Chlorococcum sp.*	21.5% ± 1.19
4. *Closterium sp.*	25.1% ± 1.36
5. *Oscilatoria sp.*	27.3% ± 2.11
6. *Monorapidhium sp.*	17.8% ± 1.89

This indicates that *Scenedesmus obliquus* and *Ankistrodesmus* sp. can be used as flocculant species because the value of settling for both types of microalgae is more than 50% for 60 minutes while other microalgae species can be categorized as non-flocculant microalgae since within 60 minutes settles less than 50% (Table 1). The fastest of settling occurs in the ratio of flocculant and non-flocculant microalgae are 4: 4 compared to 1: 4, 2: 4 and 3: 4 ratios. This suggests that the addition of more flocculant species may increase the precipitation percentage. Salim et al. [13] suggest that the addition of flocculant species with higher concentrations in harvesting will increase the settling percentage.

The results of this study also show that the difference of settling rate (%) among non-flocculant microalgae species is affected by the size of each kind of microalgae. The size of non-flocculant microalgae of *Chlorella* sp. is measuring 2.0-10.0 μm, *Monorapidhium* sp. is 2-11 μm., *Chlorococcum* sp. is 5.0-12.0 μm, *Closterium* sp. is 10-15 μm, *Oscilatoria* sp. is 10-150 μm with filament 2-20 μm while *Scenedesmus* and *Ankistrodesmus* have sizes of 15-20 μm and 12-14 μm respectively. According to [10] the rate of particle precipitation is influenced by the size of the particle, the larger the size of a particle the easier the settling.

Sathe [19] mentions that bioflocculation is spontaneous flocculation of microalgae cells occurring as a result of extracellular polymeric substances (EPS) secretion when the microalgae are under stress conditions. Lack of nutrients is a major factor causing microalgae cells to overexpress the substance of the extracellular polymer [20]. The substance of the extracellular polymer produced by the microalgae will trigger the formation of cell clumps, which then will form a precipitated biomass

The results of bioflocculation observations under a microscope with 10x magnification showed microalgae forming large networks connected to each other. At the beginning of mixing (t=0) visible cells flocculant and non-flocculant microalgae are still separated and not bounded at all. Gradually the binding process begins to occur as the cells begin to experience stress due to decreased nutrition and trigger the microalgae to secrete the extracellular polymer. The resulting extracellular polymer creates bonds between microalgae cells. The bond between the flocculant and non-flocculant microalgae cells at 5th hour has begun to appear with the bond formation between the microalgae species. More microscopically bonds begin to appear at 12th hours, when non-flocculant species are bound to flocculant species and form larger batches of biomass, causing the flocs cells to settle.

The study also proved that no greenhouse gas was formed during the bioflocculation process. Thus the method of bioflocculation with *Scenedesmus obliquus* and *Ankistrodesmus* sp. is feasible to be

applied to harvest microalgae biomass on an industrial scale.

4. CONCLUSION

It is work examined the flocculation performance of the 6 freshwater microalgae i.e *Chlorella vulgaris, Chlorella sorokiniana, Chlorococcum* sp., *Closterium* sp., *Oscilatoria* sp., *Monorapidhium* sp. induced by the bioflocculant *Ankistrodesmus* sp. and *Scenedesmus obliquus*. This research is advanced research as an effort to obtain the technique of cheap harvesting and environmentally friendly so that economic feasibility in microalgae biomass production can be improved. Thus biofloculation can be industrialized. Furthermore, further research is needed to analyze the biochemical composition of microalgae harvested by bioflocculation to produce biofuel. Further information on energy consumption and process costs should also be further investigated for the development of commercialization of microalgae-based products

5. ACKNOWLEDGMENTS

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