Harvesting of freshwater microalgae biomass by *Scenedesmus* sp. as bioflocculant

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**Abstract.** This study is particularly expected to provide information on the diversity of microalgae as the flocculant agent that gives the highest biomass yield. Bioflocculation was done by using one of the flocculating microalgae i.e. *Scenedesmus obliquus* to concentrate on non-flocculating microalgae *Chlorella vulgaris*. The freshwater microalgae *S. obliquus* tested its ability to harvest other non-flocculating microalgae, increased sedimentation rate in the flocculation process and increased biomass yield. The flocculation of biomass microalgae with chemical flocculant as comparison was done by adding alum ($\text{K}_2\text{SO}_4\cdot\text{Al}_2(\text{SO}_4)_3\cdot24\text{H}_2\text{O}$). The addition of alum ($\text{K}_2\text{SO}_4\cdot\text{Al}_2(\text{SO}_4)_3\cdot24\text{H}_2\text{O}$) as flocculant at pH 11 and *S. obliquus* sp. as bioflocculant caused significant alteration of nutrition of microalgae. Overall, the essential content produced by flocculation method with addition of alum or with bioflocculation (%, mg/100 mg dry weight) are lipid 31.64; 38.69, protein 30.79; 38.50%, and chlorophyll 0.6253; 0.8420). Harvesting with bioflocculation methods conducted at the end of the cultivation period increase the amount of biomass significantly and can accelerate the settling time of biomass. Harvesting microalgae cells by bioflocculation method becomes an economically competitive harvesting method compared to alum as a chemical flocculant because of the cheaper cost of flocculant, not toxic so it does not require further water treatment after harvesting due to the use of alum as chemical flocculants.

**Keywords:** biomass, bioflocculant, freshwater, flocculating microalgae

1. **Introduction**

The cultivation of tropical microalgae as a source of renewable energy and dietary supplements has constrained the harvesting of the microalgae itself. Small size and density make microalgae difficult to harvest. To improve the economic viability of microalgae in order to obtain a useful final product, there are three important key parameters: high biomass microalgae productivity, high metabolite compound content, and cheap and environmentally friendly harvesting methods. Harvesting of microalgae is an important part in microalgae cultivation system to produce higher biomass. The purpose of harvesting is to obtain microalgae slurry, at least 2-7% of total solids [1]. However, the harvesting of microalgae is often still a constraint. Harvesting microalgae can be done by techniques such as centrifugation, filtration, sedimentation, flocculation, flotation, ultrasonic vibration, and screening [2], [3], [4], [5].
The main disadvantage of these harvesting techniques is that it requires expensive operational costs due to high energy dependence and less environmentally friendly. In commercial industry, the best biomass harvest can reach between 0.3-0.5 g dry cell / L or 5 g dry cell / L. This makes the harvest of microalgae very difficult and expensive [6]. To minimize energy consumption in the process of harvesting microalgae, an integrated approach is needed.

Flocculation can be done in various ways. Chemical flocculation performed using Zn$^{2+}$, Al$^{3+}$, Fe$^{3+}$ or other chemical flocculants has been studied extensively [1].

1) Several chemical flocculants have been applied on an industrial scale, especially in wastewater treatment plants [7]. Although the use of chemical flocculants can be done easily but this method is expensive and not environmentally friendly because the excess caution in the flocculants needs to be removed first before the residual water (medium) of production is discharged into water bodies, thus requiring additional operational costs again [7].

2) Flocculation can also be induced by altering the culture conditions by applying extreme pH, nutrient reductions, temperature changes and changes in dissolved O$_2$ levels. The flocculation method is not appropriate for pre-harvesting of microalgae on a large scale. Most of these methods are not applicable because flocculation is difficult to control and can lead to undesirable cell composition [8]. All of these growth mediums require pre-treatment to remove chemical flocculants cations when they are reused [7].

Bioflocculation is a technique of harvesting microalgae whose principle is similar to flocculation, which is the differentiator is the flocculant used. Bioflocculation uses living things such as bacteria, fungi, or microalgae as flocculants. Flocculation using bacteria [8] and fungi [1] 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 [9].

This research is an advanced research from previous 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. By studying the speed gradient in floc formation, this study is particularly expected to provide information on the diversity of microalgae as the flocculant agent that gives the highest biomass yield. In general, it is expected that this research can provide scientific contribution to the development of microalgae biomass production technology that is more economical, efficient and environmentally friendly.

### 2. Research Method

#### 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 by incubators with a rotational speed of 100 rpm, 25 °C, fed with 2% pure CO$_2$ with a flow rate of 5 L·min$^{-1}$, illuminated by fluorescent lamps (50 μmol·photons m$^{-2}$·s$^{-1}$) with 16/8 hour light/dark cycle [11], [12]. The S. obliquus as flocculating microalgae was tested on its ability to improve the recovery efficiency and the rate of harvesting of the non-flocculating microalgae (Chlorella vulgaris and Ankistrodesmus sp.).

#### 2.2. Measurement of microalgae growth

Cell concentration was measured as the optical density at 750 nm (OD750) with an Ultraspec 2,000 spectrophotometer (Pharmacia Biotech Ltd. UK) equipped with a temperature controlled carousel cell holder with six positions. Demineralized water served as reference. The microalgal samples were diluted in a 10 × 10 × 45 mm polystyrene cuvette (Sarstedt, DE) using filter-sterilized tap water for the freshwater microalgae.

#### 2.3. The study of flocculation (%) of microalgae by flocculants and bioflocculants

The flocculation of biomass microalgae with chemical flocculant as comparison was done by adding alum (K$_2$SO$_4$.Al$_2$(SO$_4$)$_3$.24H$_2$O) with concentration of 120 ppm, pH 10. After the settling is done the
filtration or filtration process. The precipitation of microalgae with bioflocculant is obtained from the calculation of OD750 (Optical Density) data using equation 1 [13].

\[
\text{recovery} \; (\%) = \frac{\text{OD}_{750}(t_0) - \text{OD}_{750}(t)}{\text{OD}_{750}(t_0)} \times 100
\]  

(1)

\( \text{OD}_{750}(t_0) \) is the turbidity of sample taken at time zero and \( \text{OD}_{750}(t) \) is the turbidity of the sample taken at time t.

2.4. Determination of Recovery Efficiency

To determine the ability of microalgal 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 presence of flocculant microalgae (adapted from [1], [10]).

\[
\text{recovery efficiency} \; (\%) = \left[ 1 - \frac{\text{OD}_{a750}(t)}{\text{OD}_{b750}(t)} \right] \times 100
\]  

(2)

\( \text{OD}_{a750}(t_0) \) and \( \text{OD}_{a750}(t) \) are the turbidities of samples of non-flocculating microalgae with flocculating microalgae taken at time zero and at time t, respectively. \( \text{OD}_{b750}(t_0) \) is the turbidity of sample of non-flocculating microalgae taken at time zero and \( \text{OD}_{b750}(t) \) is the turbidity of the same sample taken at time t.

\( \text{OD}_{a750}(t_0) \) and \( \text{OD}_{a750}(t) \) are the turbidities of samples of non-flocculating microalgae with flocculating microalgae taken at time zero and at time t, respectively. \( \text{OD}_{b750}(t_0) \) is the turbidity of sample of non-flocculating microalgae taken at time zero and \( \text{OD}_{b750}(t) \) is the turbidity of the same sample taken at time t.

3. Results and Discussion

Increased recovery of non-flocculant microalgae added with flocculant microalgae have been evaluated by calculating percentage of recovery efficiency. For calculating the percentage of recovery efficiency (Equation 2), the measurements are seen from the average turbidity. Sedimentation rates and recovery percentages for all samples tested showed a standard deviation of less than 3.5%.

The flocculant microalgae exhibit higher recovery efficiency when added to higher concentrations, however, doubling the doubled flocculent microalgae concentration does not necessarily result in a twice higher recovery efficiency of the non-flocculant microalgae.

Sedimentation of microalgae suspension was monitored for 8 hours and recovery percentage of microalgae was determined from time to time (Figure 1). The microalgae sedimentation rate in the suspension was calculated by linear regression of the data in the curve of the recovery percentage in time and the use of the linear regression curve.

The initial sedimentation rate of flocculant microalgae measured during the first 2 hours was higher than sedimentation by non-flocculant microalgae (Table 1 and Table 2). Mixing of flocculant microalgae increases the rate of early sedimentation of non-flocculant microalgae. Large clumps formed by flocculant microalgae seem to cause the non-flocculant microalgae trapped. In addition, the sedimentation rate becomes faster than individual non-flocculant microalgae cells without mixing. Furthermore, increasing the ratio of bio-flocculent microalgae to non-flocculant microalgae causes higher sedimentation rate to be higher.
A = t0, B = after 8 hours,
mix 1 added by K₂SO₄-Al₂(SO₄)₃·24H₂O; (2) = S. obliquus; (3) C. vulgaris
(4) C. vulgaris added by S. obliquus; (5) Ankistrodesmus sp added by S. obliquus; (6) Ankistrodesmus sp.

Figure 1. Sedimentation of microalgae suspension.

Table 1. Optical densities (OD750 (t0)).

|          | A    | B    | C    | D    |
|----------|------|------|------|------|
| Mix-1    | 0.8  | 0.9  | 0.8  | 0.8  |
| Mix-2    | 0.7  | 0.5  | 0.6  | 0.8  |
| Mix-3    | 0.5  | 0.6  | 0.6  | 0.6  |

A = K₂SO₄-Al₂(SO₄)₃·24H₂O, B = S. obliquus
C = The non-flocculating microalgae (V. vulgaris) with low concentration of added S. obliquus
D = The non-flocculating microalgae (Ankistrodesmus sp.) with high concentration of added S. obliquus

Table 2. Initial sedimentation rate.

|          | Initial sedimentation rate (%) | A    | B    | C    | D    |
|----------|-------------------------------|------|------|------|------|
| (K₂SO₄-Al₂(SO₄)₃·24H₂O) | recovery.h⁻¹) | Mix-1 | 39.4 | 16.7 | 13.3 | 9.8 |
|          | Mix-2 | 34.9 | 23.7 | 20.4 | 14.6 |
|          | Mix-3 | 43.8 | 40.0 | 35.7 | 16.9 |

A = K₂SO₄-Al₂(SO₄)₃·24H₂O
B = S. obliquus
C = The non-flocculating microalgae (C. vulgaris) with low concentration of added S. obliquus
D = The non-flocculating microalgae (Ankistrodesmus sp.) with high concentration of added S. obliquus

Flocculating is a method of harvesting where microalgae linked each other and converge to form a larger mass mass, called floc. Flocculation in this study is a process of aggregating small particles into larger particles resulting from the existence of physical or chemical instability. In some literature, it is mentioned that flocculation is the same as coagulation, because the mechanism of operation is almost the same. However, for some researchers, the two methods are different, where flocculation forms aggregates due to polymers, whereas coagulation results from electrolytes. The aggregates formed are
caused by changes in pH or addition of flocculating microalgae as bioflocculants or chemical coagulants.

In the intercellular connection there are two forces involved. The first force is the repulsive force of electrostatic rejection of the cell surface because it has the same charge, which is negatively charged. This is due to the ionization of functional ionogenic groups [9]. This force occurs at a considerable distance relative. At a very close relative distance, there is an interesting attraction due to Van der Waals intermolecular forces. The intermolecular force is larger than the electrostatic force, but only occurs in very close proximity [13].

*S. obliquus* microalgae is proven to function as a bioflocculant to overcome some of the constraints that occur in the harvesting process. Thus, this study proves that total recovery and sedimentation rates of various non-flocculant microalgae will increase in each addition of different flocculant microalgae. The results showed that the addition of autoflocculant microalgae was able to induce sedimentation rate faster than sedimentation rate of non-flocculant microalgae so as to increase the efficiency of harvesting. Similar positive effects on sedimentation rates and harvesting efficiency were also observed in bioflocculation by bacteria as non-flocculant microorganisms [14].

4. Conclusion
Harvesting of non-flocculating microalgae biomass by *Ankistrodesmus* sp as bio-autoflocculating has been evaluated as an effective and promising alternative method for future harvesting of microalgae. With bioflocculation method, harvesting biomass of microalgae is possible without addition of chemical flocculant. Growth media during the cultivation process does not contain harmless chemicals so that it does not require processing before it is discharged into the environment. Bioflocculation is an alternative to energy-efficient harvesting methods.

Acknowledgment
The authors would like to thank Directorate for Research and Community Services as well as Directorate General for Strengthening Research and Development at the Ministry of Research, Technology, and Higher Education Indonesia for funding this study through Hibah Penelitian Pasca Doktor 2017

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