

Bioflocculation Activity in Harvesting System: A Biotechnology Approach for Microalgae Biomass

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Abstract—A study on a freshwater green microalgae species; *Ankistrodesmus* sp, —as a bioflocculant based on its physicochemical properties and flocculation rate has been carried out. The molecular identification via 16S rDNA showed 99% resemblance of this green microalga to the *Ankistrodesmus fumigatus* strain. The optimum batch culture condition for the bio-flocculants production was initiated by 10% inoculum (v/v). The low-concentrated bioflocculant of 10% (v/v) is considered as thermostable with a high flocculation rate to harvest the biomass of *Chlorella* sp. at a pH range of 5 to 9. The source of molasses, the mixture of yeast extract were used as the optimum sources of carbon and Ammonium sulfate were used as the optimum sources of nitrogen in the growth medium. *Ankistrodesmus* sp. bioflocculant has a high flocculation efficiency over a wide range of pH (5–9) with a low dose requirement of 10% v/v at 25°C. Hence, it is immensely competitive to promote the economic viability of the production process. Accordingly, *Ankistrodesmus* sp. bioflocculant has a high potential to be applied on an industrial scale in tropical regions as it does not require additional production cost.

Keywords: bioflocculant, flocculation activity, harvesting, competitive, production cost

Introduction

Microalgae have significant biotechnological potentials to produce valuable products such as feed, food, cosmetics, and they are greatly beneficial to pharmaceutical industries as well as biotechnological processes. Both macro- and microalgae have a crucial role in the world economy today with an estimated turnover of US\$5 billion per year (Niet *et al.*, 2011).

Microalgae are found alive in almost every biotope due to their ecological diversity and physiological adaptability. Green microalgae and Cyanobacteria are large groups of photosynthetic organisms. Being able to grow in various conditions, microalgae are considered as the most powerful organisms on earth. Algae are usually found to be distributed throughout the biosphere over a wide range of environments, ranging from freshwater and hypersaline ecosystems to humid water bodies or terrestrial sites (Zaki, S., *et al.*, 2011). The fundamental differences between algae and terrestrial plants include the lack of phyllids (leaves) and rhizoids of non-vascular plants in algae, or leaves, roots, and other organs commonly found in tracheophytes (vascular plants).

Microalgae are photosynthetic microorganisms with straightforward growing requirements of light, glucose, CO₂, N, P, and K that can yield large amounts of lipids, proteins, and carbohydrates over a short period of time. These products can be processed into biofuels and other economical co-products such as raw materials of medicines, beauty, as well as human food and fodder. The plain diversity, abundance, and growing requirements explain why microalgae cultivation has been massively researched nowadays to obtain high microalga biomass (Li *et al.*, 2007).

The final stage of the microalgae production—cultivated in either open-air pools or controlled photobioreactors—is the harvesting process. Various methods of harvesting have been widely acknowledged, but they generally require a considerable amount of energy. One common approach to harvesting the controlled microalgae culture is the flocculation method by adding chemical flocculants, such

as alum ($K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$), polyaluminium chloride (PAC), and polyacrylamide. These chemical flocculants are often used in the harvesting process due to their cost-effectiveness and high flocculation efficiency (Li *et al.*, 2007). However, eventually, it has been widely reported that the use of chemical flocculants in microalga biomass harvesting systems as animal feed, food industry supplies, and medicines has negative effects on the health of livestock and humans. Among those adverse impacts, the polyaluminium salts can cause Alzheimer's disease (Arezoo, 2002). The residual water of microalgae production also contains chemical flocculants and still requires further processing before being discharged into water bodies. Indeed, it entails an additional cost that needs to be taken into account in industrial-scale production. This inevitable disadvantage of using chemical flocculants has shifted the focus of research on the utilization of various potential microorganisms as flocculants (Nieet *al.*, 2011; Zhiet *al.*, 2010). Researchers have studied the prospects of several types of bacteria and microalgae as bio flocculants.

Unlike chemical flocculants, bioflocculant has additional benefits such as being eco-friendly, biodegradable, free from secondary pollution risks, non-toxic, and harmless to humans, animals, as well as the environment (Nieet *al.*, 2011; Zhang *et al.*, 2002; Yang *et al.*, 2012). Microalgae that have potentials as bioflocculants have been isolated from almost all different types of environments such as soil, wastewater, activated sludge, and rivers (Deng *et al.*, 2005; Yimet *al.*, 2007; You *et al.*, 2008; Wan *et al.*, 2013).

The utilization of bacteria as bioflocculants is considered more promising than chemical flocculants. Bioflocculant bacteria are not only useful in wastewater treatments but also beneficial for a wide range of applications. In an instance, bioflocculants produced by *Sobacillussilvestries* W01 and *Paenibacillus* sp. AM49 are used to harvest marine microalgae of *Nannochloropsisocceanica* and *Chlorella vulgaris*, respectively (Wan *et al.*, 2013; Zhaoet *al.*, 2013). Nonetheless, the cultivation of flocculant bacteria in the microalgal biomass harvesting system surely has different nutrient requirements which result in the additional cost of the production process. Therefore, currently, research on the harvesting of economically competitive microalgae is more focused on increasing the utilization of other auto-flocculants microalgae since both the flocculants and the harvested microalgae need relatively equivalent nutrients. This research is a part of consecutive studies to investigate the ability of freshwater green microalga called *Ankistrodesmus* sp. to harvest the biomass of another freshwater green microalga, *Chlorella* sp., in a controlled environment using batch cultures.

Materials and Methods

Cultivation of *Ankistrodesmus* sp. and *Chlorella* sp. in Batch Cultures

Ankistrodesmus sp. and *Chlorella* sp. microalgae derived from the collection of the Environmental Biology/Microbiology Laboratory, Department of Environmental Engineering, Universitas Trisakti were utilized in this research. They were isolated from the water of Setiabudi Reservoir, Jakarta. Both types of microalgae were cultivated separately in a 1.5L batch culture photobioreactor filled with Provasoli Haematococcus Media (PHM) as the artificial growth medium (**Figure 1**). The cultivation environment was controlled during the study period, comprising a room temperature of $\pm 27^\circ C$, pH 6, a light intensity of 4000 lux, 24-hours bright condition, and continuous aeration (Rinanti *et al.*, 2013).

Determination of Flocculation Activity

The determination of flocculation rate was conducted by calculating the percentage value of flocculation (recovery percentage) obtained from the quantification results of OD₇₅₀ data (optical density) using **Equation 1** (Salim *et al.*, 2010).

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

OD₇₅₀(t₀) is the turbidity value at the beginning of mixing process, while the OD₇₅₀(t_n) is the turbidity value at the measurement time of *n*.



Figure 1. Batch culture cultivation

Optimization of Environmental Conditions for the Bioflocculant Production

Benefits of Carbon Sources

Carbon and nitrogen sources are key factors that greatly affect the production of bioflocculants by microalgae (Zhi et al., 2010). The impact of adding different sources of carbon and nitrogen to the production of bioflocculants follows the Lachhwani's procedure (Zhang et al., 2002). Microalgae were inoculated to the growth medium of PHM contained in separate flasks, each equipped with different carbon sources such as glucose (20 g/L), sucrose (20 g/L), starch (20 g/L), lactose (20 g/L), maltose (20 g/L), as well as fructose (20 g/L), and incubated in a rotary shaker (120 rpm) at 28°C for 5 days. The flocculation rate was calculated according to the previous formula (1).

Benefits of Nitrogen Sources

Several nitrogen sources comprising a) organic nitrogen sources such as yeast extract, urea, peptone, and tryptone; b) inorganic nitrogen sources, e.g., ammonium sulphate; as well as c) combined nitrogen sources consisting of yeast extract, ammonium sulphate, and urea were added as much as 10% (v/v) to the culture of *Ankistrodesmus* sp. to optimize the effect of nitrogen sources on the bioflocculant production. The flocculation rate was calculated based on the mentioned formula (1).

Roles of the Initial pH of Growth Medium

The variation of acidity level (pH), ranging from 5 to 9 on the PHM growth medium with the optimum sources of carbon and nitrogen, was adjusted by adding NaOH (0.1 M) or HCl (0.1 M). The cultures were incubated at room temperature for 5 days, and the flocculation rate was calculated according to the previous formula (1).

Optimization of Flocculation Activity by *Ankistrodesmus* sp.

Bioflocculant Concentration on the Flocculation Activity

A bioflocculant suspension in the exponential phase with a density of 10^6 cells per mL was prepared and used to determine the optimum bioflocculant dose. A total of 10%, 30%, 50%, and 70% suspended bioflocculants were added to the culture of *Chlorella* sp. The mixture of both suspensions was stirred rapidly, and the flocculation activity was observed at room temperature for 2–8 hours. The flocculation activity was measured at 650 nm, and the flocculation rate was calculated based on the Equation 1.

Roles of the Temperature on the Flocculation Activity

The influence of temperature on the flocculation activity was observed by assigning the mixture containing flocculants with the optimum concentration into the culture of *Chlorella* sp. at temperature variations of 20°C, 25°C, 30°C, and 35°C. The flocculation activity was measured at room temperature, and the flocculation rate was calculated according to the previous formula (1).

Roles of pH on the Flocculation Activity

The mixture pH was adjusted to the values of 5, 6, 7, and 8 in a 250 ml flask to test the effect of pH on the flocculation activity. The flocculation activity was then measured at room temperature and calculated based on the Equation 1.

Results and Discussion

Optimization of Culture Conditions for the Bioflocculant Production

Benefits of Carbon Sources

The selection of carbon sources has a major influence on the production of bioflocculants by the bacterial strain. The effect of various carbon sources on the bioflocculant production is shown in Figure 2.

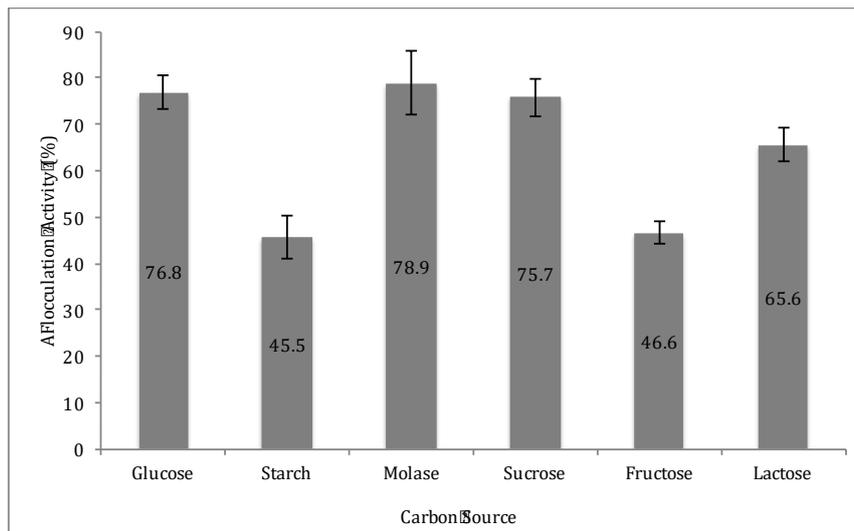


Figure 2. Effect of carbon sources on the production of bioflocculants

Among the tested carbon sources, molasses is the preferred carbon source for bioflocculant production with the highest flocculation activity of 78.9%, followed by glucose and sucrose with a flocculation activity of 76.8% and 75.7%, respectively. Lactose provided a flocculation rate of 65.6%, while fructose and starch showed the lowest flocculation activities of 46.6% and 45.5%, respectively. Molasses were used as the optimum carbon source in the subsequent experiments.

Dearfield *et al.* (1988) and Yimet *et al.* (2007) reported that molasses was the optimum source of carbon in the bioflocculant production by *Solibacillus silvestris* with a flocculation activity of 88.7% (Wan *et al.*, 2013). Glucose, sucrose, and fructose were carbon sources preferred by *Corynebacterium glutamicum* on the other hand (Zhao *et al.*, 2013). Many bioflocculant-producing microorganisms prefer organic carbon sources for bioflocculant production (Li *et al.*, 2007).

Benefits of the Nitrogen Sources

Nitrogen sources play a crucial role in the production of bioflocculants (Li *et al.*, 2007). Different microorganisms use organic or inorganic nitrogen sources, or even both, to produce bioflocculants (Dearfield *et al.*, 1988). The effects of organic, inorganic, and combined nitrogen sources on the bioflocculant production by the tested microalgae were evaluated. Figure 3 shows that the complex nitrogen source—a mixture of yeast extract, urea, and ammonium sulphate—is a better source of nitrogen that can increase the production of *Ankistrodesmus* sp. as bioflocculants compared with the single nitrogen sources that were added separately one by one. Urea preferably supported the bioflocculant production as well by 76.8% compared with the other single nitrogen sources, i.e., peptone, yeast extract, tryptone, and ammonium sulphate.

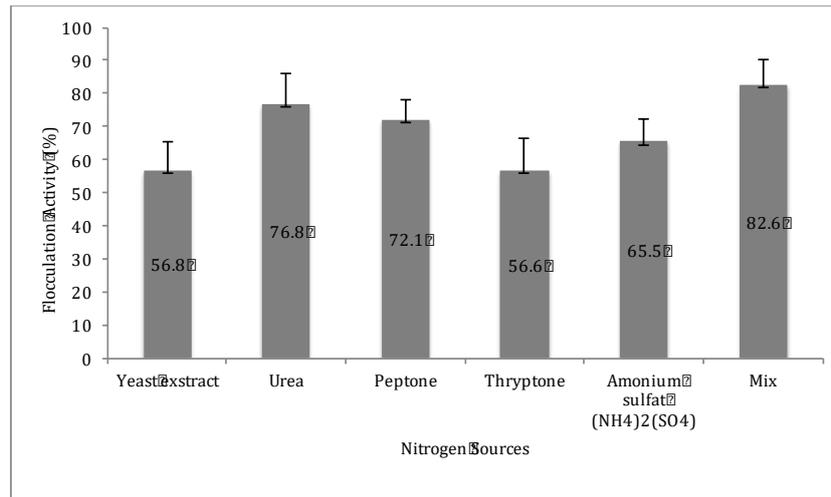


Figure 3. Effect of the nitrogen sources on the bioflocculant production

Zakiet al. (2011); Yimet al. (2007); You et al. (2008) observed that some sources of nitrogen (meat extract and urea) could promote the flocculation activity of up to 94.1% for *Serratiaficaria*. Tryptone emerged as the optimum source of nitrogen that showed a flocculation activity of more than 90% (Zhiet al., 2010). Organic nitrogen sources are more suitable for bioflocculant production and more readily absorbed. Therefore, organic nitrogen sources are more desirable for microalgal cell growth compared with the inorganic ones (Zhang et al., 2002).

Roles of the Initial pH of Growth Medium

The initial pH of the culture medium affects not only the cell electrical charge and the reduction potential of oxidation but also the absorption of nutrients and enzymatic reactions in cells (Li et al., 2007). Thus, the effect of initial pH of the culture medium on the bioflocculant production was examined at pH values ranging from 5 to 9. The results are presented in Figure 4. It can be seen that the optimum bioflocculation activity occurred at pH 7 with a flocculation rate of 52.4%. At an acidic level (pH 5) and alkaline condition (pH 9), the flocculation activity was reduced. Hence, pH 7 was used for the growth medium since the anticipated production cost would be lower for a neutral pH.

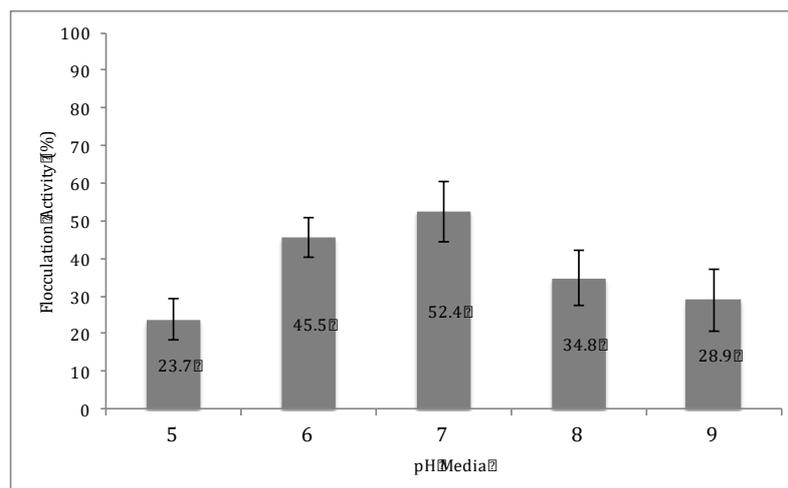


Figure 4. Effect of the initial pH on the growth medium of bioflocculant cultures

Yang et al. (2012); You et al. (2008); Wan et al. (2013) observed the similar neutral pH value on the cultivation by *Halomonas* sp. and *Aspergillus flavus*. The bioflocculant produced by *Klebsiella pneumonia* also achieved the highest flocculation activity at pH 7 (Zhao et al., 2013). However, the required initial pH of the medium to attain the optimum production of bioflocculants varies for different microorganisms. For the

production of biofloculants by *Arthrobacter* sp., the highest flocculation activity was reached at a pH value of above 12.

Biofloculant Concentration on the Flocculation Activity

The primary objective of this study is to determine the lowest concentration of biofloculant that can attain the highest flocculation efficiency. As depicted in Figure 5, the flocculation activity of above 80% occurred in the addition of biofloculant with a concentration of 10%. The maximum flocculation activity (92.3%) took place in the addition of 70% biofloculant. The flocculation activity increased along with the addition of biofloculant as much as 10% (v/v) to 70% (v/v), indicating that there is no major difference in the flocculation activity due to the addition of biofloculant concentration. The results of this study showed that *Ankistrodesmus* sp. as biofloculant exhibited a high flocculation activity with a fairly low concentration (10% v/v)—enough to support the economic feasibility of the production process. Low-dose biofloculants with a high flocculation activity can reduce the production cost simultaneously.

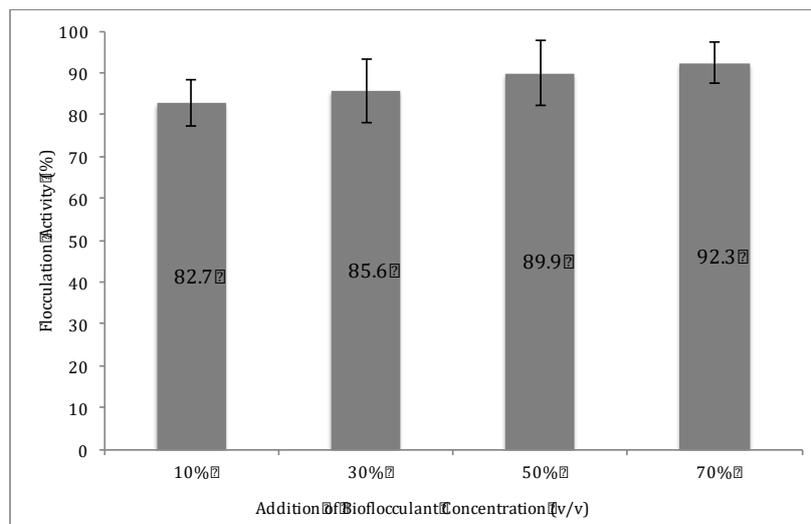


Figure 5. The addition of biofloculant

Roles of the Temperature on the Flocculation Activity

The study of temperature effect on the flocculation activity by the biofloculant denoted that the biofloculant could retain more than 70% of its activity at a temperature of 25°C to 30°C by decreasing only 5% of the flocculation activity after being exposed to heat (35°C) for 1 hour, thereby demonstrating a characteristic of thermostability (Figure 6).

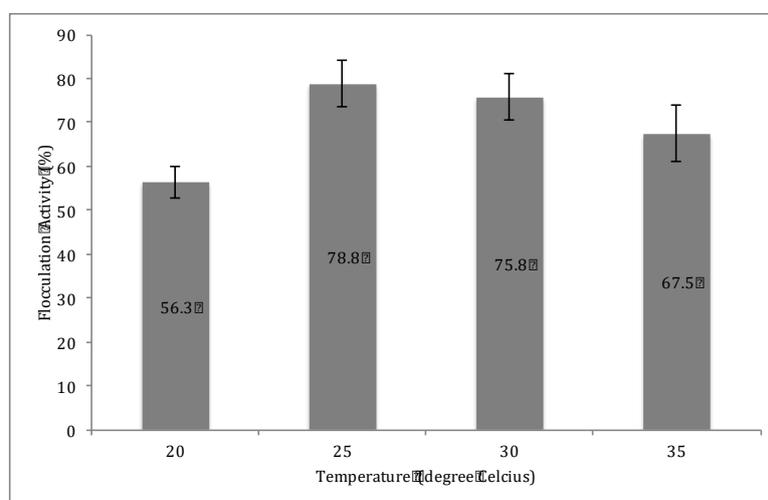


Figure 6. Effect of temperature

Although the results of the previous study (Wan *et al.*, 2013) mentioned that the bioflocculant produced by *Aeromonas* sp. retained its flocculation activity with only 9.2% decrease after a heating process at 100°C for 60 minutes, the utilization of this thermostable bioflocculant on an industrial scale is more expensive than the bioflocculant showing a high flocculation activity at room temperature in tropical regions as seen in the use of *Ankistrodesmus* sp. in this study as bioflocculants.

Roles of pH on the Flocculation Activity

Figure 7 illustrates that bioflocculants are active in the pH range of 7 to 8, indicated by the remarkable flocculation rates of above 75%. Presumably, it is due to the fact that the electrical state of the bioflocculant may vary at different pH conditions, which in turn affects the flocculation efficiency of the bioflocculant.

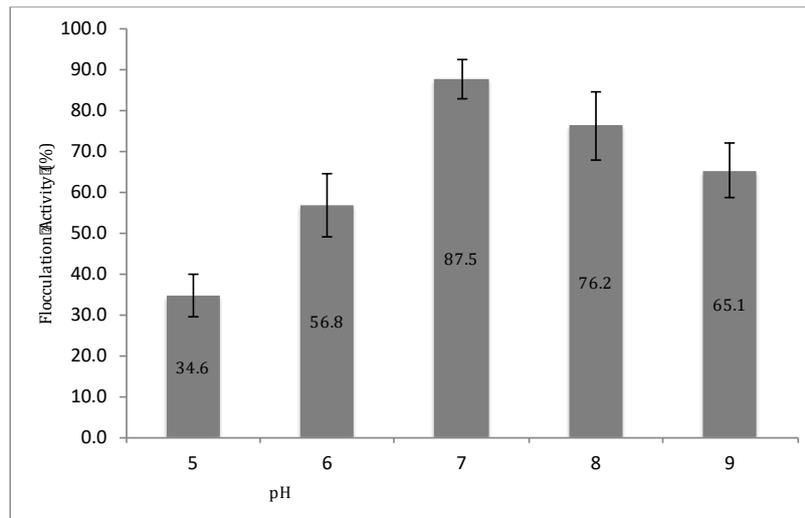


Figure 7. Effect of pH after the addition of bioflocculant

The highest flocculation activity of 95.9% was achieved at pH 8. There was an inconsistency of bioflocculant activity in the acidic environment, indicated by the decreased flocculation activity at pH 5. It is probably due to the fact that the spatial arrangement of surface charge depends not only on pH but also on temperature (Dearfield *et al.*, 1988). The significant rate of flocculation activity in a fairly wide range of pH signifies the applicability of the bioflocculant on an industrial-scale harvesting system, without having to adjust the pH of the culture to economize on production costs.

Conclusion

This study shows that *Ankistrodesmus* sp. is a competitive bioflocculant for harvesting microalgal biomass. The bioflocculant optimally produces polysaccharides when the source of molasses and the mixture of yeast, as well as urea and ammonium sulfate, are used as the carbon and nitrogen sources in the growth medium, respectively. The bioflocculant has a high flocculation efficiency over a wide range of pH (5–9) with a low dose requirement (10% v/v), making it highly competitive to be applied on an industrial scale.

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