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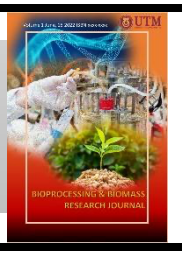

Research Article

Application of Membrane Filtration for Microalgae Harvesting and Protein Separation

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ABSTRACT

The seek for sustainable protein sources has led to the exploration of microalgae as an alternative. Membrane filtration, known for its environmental friendliness, holds promise for purifying protein from microalgae. This research focuses on the protein purification from *Chlorella vulgaris* microalgae using polyethersulfone (PES) membrane. This research aims to investigate the effect of membrane composition for enhanced microalgae harvesting and protein purification, as well as evaluating the effects of membrane pore sizes and porosity on the performance. Three membrane compositions were evaluated, which are 18% PES, 15% PES, and 12% PES. The membranes were tested for efficiency in microalgae harvesting and protein filtration through dead-end filtration. SEM analysis, contact angle analysis, and theoretical calculations were used to assess membrane characteristics. In terms of algae harvesting, both 18% PES and 15% PES were better than 12% PES in terms of retention of algae. Lowest protein rejection or high protein recovery in the permeate was achieved using 18% PES while 12% PES gave the highest rejection or low protein recovery. Our result can provide valuable guidance for optimizing PES membrane compositions to enhance microalgae-based processes.

INTRODUCTION

In recent years, the interest in microalgae proteins has surged due to their versatility and nutritional richness. Microalgae, such as *Chlorella vulgaris*, are known for their high protein content, which can meet the nutritional needs of both humans and animals. Additionally, microalgae proteins offer various health benefits, including immune support and disease prevention, owing to their abundance in vitamins, minerals, and antioxidants (Andreeva et al., 2021). Moreover, the sustainable cultivation of microalgae, which thrives in diverse conditions including wastewater treatment plants, presents an environmentally friendly protein source that reduces pressure on land and freshwater resources (Dolganyuk, V. et al., 2020).

Chlorella vulgaris stands out among microalgae species due to its robustness and adaptability, making it an ideal candidate for protein extraction (Coronado-Reyes et al., 2020). Its high productivity and simplicity in cultivation make it a preferred option for biomass production and protein extraction. Research efforts have focused on refining extraction techniques and optimizing growth conditions to increase protein yield and biomass concentration, enhancing its feasibility as a protein source. Innovative extraction methods, such as ultrasound-assisted extraction and membrane filtration, show promise in improving protein recovery and synthesis efficiency (Han et al., 2017).

While conventional protein purification methods like spray drying, freeze drying, and phase separation have their limitations, membrane filtration offers a promising alternative. Membrane filtration separates proteins based on their molecular weight and size, ensuring precise isolation and purification (Corrêa et al., 2020). It is a simple, scalable, and eco-friendly method that requires less energy and produces minimal chemical waste. This research aims to identify the suitable composition of polyethersulfone (PES)

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membrane for both harvesting of microalgae and purification of proteins, as well as assessing the impact of pore sizes and porosity on membrane performance (Castro-Muñoz & García-Depraect, 2021; Xia et al., 2021).

MATERIALS AND METHOD

Materials and chemicals

Chlorella vulgaris was obtained from Bioprocess Engineering laboratory at the Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia. The strains were maintained on Bold Basal Medium agar plates cultivated at the temperature of 30±2°C and light illumination at 40 µmol/s/m^2. The chemicals used for the membrane fabrication were N-methyl pyrrolidone (NMP) as solvent and polyether sulfone (PES) in pellets, as well as acetone for dope solution cleaning. Then, to detect protein presence in sample, Bradford reagent was mixed with the centrifuged sample. Lastly, the microscopic observation by compound microscope used safranin o as staining purposes.

Cultivation of *Chlorella vulgaris*

Chlorella vulgaris cultivation began with modified Bold's Basal Medium (BBM) consists of 2.5×10^{-1} g $NaNO_3$; 7.5×10^{-2} g $MgSO_4{\cdot}7H_2O$; 2.5×10^{-2} g NaCl; 7.5×10^{-2} g K_2HPO_4; 1.75×10^{-1} g KH_2PO_4; 2.5×10^{-2} g $CaCl_2$; 8.82×10^{-3} g $ZnSO_4{\cdot}7H2O$; 1.44×10^{-3} g $MnCl_2{\cdot}4H_2O$; 2.04×10^{-3} g $Na_2MoO_4{\cdot}2H_2O$; 1.57×10^{-3}g $CuSO_4{\cdot}5H_2O$; 4.9×10^{-4} g $Co(NO_3)_2{\cdot}6H_2O$; 1.14×10^{-2} g H_3BO_3; 5×10^{-2} g Na_2-EDTA; 3.1×10^{-2} g KOH; 4.98×10^{-3} g $FeSO_4{\cdot}7H_2O$; and 1×10^{-6} mL concentrated H_2SO_4. The composition for 1 L of BBM agar (1.6 %) consists of the mentioned components added with 16 g agar powder. Cultivation occurred indoors in batch mode under controlled conditions: 30 °C temperature, pH 7, aeration with plain air, and 120 µmol/s/m^2 cool white light illumination at 24:0 (h:h) light:dark photoperiod. Inoculum at concentration of 3×10^6 units per millilitre size, was inoculated at 4% in volume into five units of 5-L cultivation glass bottles. Optimum harvesting was determined by nitrate nutrient depletion in the cultivation medium, monitored daily with a UV-Vis spectrophotometer at 220 nm. At the end of cultivation, approximately 35 g of wet biomass harvested (Hotos et al., 2020).

Polyethersulfone (PES) membrane fabrication

The process began by preparing a 30 g membrane dope solution by dissolving 5.4 g of PES pellets in 24.6 g of N-methyl pyrrolidone (NMP) solvent. Three membrane compositions were created: 18% PES + 82% NMP, 15% PES + 85% NMP, and 12% PES + 88% NMP. After heating the solvent at 40 °C for 15 min, PES pellets were gradually added and stirred at 60 °C for 24 h until fully dissolved. The solution was then degassed for approximately 3 h to remove air bubbles. This procedure was repeated for membranes with 15% and 12% PES compositions. Membranes of uniform thickness (200 µm) were cast using a casting machine with the speed set to 2, ensuring horizontal spreading of the solution. After solidifying in distilled water, the PES membranes were stored in containers filled with distilled water, at room temperature to maintain their structure.

Characterization of Polyethersulfone (PES) membrane

The PES membrane characterization involved SEM and contact angle analysis at the AMTEC laboratory, Universiti Teknologi Malaysia. SEM analysis assessed structural differences between membranes, examining cross-sectional and top surface views at magnifications of 1.0k, 3.0k, and 8.0k. Thickness measurements and average pore size were estimated for membranes with different compositions. Contact angle analysis was conducted to determine differences in contact angles between the experiment's PES membrane (PES 18%) and a commercial PES membrane with molecular weight cut-off of 30 kDa.

Distilled water flux

The distilled water rejection test was performed on all membranes (PES 18%, PES 15%, and PES 12%) using dead-end filtration. A constant pressure of 5 bar was applied, and the feed water volume was set to 20 mL. The time taken for the permeate to reach volumes between 2 to 10 mL in 1 mL increments was recorded after releasing pressure into the filtration system. Water flux (J_w) was calculated by dividing the volume of permeate (V) by the membrane contact surface area (A) and time (t) using (**Eq. 1**):

$$J_w = \frac{V}{A\Delta t} \qquad \textbf{(Eq. 1)}$$

Standard curve preparation

The microalgae culture was diluted to six different concentrations, yielding 2 mL samples each, to create a standard curve. These concentrations were precisely set at ratios of 1:3, 1:6, 1:9, 1:12, 1:15, and 1:18. The absorbance values of these samples were then measured at a wavelength of 550 nm using a UV-vis spectrophotometer. Subsequently, each sample was dried in a 60°C oven for three days to remove all moisture content. The weight of the dried algae was measured, and a standard curve was constructed by plotting the dry weight of the algae (g/L) against their corresponding absorbance values at 550 nm.

Harvesting microalgae by membrane

The microalgae harvesting process utilized a dead-end filtration system with a consistent pressure of 5 bar during each membrane test. Initially, 20 mL of microalgae culture was introduced into the filter system as feed, and the time taken for the permeate to reach volumes between 2 to 10 mL in 1 mL increments was recorded upon pressure release. The harvest flux was determined using (**Eq. 1**). Subsequently, samples of feed, permeate, and retentate were collected and diluted with distilled water at a 1:9 ratio. The absorbance of the diluted samples was measured using a UV-vis spectrophotometer with a wavelength of 550 nm. Membrane rejection (%) was calculated using (**Eq. 2**).

$$R(\%) = \left(1 - \frac{C_p}{C_f}\right) \times 100 \qquad \textbf{(Eq. 2)}$$

Where C_p is the permeate concentration and C_f is the feed concentration. To ensure accuracy, this process was repeated twice, and the standard deviation error was calculated using (**Eq. 3**) where x is the data set values, µ is mean of the data set, σ is standard deviation, N is the number of values in data set, and n is the number of repeated experiments that was being conducted.

$$\mathrm{SD} = \sqrt{\frac{\sum |x-\mu|^2}{N}} \quad SE = \frac{\sigma}{\sqrt{n}} \qquad \textbf{(Eq. 3)}$$

Disruption of microalgae cells by ultrasonication

The microalgae cells were disrupted using an ultrasonic cell disruptor. Initially, 200 mL of microalgae culture was prepared in a 500 mL beaker, which was then submerged in a basin of ice to prevent temperature rise during sonication. The beaker was positioned in the disruptor with the tip placed at the centre without touching the bottom to ensure effective sonication. The disruptor was set to 30 °C for 10 min, with a pulse of 10 seconds on and 10 seconds off, and an amplitude of 20 kHz. After sonication, the sample was allowed to cool for approximately 30 min. Observation of microalgae cells before and after disruption was carried out using a compound microscope at magnifications of 40x and 100x to analyse the structure and constituents of the cells released after ultrasonication.

Protein purification by membrane filtration

The purification of microalgae protein involved utilizing a dead-end filtration system with a constant pressure of 5 bar across all membrane tests. Initially, 20 mL of sonicated microalgae culture was poured into the system as feed and the time taken for the permeate to reach volumes between 2 to 10 mL in 1 mL increments was recorded upon pressure release. Protein flux was then calculated using (**Eq. 1**). Subsequently, 2mL samples of feed, permeate, and retentate were collected and centrifuged for 6 min at 10krpm. After centrifugation, 0.05 mL of supernatant from each sample was mixed with 1.5 mL of Bradford reagent, and the absorbance values were measured using a UV-vis spectrophotometer at 550 nm, which were then compared to a standard curve to determine protein concentration. This process was repeated twice for accuracy, and standard deviation was calculated using (**Eq. 3**). Protein presence in the samples was observed using a compound microscope at 40x and 100x magnifications, facilitated by staining with safranin O to enhance visibility.

RESULTS AND DISCUSSION

In this section, the results of this research were presented in three main sections which are the characterization of PES membranes, evaluation on the efficiency of PES membrane in microalgae harvesting, and determination of most effective PES membrane for purification of microalgae's protein by membrane filtration.

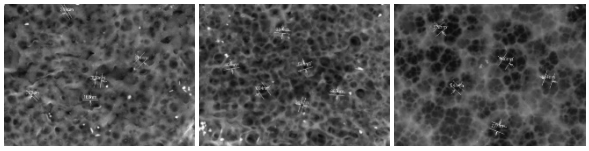
Polyether sulfone (PES) membrane characterization

Scanning Electron Microscopy (SEM) analysis

SEM analysis depicted structures of the PES membrane, shown in **Figure 1** and **Figure 2**. The pores of the PES 18% membrane appeared smallest and exhibited a more uniform distribution compared to the other membranes. Specifically, the PES 18% membrane displayed the smallest average pore size at 407.2 nm, while the PES 12% membrane showed the largest average pore size at 742 nm, with the PES 15% membrane falling in between at 487.33 nm.

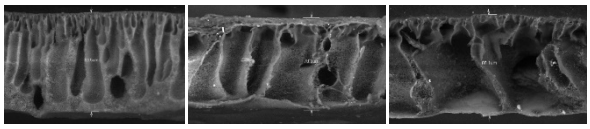
The composition of polymers significantly impacted the membrane structure, with higher polymer content resulting in smaller and more uniformly distributed pores due to increased entanglement and packing of polymer chains (Jiang et al., 2018). Moreover, a higher polymer content limited polymer chain mobility, reducing irregularities in the membrane structure, and contributing to a denser and more uniform membrane.

SEM analysis also revealed slight differences in membrane thickness compared to the constant 200 µm thickness set during casting. This variance can be attributed to factors such as compression, solvent evaporation, polymer chain alignment, and pressure application during casting, collectively resulting in a thinner membrane with a more compact and dense structure than initially designated.



(a) (b) (c)

Figure 1 SEM images that show the top surface view of membrane in 8.0k magnification for (a) PES 18%, (b) PES 15%, and (c) PES 12%



(a) (b) (c)

Figure 2 SEM images that show the cross-section view of membrane in 1.0k magnification for (a) PES 18%, (b) PES 15%, and (c) PES 12%

Contact angle analysis

It is revealed that the contact angle of the PES 18% membrane ranged from 73° to 77.5°, whereas the contact angle for the commercial PES membrane with a molecular weight cut-off of 30 kDa varied between 79.5° and 82.5°. These results indicate that the PES 18% membrane exhibited a smaller contact angle.

The contact angle analysis provides valuable insights into the interaction between a liquid and a solid surface, such as a membrane. A lower contact angle suggests improved wetting and absorption, facilitating efficient liquid penetration. Conversely, a higher contact angle indicates reduced wettability and limited liquid absorption, making membranes less effective for applications requiring efficient liquid permeation. Therefore, membranes with smaller contact angles are preferable for filtration processes and membrane-based separations where efficient liquid absorption is essential.

Calculation of membrane porosity and pore sizes

Porosity, ε and average pore size, r_m of the membranes were determined theoretically using (**Eq. 4**) and (**Eq. 5**).

$$\text{Porosity, } \varepsilon : \frac{m_w - m_d}{\rho A l} \qquad \textbf{(Eq. 4)}$$

$$r_m : \sqrt{\frac{(2.9 - 1.75\varepsilon)8\eta l Q}{\varepsilon A \Delta P}} \qquad \textbf{(Eq. 5)}$$

Table 1 shown that PES 18% membrane had the lowest porosity and smallest average pore size, while the PES 12% membrane had the highest porosity and largest average pore size. The PES 15% membrane had intermediate values for both parameters. These findings aligned with experimental methods, particularly SEM analysis, indicating consistency in pore size assessment. However, there are significant difference in average pore size values between experimental and theoretical approaches. This may happen as SEM analysis provides detailed images of the membrane's

surface and cross-sections, identifying irregularities contributing to larger observed pore sizes. Additionally, SEM may capture a range of pore sizes, leading to broader pore size distribution and larger average pore size values compared to theoretical calculations.

Table 1 Result of porosity and average pore size of each membrane determined by calculation

Membrane	Wet weight (g)	Dry weight (g)	Porosity (%)	Average pore size (nm)
PES 18% + NMP 82%	0.3857	0.0847	76.86	3.91
PES 15% + NMP 85%	0.3619	0.0443	81.10	6.76
PES 12% + NMP 88%	0.3312	0.0083	82.45	11.99

Membrane water flux (distilled water)

From **Table 2**, PES 12% membrane have the highest water flux in which it achieved 10 mL in a very short duration, whereas the PES 18% membrane required the longest time to reach the same volume, with PES 15% exhibiting intermediate performance.

The observed differences in water flux also affect the volumetric flow rate of water passing through the membrane. Membranes with higher porosity and larger pore sizes tend to exhibit higher water flux rates. This occurs due to the presence of larger void spaces and openings in the membrane structure, which facilitate the easier passage of water molecules, thereby increasing water flux. Conversely, membranes with lower porosity and smaller pore sizes restrict the flow of water molecules, resulting in lower water flux rates.

Table 2 Data for average water flux and volume flowrate of each PES membranes

Membrane	Average water flux ($L/m^2.hr$)	Volume flowrate (L/hr)
PES 18% + NMP 82%	10.4913	0.0188
PES 15% + NMP 85%	38.0502	0.0622
PES 12% + NMP 88%	123.1116	0.2023

Harvesting microalgae by membrane filtration

Harvesting microalgae

Similar to membrane water flux test, harvesting microalgae also employed dead-end filtration under constant pressure of 5 bar and 10 mL of permeate collected. **Figure 4** distinct the harvest algae flux for each membrane for 2 tests in which it shows that PES 12% membrane have the highest flux for both tests.

However, **Figure 5** shows significant differences on the appearance of the harvested permeate sample from the PES 12% membrane compared to the other two membranes. The permeate from PES 12% appears cloudy, indicating the presence of microalgae cells, suggesting lower efficiency in microalgae harvesting. In contrast, the permeate samples from PES 18% and PES 15% membranes appear clearer, indicating minimal presence of microalgae cells, aligning with the desired outcome. This difference in performance can be attributed to membrane porosity (Sharabati et al., 2019). The PES 12% membrane, with its higher porosity, has larger pores prone to fouling, reducing harvesting efficiency. Lower porosity membranes like PES 15% and PES 18% are more effective in microalgae harvesting because they have smaller pores that retain microalgae cells without pore obstruction and reducing fouling risks.

These results can also be proven by **Table 3**, that shows the concentration of microalgae cells present in each sample by the membranes' performance. It highlights that PES 15% and PES 18% are the most efficient for harvesting microalgae as it has the highest rejection profile, and the lowest concentration of microalgae cells present in permeate sample.

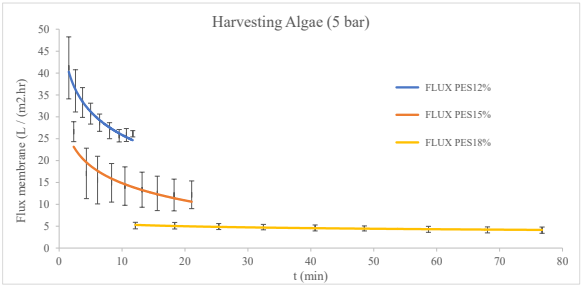
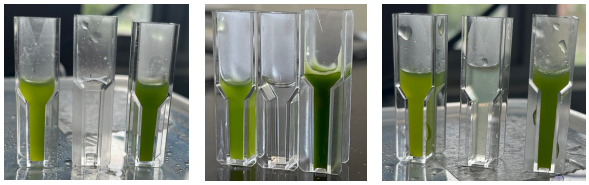


Figure 4 Harvesting algae flux for PES 18%, PES15%, and PES 12% membranes



(a) (b) (c)

Figure 5 Samples obtained for harvesting microalgae by (a) PES 18% membrane, (b) PES 15% membrane, and (c) PES 12% membrane

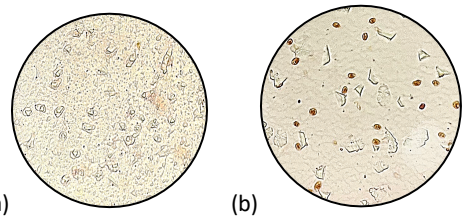
Table 3 Performance of membranes during algae harvesting

Membrane	Rejection of microalgae (%)	Flux ($L/m^{-2}h^{-1}$)
PES 18%	92.86 ± 1.36	6.59 ± 1.29
PES 15%	95.11 ± 2.79	29.69 ± 1.28
PES 12%	89.86 ± 0.23	34.15 ± 0.89

Protein purification by membrane filtration

Disruption of microalgae cells

Figure 6 presents microscopic observations of the microalgae cells before and after disruption. Before ultrasonication, the microalgae constituents were contained within intact cell walls. Conversely, after ultrasonication, the cell walls appeared broken, leading to the release of constituents into the surrounding culture medium (Liu et al., 2021; Ursu et al., 2014).



(a) (b)

Figure 6 Images captured from compound microscope at 100x magnification for (a) before ultrasonication, (b) after ultrasonication

Purification of microalgae's protein

Protein purification also utilized dead-end filtration under constant pressure of 5 bar and 10 mL of permeate collected. Using the ultrasonicated algae solution as the feed, membrane filtration was executed. **Figure 7** shows the protein flux for each membrane for 2 tests in which it shows that PES 12% membrane have the highest flux for both tests, similar to the result obtained from distilled water flux and harvest algae flux.

Analysing the data in **Table 4**, it's clear that the PES 18% membrane yielded the highest protein concentration in the permeate sample with lowest rejection percentage, while the PES 12% membrane showed the lowest concentration in permeate sample with highest rejection value. This contrasts with microalgae harvesting, where a higher rejection rate is desired to ensure efficient harvesting by retaining microalgae cells within the filtration system (Safi et al., 2015). Conversely, in protein purification, where smaller protein molecules need to permeate the membrane, a lower rejection rate indicates higher effectiveness (Gifuni et al., 2020).

The PES 18% membrane shows the highest efficiency in purifying protein from microalgae, despite the PES 12% membrane having the highest porosity. This difference is due to cell debris presence in the PES 12% membrane permeate sample, resulting in a lower percentage of protein molecules compared to the PES 18% membrane. The latter effectively filters out cell debris, leading to a higher percentage of protein molecules in the permeate sample thus facilitate recovery.

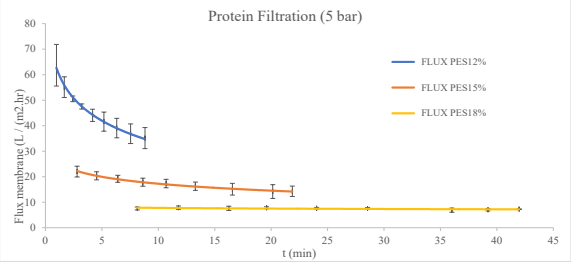


Figure 7 Protein flux for the purification of protein by membrane filtration

Table 4 Performance of membranes during protein purification

Membrane	Rejection of protein (%)	Flux (L/m^{-2}h^{-1})
PES 18%	-108.18 ± 10.03	8.06 ± 0.085
PES 15%	-16.40 ± 1.1	20.72 ± 1.45
PES 12%	23.99 ± 0.20	44.45 ± 0.90

CONCLUSION

The research successfully achieved its objectives, providing insights into suitable PES membrane compositions for microalgae harvesting and protein purification. This research found that membranes with higher polymer content, like PES 15% and PES 18%, exhibited greater efficiency in microalgae harvesting, with rejection profiles of 97.89% and 94.21% respectively. Clear permeate samples from PES 18% and PES 15% membranes contrasted with the cloudy appearance of the PES 12% permeate, indicating better cell retention. For protein purification, the PES 18% membrane showed the highest protein concentration in the permeate and the lowest rejection profile for protein molecules, while the PES 12% membrane performed poorly, struggling to separate protein molecules from cell debris. It was also highlighted the importance of porosity and pore sizes, with the PES 12% membrane's larger pore size hindering its performance. Membranes with lower porosity and smaller pore sizes demonstrated better efficiency in both microalgae harvesting and protein purification processes. Overall, the research provides valuable guidance for optimizing PES membrane compositions to enhance microalgae-based processes.

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