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Marine microalgae *Nannochloropsis oculata* biomass harvesting using ultrafiltration in cross-flow mode

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Abstract. Microalgae is a potential bioenergy source. It can grow rapidly, even it could be harvested within 7 days. Harvesting is an important part of microalgae cultivation due to the method used. It should be undamaging toward essential content of microalgae and should produce high yields of biomass. In this study, the harvesting of *Nannochloropsis oculata* was carried out using capillary ultrafiltration in cross flow mode. This study aims to test ultrafiltration membrane performance in *Nannochloropsis oculata* harvesting accompanied by *Backwash* and *Non-Backwash* modes and to analyse its total lipid content. The harvest was done under 1; 1.5; and 2 bar of trans membrane pressure. Some observed parameters were permeate flux, cell density, biomass recovery, microalgae's dry weight, yield, and total lipid content. The application of high pressure and backwashed treatment have boosted slurry production time which lead to microalgae's biomass abundance. The result showed that the best treatment of *Nannochloropsis oculata* harvesting using capillary ultrafiltration membrane in cross flow mode is under 2 bar of pressure with backwashed treatment. This is the fastest condition to produce slurry within 1800 s with the highest recovery percentage 79.50%, 16.05×10^6 cell/ml of post-treatment cell density, 6.8 grams of biomass' dry weight, 22.66 % of yield, and 2.52 % of total lipid content.

1. Introduction

Microalgae has been considered as one of biodiesel production raw material due to its high content of lipid and rapid growth compared to other biodiesel raw material crops [1]. Microalgae has to undergo additional treatment prior to biomass acquirement; harvesting. It is a separation process between microalgae and its medium through liquid-solid separation.



Harvesting is an essential process in microalgae cultivation due to the method used. It should not damage essential content of microalgae and should produce high yields of biomass. It is difficult to choose suitable separation method considering microalgae's tiny size of 3 to 30 μm . Membrane technology is promising methods for separation of microsize particles in aqueous solution [2], including separation of microalgae biomass from its liquid growth medium [3].

Despite its potential, membrane technology is suffered by fouling [4,5]. Although chemical approach such as modification of surface properties promotes low fouling [6], yet the physical cleaning is still effective [7]. In this work therefore, a utilization of ultrafiltration (UF) membrane accompanied by backwash treatment as physical cleaning method have been conducted in marine microalgae harvesting process. The use of UF membrane is hoped that it could produce high lipid content within the acquired dry biomass without damaging essential substances of microalgae.

2. Materials and Method

2.1. Materials

Marine microalgae species *Nannochloropsis oculata* obtained from Centre for Development of Brackish Water Aquaculture at Situbondo, cultivated in sea water sequentially in glass carboy of 3-4 L, jars of 10 L and fiber container of 500 L. Prior to microalgae cultivation, the growth medium sterilized to prevent any contaminants. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) was used for deactivation of microalgae solution after sterilization. Afterwards, the water was enriched to provide the culture with nutrients. In this step, aeration applied and pro analyse-grade diatomic fertilizer and Technical Growth (TG), were added. Furthermore, mass culture was conducted in an open pond.

Polysulfone (PS) UF membrane with pore size of 0.01 μm was used for microalgae harvesting, driven by water pump Shimizu PN-125 BIT to pressurize the microalgae solution prior to the membrane modules. The membranes were used for another experiment run after splashed with 0.1% of Sodium hydroxide (NaOH). For calculating cell density, a centrifuge Nesco 80-2 (8 hole), microscope and haemocytometer were used.

2.2. Method

2.2.1. Observed factors

There were two observed factors in filtration process: operation mode and operation pressure. Operation mode was divided by two treatments: non-backwash and backwash treatment. And three variations were used in pressure: 1 bar, 1.5 bar, and 2 bar. Backwash process was done for 10 seconds in every 10 minutes interval at 2 bar pressure.

2.2.2. Microalgae harvesting.

Readily harvested *Nannochloropsis oculata* was distinguished by rather deep brown appearance, 1-2 million cell/ml of cell density reached, and was cultivated for 5-6 days. *Nannochloropsis oculata* was harvested while in stationary phase due to higher lipid total content compared to exponential phase. In the beginning of 9 days of *Nannochloropsis oculata* feed fetching in the mass culture pool, it was known that the average cell density was $\pm 3.38 \times 10^6$ cell/ml.

Thirty liters of 5-6 days-growth microalgae were harvested on each treatment variation. UF membrane used had inside-out system of hollow fiber module. A cross flow mode was used instead of dead end mode due to low fouling potential. Liquid feed was pushed entering the membrane channel by using a gear pump, while the trans membrane pressure set at 1, 1.5 or 2 bar. Permeate would pass through membrane while concentrate would be pushed back to reservoir by feed flow. Filtration process produced microalgae biomass concentrate slurry and sea water permeate which then were collected using measuring cup. The volume were measured in every minute.

2.2.3. Membrane washing

UF membrane washing was done in every end of harvesting process using \pm 20 liters of 0.1% NaOH which was continually circulated every 10 minutes at 2 bar. It then rinsed using distilled water for 10 minutes.

2.2.4. Membrane performance testing

Membrane performance was tested using flux by measuring permeate volume every minute per unit area of membrane.

2.3. Data Analysis

Data analysis was conducted by measuring cell density, biomass recovery percentage, dry weight, yield, and fatty acid content.

3. Results and Discussion

3.1. Water flux test

One micron-filtered tap water was used in this test, to check the performance of newly used PS membranes. The test was carried out every minute for 30 minutes with three pressure variations: 1 bar, 1.5 bar, and 2 bar. The water flux test result showed a stable flux flow which meant there was no fouling found. At 1 bar, 1.5 bar, and 2 bar of pressure, the flux value were stable on 0.018, 0.030, and 0.037 L/m²s.

3.2. Backwash effects

Flux value test was carried out in order to determine the best treatment to generate cross flow permeate flux on UF membrane. *Nannochloropsis oculata* backwash harvesting was done first followed by non-backwash one. Backwash was done to reduce biomass fouling effect which might decrease cross flow permeate flux. Besides, flux value showed membrane capability to flow permeate through UF membrane in certain time unit. The tight membrane pores were supposed to optimally hold microalgae biomass. Backwash efficiency in minimizing flux decline caused by fouling were affected by several factors such as, pressure, interval, and backwash duration.

Different operation pressure used in microalgae harvesting has caused filtration time difference, 2,880 seconds, 2,040 seconds, and 1,800 seconds at 1 bar, 1.5 bar, and 2 bar respectively. Given that condition, backwashing was done differently for each pressure, five times of backwashing at 1 bar and three times for 1.5 and 2 bar.

The result showed that the fastest and most stable process was occurred at 2 bar of pressure. Meanwhile, at 1 bar, the slowest one has occurred, additionally, at the end of process the flux was sharply down. Overall, fluctuated fluxes were always found during all three treatments. Nevertheless, at 2 bar, the process occurred faster and more efficient. The flux value at 2 bar also indicated that the best filtration process has occurred among three variations. Backwashing effect can be seen on Figure 1.

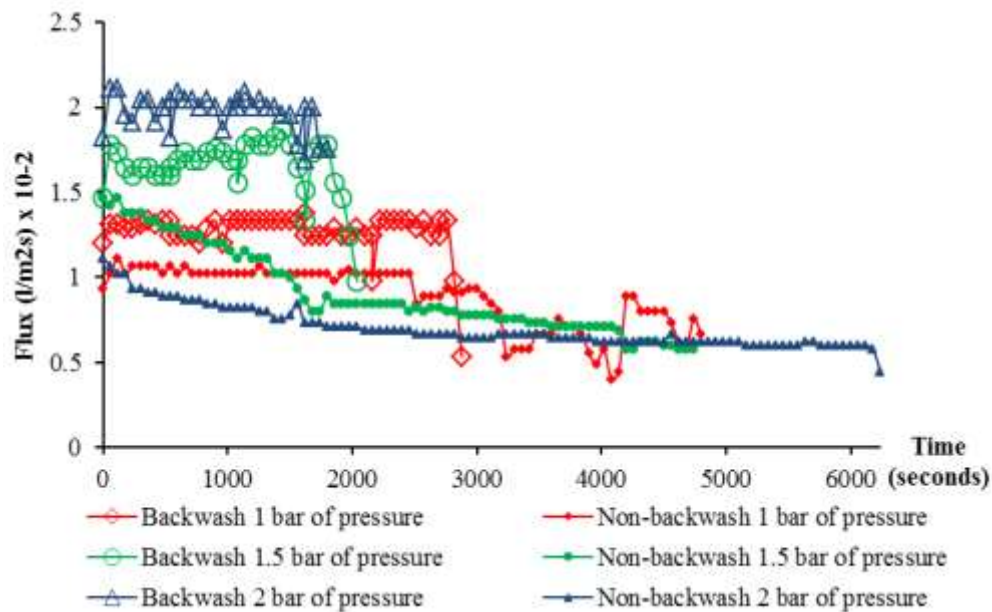


Figure 1. Effect of backwashing toward flux behaviour

It was known that backwashed process at 2 bar of pressure considered to be the best treatment, followed by 1.5 bar and 1 bar respectively using the same method. Besides offered faster process, backwashed operation also showed steady flux compared to non-backwashed one which showed declined trend caused by accumulated fouling inside the membrane. As for non-backwashed harvesting, the highest flux value was found at 1.5 bar of pressure. Unfortunately, it could not keep the stability. Nevertheless, 1.5 bar of pressure was proved to be the fastest harvesting pressure among others.

Short backwash interval has caused higher flux and flow in every filtration cycle. This indicated that formed fouling was better treated frequently by backwash cycle [8]. Ninety percent of permeate flux could be kept by backwashing for 10 seconds at 1 bar of pressure and 20 minutes interval. The result was still lower than was by 10 minutes backwashing, which was 95%.

However, Bhawe et al. [9] shows that harvesting with two-step membrane filtration of *Nannochloropsis sp.* 2 g/L-150 g/L in batch system with cross flow using microfiltration have constant TMP at 2.3-25.6 Psi at 2.5 hour/cycle with flux 35-684 L/m²h. Zhang et al. [10] using membrane ultrafiltration in batch system with cross flow mode harvest *Scenedesmus quadricauda* with constant TMP at 34.5 kPa at 30 min/cycle with flux 45 L/m²h.

3.3. Cell density during backwash and non-backwash modes

In backwashed treatment (Figure 2), cell density measurement was done once at the start of feeding and five times at post-backwash. On the 1 bar pressure harvesting, the initial cell density was 3.31×10^6 cell/ml and 7.37×10^6 cell/ml; 8.46×10^6 cell/ml; 8.99×10^6 cell/ml; 10.7×10^6 cell/ml; and 14.31×10^6 cell/ml at the post-backwash. Backwashing was occurred five times at the 1 bar pressure due to the longer harvesting time. At 1.5 bar, backwashing was done three times and resulted: 8.22×10^6 cell/ml; 11.59×10^6 cell/ml; and 14.93×10^6 cell/ml accompanied by initial measurement which was 3.56×10^6 cell/ml. While at 2 bar, backwashing was also done three times and resulted: 11.25×10^6 cell/ml; 13.52×10^6 cell/ml; 16.05×10^6 cell/ml, followed by the initial cell density: 3.29×10^6 cell/ml.

Harvesting process at 1.5 bar and 2 bar was completed faster compared to 1 bar process so that there were only three backwashing. At the 2 bar process, cells would become dense faster than those at 1 and 1.5 bar. At the first backwashing, the cell density was 11.25×10^6 cell/ml and sharply increased at the last which reached 16.05×10^6 cell/ml. Cell density at the 1.5 bar process was also relatively

high, the first one was 8.22×10^6 cell/ml and the last was 14.93×10^6 cell/ml. The correlation among flux, backwashing, and cell density at the single pressure can be observed in figure 2.

In non-backwashed treatment cell density measurement was done before and after harvesting. Initial cell density at 1; 1.5; and 2 bar were 2.74×10^6 cell/ml; 2.72×10^6 cell/ml; 2.41×10^6 cell/ml respectively and the post-harvest one were 7.924×10^6 cell/ml; 8.521×10^6 cell/ml; 9.43×10^6 cell/ml also respectively.

Non-backwashed process cell density was concluded to be relatively low. This was caused by the microalgae had started to enter their death phase considering the fact that the harvesting process took a lot of time. Backwashed harvesting was done before the non-backwashed one. High temperature on the tank 35°C caused by improper water circulation, killed the microalgae faster.

Although the cell density at 2 bar treatment was higher, it was feared that microalgae cell would be broken in enormous number due to the high pressure. One of the aim of this research is to achieve maximum yield of total lipid extracted from dried microalgae. In this study, the number of broken cell analysis was not carried out. The amount of broken cells were rising as the increasing TMP. Therefore, maximum TMP 1.5 bar will be used in future study. High temperature would inhibit microalgae's growth and eventually leads to their death.

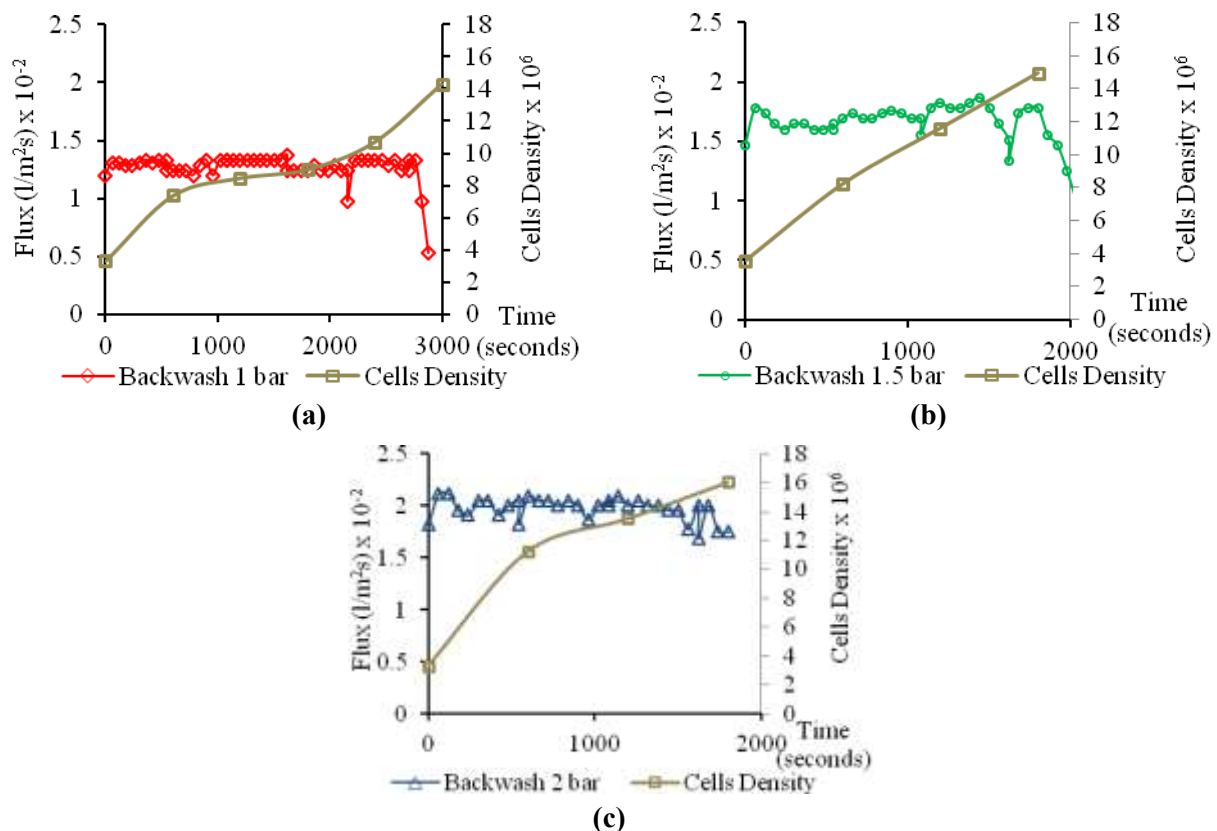


Figure 2. Flux, backwashing, and cell density at different TMP: (a) 1 bar; (b) 1.5 bar; (c) 2 bar.

3.4. Biomass recovery, dry weight and harvesting yield

Biomass recovery percentage describes the amount of biomass which has been successfully acquired at the end of harvesting process. At 1; 1.5; and 2 bar backwashed treatment, 76.18%; 76.15%; and 79.5% of pre-harvest product has been successfully retrieved, respectively. Highest recovery percentage at 2 bar process resembles the high cell density which was 16.05×10^6 cell/ml. On the other hand, non-backwashed treatment generated lower recovery percentage compared to backwashed one. In consecutive order, the recovery percentage at 1; 1.5; and 2 bar non-backwashed treatment were

65.42 %; 68.16%; and 74.44%. The recovery percentage difference between backwashed and non-backwashed process is proportional to cell density (slurry). However, Zhang et al. [10] in the previous study shows results that harvesting can be achieved up to 98% recovery of *Scenedesmus quadricauda* with backwash and membrane cleaning using 2% NaOH, 0.5% citric acid and 200 mg/L NaOCl, for 1 h at 20 °C [10].

Dry weight described as the final steady biomass weight after being put in the 80° C oven for 24 hours. At 1; 1.5; and 2 bar backwashed treatment, the dry weight were: 5.78 gram; 6.33 gram; and 6.8 gram respectively. Highest dry weight at 2 bar process resembles the recovery percentage which was 79.50 %. Biomass' dry weight at backwashed process was affected by the pressure that was used which might affect slurry's weight at the end of harvesting.

Non-backwashed treatment resulted lower dry weight compared to backwashed one. In consecutive order, the recovery percentage at 1; 1.5; and 2 bar non-backwashed treatment was 3.83 gram; 4.15 gram; and 4.79 gram. This relative low dry weight might have been caused by the microalgae had started to enter their death phase so that there was not much slurry to be produced which might affect the recovery percentage that was going to be used in dry weight calculation later on.

Harvest yield defines the amount of acquired dry weight each liter of harvested microalgae. Backwashed treatment yield was higher than non-backwashed one, they were: 19.26% at 1 bar pressure; 21.1 % at 1.5 bar; and 22.66% at 2 bar. Yield value at backwashed process was affected by the pressure that was used which might affect slurry's weight and might affect its dry weight as well. As for non-backwashed treatment, in consecutive order, the yield value at 1; 1.5; and 2 bar non-backwashed treatment 12.76 %; 13.83 %; 15.96 %. The lower yield might have been caused by the microalgae had started to enter their death phase so that there was not much slurry to be produced.

3.5. Lipid content

Total lipid content resembles the amount of fatty acid content in the microalgae as biofuel's raw material. The sampling for this test was done at the end of harvesting process by extracting 2 grams of *Nannochloropsis oculata* biomass using soxhlet.

Extracting result showed that total lipid content was 2.52%. This percentage is quite low since according to previous study that was done by another researcher that *Nannochloropsis sp* is one species of microalgae which has considerable high total lipid content ranging from 37% to 60% of its dry weight, higher than other microbial strain [11]. This phenomenon might have been caused by lipid content dependence on harvesting time and extraction method. Besides, 2 bar of pressure seemed too high for microalgae so that they would be collapse and leak their cytoplasm which contains the fatty acid itself. Therefore, there was only little amount of available lipid to be extracted.

4. Conclusion

Nannochloropsis oculata harvesting using UF membrane in cross flow mode shows that backwashed treatment under 2 bar of pressure would do the fastest harvesting process which is 1,800 seconds. Backwashing was done three times for 10 seconds every 10 minutes. At this particular condition, 79.50% of recovery percentage, 6.8 grams of biomass' dry weight, and 22.66 % of yield were successfully acquired. But there was only 2.52 % of total lipid content which is relatively low.

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