Flocculation-filtration method for harvesting *Euglena* sp.

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Abstract. *Euglena* sp. is a type of microalgae that can produce various biomolecules including proteins, vitamins, carbohydrates, and can also be the best source of biomass because it contains lipids, which are especially useful for extracting and converting it into biodiesel. Unfortunately, harvesting *Euglena* sp. biomass is a challenge. Most of the production costs are occur in the harvesting process. The primary concerns revolve around efficiency levels and the operational costs. The methodology used in this study is by combining the flocculation method using *Poly Aluminium Chloride* (PAC) with the filtration method to answer the existing challenges. The purpose of this study is to provide scientific information related to efficient and effective methods for harvesting *Euglena* sp. The parameters measured in this study are Cell Density and dry weight of biomass. This study showed that by using the flocculation method first before the filtration method, through optimal settling time treatment, even by using cheap filtration materials.This study indicated that the flocculation method with optimal settling time treatment as a pretreatment before the filtration method is an efficient and effective method for harvesting Euglena sp.

1 Introduction

Microalgae biotechnology provides a sustainable solution for multiple businesses by utilizing the potential of microalgae to generate bioactive substances that can be used in food, pharmaceuticals, biofuels, and other sectors[1], [2]. These minuscule creatures are abundant in proteins, carotenoids, fatty acids, and other important chemicals that have positive effects on human health [1].Microalgae have the potential to serve as a sustainable and renewable energy source that can fulfil the world energy requirements [3].Microalgae biomass is a significant supply of lipid, which can be utilised as a future biodiesel source [4]. Microalgae demonstrate accelerated growth rates compared to other plants and can flourish without the need for extensive land areas. This leads to the absence of competition between the agricultural industry, residential regions, and livestock for biofuel requirements [5].

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One type of microalgae that is being widely researched is *Euglena* sp. Microalgae, such as *Euglena* sp., show great potential as sources of biomass, bioproducts, and biofuels because of their high carbohydrate content and exceptionally high lipid synthesis, which is approximately ten times more than that of palm oil [6]. *Euglena* sp. can produce commercially valuable bioproducts consisting of proteins including essential amino acids, pro(vitamins), lipids, and β-1,3-glucan paramylon. *Euglena* sp. has the ability to store significant amounts of paramylon β-1,3-glucan polysaccharides. These polysaccharides can reach more than 80% (weight) of dry weight (biomass that has been dried to a constant weight without undergoing oxidation)[7]. In addition, β-1,3-glucan has shown the ability to reduce *cholesterol* levels and exhibits antidiabetic, *antihypoglycemic*, and *hepatoprotective* properties. They have also been used in the treatment of colorectal and gastric malignancies [8].

As the harvesting process consumes a large part of the total cost of microalgae production, it is the most important stage in terms of economic calculation of microalgae biofuel production [9]. The efficiency, time and energy requirements, investment, operational considerations, and chemical expenses are factors that define the advantages and disadvantages of different microalgae harvesting processes [10]. Efficient harvesting microalgae is essential for diverse industrial applications; nevertheless, conventional methods frequently encounter obstacles such as exorbitant expenses and limited effectiveness [11].Three commonly employed methods for extracting biomass from microalgae include flocculation, centrifugation, and filtration. Each of the three options has its own set of benefits and drawbacks [12]. The efficiency and economics of the microalgae harvesting process have improved significantly when flocculation and filtration are combined [13]. Using Polyaluminum Chloride (PAC) of flocculants, the dynamic dewatering process that combines filtration and flocculation has been investigated [14].

This research aims to identify effective and efficient methods for harvesting *Euglena* sp., considering its small cell size, which poses challenges for conventional physical filtration techniques. The feasibility of combining flocculation and filtration will be assessed by selecting efficient flocculants, determining optimal dosages, using cost-effective filtration materials, and optimizing settling times. The goal is to minimize total harvesting costs while achieving optimal biomass yield. The study will evaluate dead-end filtration performance, including cell counts before and after filtration, providing comprehensive insights into the synergistic effects of flocculation and filtration in microalgae harvesting.

2 Material and Method

2.1 Strain and culture condition

Euglena sp. was chosen for this experiment because it has rapid exponential growth, reaching a stationary phase before declining. *Euglena* sp. growth began to stabilise on the 9th day of cultivation [15]. The algae used for this study were obtained from the Biotechnology Laboratory Building A, Faculty of Biology, Universitas Gadjah Mada Indonesia. The algae were first cultivated in 0.5 L flasks using distilled water media with constant stirring and fluorescent lamps with a 24-hour irradiation period and room temperature of 28-35 centigrade, and after 9 days they were then transferred into 40 L containers indoors. The composition of the nutrients used is shown in Table 1.

Table 1. The composition of macronutrients in the cultivation of 1000 mL Euglena sp.

Composition	**Consentrate**
ZA $(NH_4)_2SO_4$	1g/L
KCI	0.02 g/L
TSP	0.99 g/L
$MgSO_4$	0.2 g/L
Vit B1	1 mL/L
Vit B2	1 mL/L
Sodium molybdenate	1 mL/L

2.2 Flocculation of algae

The flocculant used in this study was Polyaluminum Chloride (PAC) due to its wide application in industry and low cost. The efficacy of PAC in promoting algal flocculation during water treatment procedures has been thoroughly investigated. It has been discovered that PAC is useful for filtering microalgae, particularly *Chlorella vulgaris*.PAC coagulation can effectively dehydrate microalgae by reducing pore obstruction and increasing filtration flux [16]. Flocculant stock was prepared in advance with a concentration of 100 ppm from 500 mL of media. The prepared flocculant stock was then added into the algae culture and stirred rapidly for 30 minutes to make the mixture homogeneous. The mixture was then allowed to stand for 2 hours, 4 hours, and 6 hours for flocculation and sedimentation.

2.3 Filtration of algae

The best technique for collecting microalgae is filtration since it produces high-quality biomass with little harm to the cells and doesn't require the use of chemicals. Applying filtration by vacuum, pressure, or gravity allows for flexibility in meeting different harvesting needs. In order to minimize harvesting expenses, filtration technology is appropriate for biofuel applications [17].

Plas chamois and polyester screen mesh with a mesh count of 200 or higher (T200) materials were selected for this research. Studies have shown that plas chamois offer better antifouling, hydrophilicity, and permeability properties, making them suitable for screening microalgae species with different characteristics. Overall, PVA sponge materials for plas chamois show great potential for efficient and sustainable algae screening in various environments and industries[18]. Meanwhile much research has been conducted on polyester screen mesh for filtration uses, such as growth suppression and algae removal. Research on microalgae harvesting techniques shows that polyester screen mesh is a viable option for filter algae harvesting. Studies have shown that various fabric filters, such as those made of polyester, can effectively collect microalgal biomass using physical filtration techniques [19].

2.4 Data collection

In this study, The cell density of microalgae Euglena sp. Is calculated. It was carried out through observation of the number of cells using a Yazumi Model XSZ-107BN microscope and a Neubauer Improved Haemocytometer before and after harvest. Observation was carried out by taking a culture sample of 2 ml using a syringe, then adding 0.3 ml of alcohol liquid to be dripped into the Haemocytometer which had been placed under the objective lens of the microscope with 10x magnification. The calculation of the number of microalgae cells

was carried out by calculating the number of cells in a large box with a width of 1 mm, an area of observation area of 1 mm2, and a volume of 0.1 mm3 (1x10-4 mL) [20].

The biomass obtained is harvested through several stages, initially the *Euglena* sp. as much as 500 ml were given PAC coagulant and stirred using an aerator for 30 minutes, then let it sit for 2 hours, 4 hours, 6 hours. Furthermore, the sample was filtered using 2 different materials to determine the effectiveness of each. Then the biomass is dried using an oven for 30 minutes at a temperature of 60°C so that the dry weight of *Euglena* sp. biomass is obtained and weighed.

3 Result and Discussion

3.1 Effects of Settling Time and Filtration Material on Cell Density

Figure 1 shows the effect of different settling times and filtration materials used on cell density before and after the harvesting process of *Euglena* sp. which is cultivated in aquadest water with the addition of nutrients. It was found that there was no significant difference in the type of material used in this study, namely Plas Chamois and Screen Mash T200, both materials could only filter *Euglena* sp. after the previous flocculation process. In the absence of a flocculation method first, the two materials cannot filter *Euglena* at all.

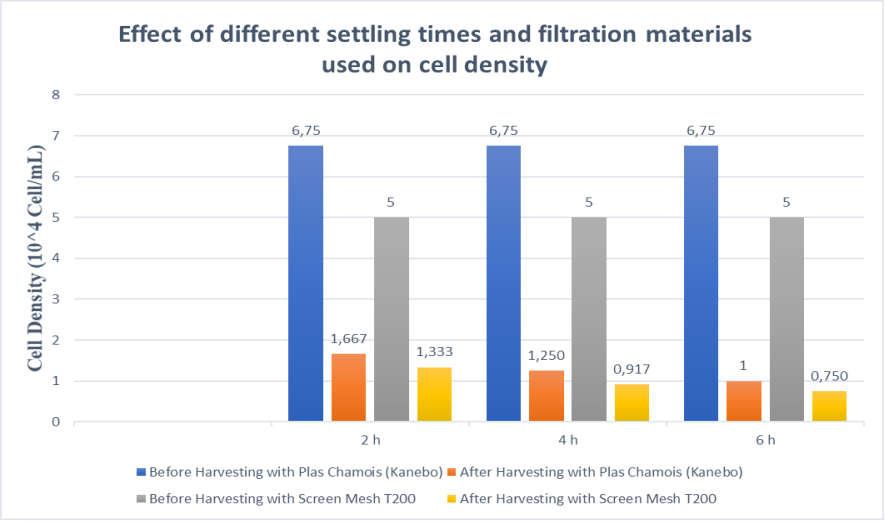

Fig. 1. Effect of different settling times and filtration materials used on cell density.

Figure 1 illustrates the difference in the number of Euglena sp. cells before and after harvesting in the 100 ppm coagulant dose treatment with plas chamois filter material and screen mesh T200 . In the harvesting group using plas chamois material, the bar chart in blue color illustrates the number of Euglena sp. cells before harvesting which is 6.75 cells/mL. The number of cells after harvesting can be seen in the orange bar chart which shows that the number of cells decreased to about 1,667 cells/mL after two hours, 1,250 cells/mL after four hours, and 1 cell/mL after six hours. Whereas in the harvesting batch using the screen mesh T200, the number of cells before harvesting (gray bar chart) was 5 cells/mL and after harvesting (yellow bar chart) decreased over time, where after 2 hours, 4 hours, and 6 hours of precipitation, the remaining number of cells was 1.33; 0.917; and 0.75 cells/mL, respectively.

Meanwhile, the length of settling time significantly affects the efficiency of harvesting results. Cell density was significantly reduced after harvesting with a settling time of 6 hours with the same concentration of coagulant, by 100 ppm for every 500 mL of sample. The formation of flocs and turbidity of the treated can be greatly affected by the duration of the waiting time and the intensity of flocculation. Nonetheless, the intensity and scheme of flocculation may not be important for overall effectiveness [21]. And that is why the harvesting efficiency seen from the cell density increases with the flocculation waiting time.

Figure 2 shows the efficiency before and after harvesting in the form of the measured percentage of cell density. It was found that the cell density with the longest time was able to get the highest level of efficiency, from the figure the efficiency of the 6-hour waiting time can reach the range of 85%, while the efficiency of harvesting with a waiting time of 4 hours is 81%-82%, and with a settling time of 2 hours is only 73%-75%. The application of cationic coagulants, such as Poly Aluminum Chloride (PAC), helps to balance the negative charge present in the culture medium, thus facilitating effective floc formation [22]. Higher doses will result in greater cation release, which is required to offset the negative charge in the culture medium. settling period has a major impact on microalgae harvesting effectiveness of up to 85% [23].

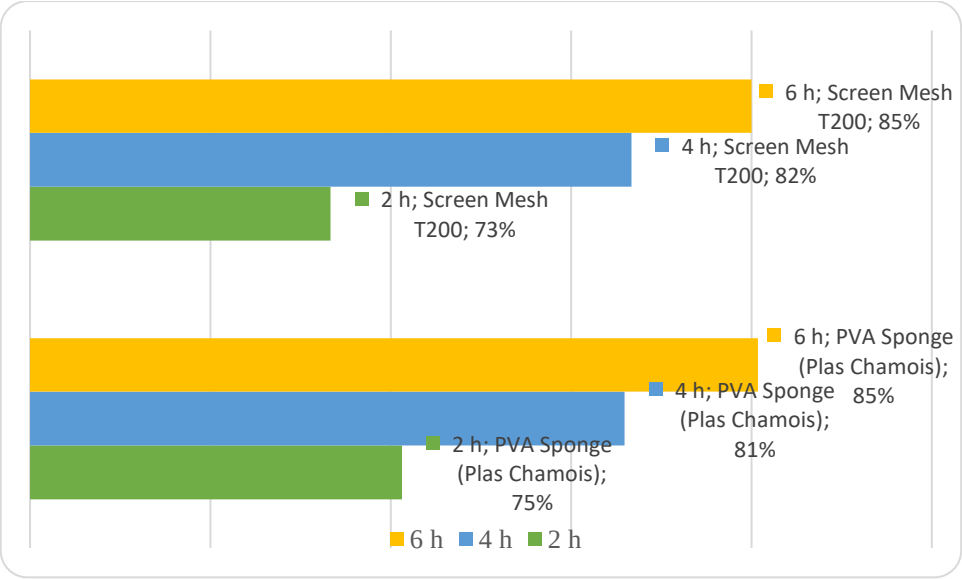

Fig. 2. Efficiency of cell density before and after harvesting.

3.2 Effects of Settling Time and Filtration Material on Dry Biomass Yield

The effect of sedimentation time is still the most influential variable in this study when viewed from the dry weight of the biomass (g/L). Figure 3 presents data on the dry weight of Euglena sp. biomass in grams and its harvesting effectiveness of each harvesting batch under various treatments. The two main variables analyzed were the coagulant dose of 100 ppm and the settling time (2 hours, 4 hours, and 6 hours).

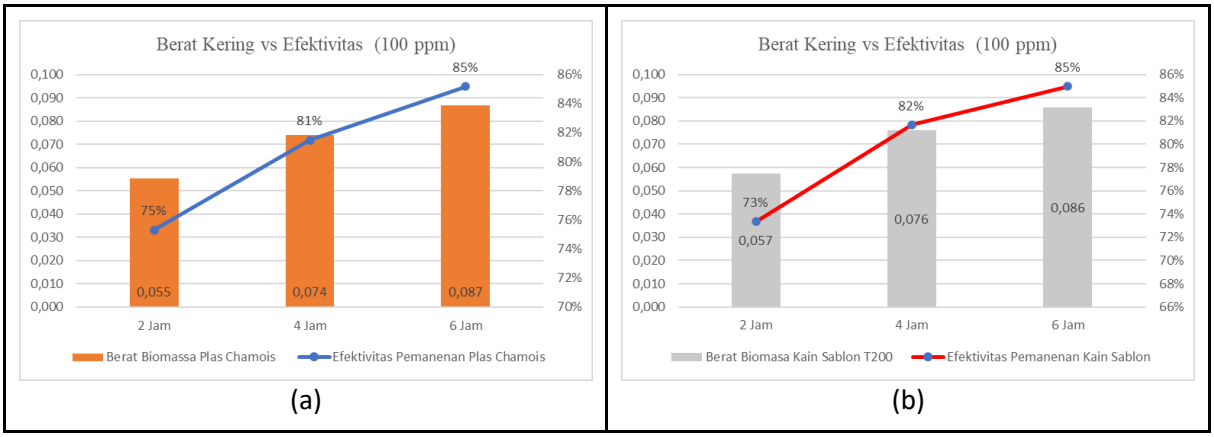

Fig. 3. Biomass dry weight and harvesting effectiveness of various treatments. (a) 100 ppm coagulant dosage with a chamois cloth filter, (b) 100 ppm coagulant dosage with a mesh screen.

The dry weight of biomass produced varied according to the length of deposition time. At 2 hours of settling, the dry weight of the biomass reached 0.055 grams using pas chamios and 0.057 grams using mesh screen T200. The harvesting effectiveness value in these two treatments was 75% in the plas chamois batch, and 73% of the T200 screen printing cloth batch. After 4 hours of settling, the biomass dry weight increased to 0.074 grams for the plas chamois and 0.076 grams for the mesh screen T200, with the harvesting effectiveness values being 81% and 82% for each batch. At 6 hours of deposition, the biomass dry weight reached 0.087 grams of 85% effectiveness value using plas chamios and 0.086 grams using mesh screen T200 with 85% harvesting effectiveness value as well.

The fact that the filtration materials used in this study have equivalent performance, the difference is not so obvious for the type of filtration material used. Both can be used for harvesting with flocculation done first, this is obtained because applying flocculation increases efficiency by forming larger clumps of algae, which reduces the need for smaller filtration pore sizes. As a result, the use of filter materials with large enough pores is able to filter them [24].

4 Conclusion

The conclusion of the study showed that the highest effectiveness was under the condition of 6 hours of sedimentation time with the use of plas chamois filtration material. The treatment of the type of filtration material used, both plas chamois (chamois) and screen mesh T200, did not have a significant effect on the harvest of Euglena sp. microalgae because both have a large amount of both capable of being used to harvest microalgae with this method, but did not experience significant differences. Only in some variations the screen mesh T200 material was able to outperform the chamois plas material. But the selection of this material can be a viable alternative for harvesting *Euglena* sp. massively because the price of the material is much cheaper than membrane materials that are often used directly for filtration.

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