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Pilot scale microalgae harvesting by a membrane: cross flow vs. submerged membrane

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ABSTRACT

Harvesting microalgal biomass is a major challenge because of their small size, their density, and their low concentration in the culture medium. Thus, the energy inputs to the cultivation/harvesting processes are high, and often exceed the energy content of the microalgal biomass itself. In this study, the cross-flow membrane and submerged membrane systems were installed to raceway ponds, and the harvesting yields were compared. The highest algal concentration reached by submerged membrane system which was 10.44 g/L; and by cross-flow membrane system maximum biomass reached was 6.99 g/L, which corresponds to concentrate 6 and 4.2 fold, respectively.

Keywords: Cross-Flow membrane; Digestate; Microalgae; Raceway ponds; Submerged membrane; Wastewater

1. Introduction

In the last decades, microalgal biomass did draw attention as a promising alternative source of feedstock for feed, food, fertilizer, and/or biofuels. The major obstacle to industrial microalgae production and recovery is the dewatering process, which accounts for approximately 20%–30% of the total cost [1]. In pilot-scale studies, comparative analyses should be performed to determine the most accurate and appropriate dewatering method for harvesting and concentrating algae biomass economically. Most existing large-scale microalgal plant systems still use energy-intensive centrifuges to harvest microalgae [3]. Al Hattab et al. [2] compared nine different physical microalgae separation methods which were sedimentation (S), vacuum filtration (VF), pressure filtration (PF), cross-flow filtration (CFF), disk stack centrifugation (DSC), decanter centrifugation (DC), dispersed air floatation (DVF), dissolved air flotation (DVF), and fluidic oscillation (FO). According to his research, CFF and DC have the highest dewatering efficiency, followed by VF, PF, DSC, and DAF. Bilad et al. [3] investigated the applicability of submerged microfiltration for *Chlorella vulgaris* and *Phaeodactylum tricornutum* using three different porosity and suggested that submerged microfiltration for algal harvesting is economically feasible. Membrane separation is used to perform a separation under the influence of a driving force [3] that is generally cheaper than applying centrifuges and is known to be not energy-intensive. Therefore, it becomes a very promising technology for algal harvesting [4]. It is accepted as a promising technique for microalgae harvesting due to its advantages, such as a lack of chemical additives which is especially suitable for reducing water pollution and increasing water recycling, low energy requirement, the feasibility of incorporation in hybrid harvesting processes, and ease of scale-up [5]. There are some studies using membrane systems for harvesting pure microalgae culture successfully [6–10], but there is no any other study for harvesting mixed algal culture to optimize the harvesting efficiency, since membrane material selection in the algal industry may depend on culture concentration, species characteristics and flow parameters [11].

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Anaerobic liquid digestate is a nutrient rich by-product produced after anaerobically decomposition of organic materials. The use of liquid digestates in microalgal cultivation has gained increasing attention in recent years: it both recycles nutrients and produces valuable biomass, which can be further valorized. The ability to inoculate microalgae in wastewater for lowering the cost of the nutrient for growth (which digestate is an excellent source) and then to use the biomass either whole or fractionated as a commodity is an attractive proposition [12]. However, complex matrix of digestate makes the membrane selection step crucial.

In this study, the cost-effective strategy toward the cultivation of mixed microalgae consortia was provided that could be further converted into biofuels and/or high value added chemicals with a biorefinery understanding, along with the digestate treatment. Moreover, mixed microalgae grown in pilot-scale raceway ponds were harvested by membrane systems, and different membrane structure and system configurations were compared in terms of the performance on the harvest yield of microalgae and the effect of membrane material on the process of harvesting the microalgae.

2. Material and methods

2.1. Wastewater collection and analysis

The liquid digestate was obtained from a full-scale plant decomposing the waste mixture of mechanically/manually separated organic fraction of municipal solid waste (50%), cattle manure (17%), leaching water from solid waste collection vehicles (8%), expired market wastes (4%), and chicken manure (4%). The characterization of ALD was given in a previous work by Ermis and Altinbas [13]. The PerkinElmer® Optima™ 7000 DV (USA) inductively coupled plasma (ICP) optical emission spectrometer was used to analyze the wastewater elements using the standard solutions where calcium (Ca) was 58.1 mg/L, magnesium (Mg) was 19.1 mg/L, iron (Fe) was 27.8 mg/L, manganese (Mn) was 3.9 mg/L, aluminum (Al) was 9.5 mg/L, silicon (Si) was 46.7 mg/L, lead (Pb) was 1.1 mg/L, boron (B) was 5.8 mg/L, chromium (Cr) was 5.3 mg/L, cadmium (Cd) was 0.4 mg/L, nickel (Ni) was 3.1 mg/L, silver (Ag) was 4.8 mg/L, sulfur (S) was 619.7 mg/L, zinc (Zn) was 0.9 mg/L, Sr was 1 mg/L, and sodium (Na) was 871.4 mg/L.

2.2. Isolation and identification of mixed microalgae

The isolation of mixed culture of microalgae was performed as described in a previous study by Ermis and Altinbas [13]. Isolated wild-type microalgae culture was firstly checked by light microscopy, and mixed culture was morphologically characterized by using microalgae systematics books. Afterward, next generation sequencing was performed.

2.3. Analytical methods

Nitrogen was measured as total Kjeldahl nitrogen (TKN) and ammonia (NH_3–N); whereas phosphorus was measured as total phosphorus (TP) and orthophosphate (PO_4). Suspended solid, TKN, NH_3–N, TP, and PO_4–P values were analyzed as mg/L according to Standard Methods [14]. According to the characterization of ALD used in this experiment: COD (mg L^{-1}) was 12,600 ± 300, TKN (mg L^{-1}) was 1,692 ± 256, NH_3–N (mg L^{-1}) was 900 ± 62, NO_3–N (mg L^{-1}) was 0.13 ± 0.02, TP (mg L^{-1}) was 105 ± 7.5, PO_4–P (mg L^{-1}) was 64 ± 6, TSS (mg L^{-1}) was 15,880 ± 932, and pH was 9.00–9.15. Particle distribution analysis was performed by Master Sizer 2000 (Malvern, UK) working on the principle of refraction of laser lights; to observe the effect of particles on the membrane harvesting.

Fourier-transform infrared spectroscopy (FT-IR analyses) was performed by Perkin Elmer spectrum100 Attenuated Total Reflectance model (USA).

2.4. Filtration studies

The amount of concentrated microalgae varies according to the type of membrane material. Different membranes have different electrostatic properties and affect the passage through the membrane pores. Polypropylene membranes are positively charged while nylon (NY) membranes are negatively charged. In order to observe this difference, polyethersulfone (PES) and polyvinylidene difluoride (PVDF) membranes with the same membrane charge were tested and compared NY membranes. The dead-end filtration of the nylon and PES membranes with four different pore sizes (0.45, 0.65, 0.8, and 1.2 microns) each 47 mm diameter were investigated. Sterlitech HP4750 model stirred cell (USA) was used in membrane experiments. In the experiments, 99.99% high purity nitrogen mixture was used for the required pressure.

Clean water resistance values are used to determine the resistance of new membranes to water. The clean water resistance test is carried out using pure water. It can, therefore, be considered as a reference for the resistance value of the membrane to any wastewater. Before the clean water resistance test, the membranes were kept in clean water for 1 h to ensure the saturation of the membranes in water and to obtain the correct results. The clean water resistance was tested under three different pressures (1, 2, and 3 bar) by passing pure water in the specified volume (250 mL), in four different pore sizes (1.2, 0.8, 0.65, and 0.45 μm). With time-dependent mass flow rate obtained using pure water. Flux values at different pressures were calculated and permeability values were recorded.

In this study, the determined volume of microalgae solution (100 mL) was placed in Sterlitech HP4750 mixing cell and the flux value was obtained by measuring the time-dependent filtrate volume under constant pressure (1 bar).

$$J\left(\mathrm{LMH}\right)=\frac{Q\left(\mathrm{L}\right)}{A\left(\mathrm{m}^2\right)\times t\left(\mathrm{h}\right)} \tag{1}$$

where J is the flux (L/m^2 h), Q is the total volume (L), A is the membrane area (m^2), t is the time to collect the sample (h).

The permeability values were maintained and recorded until the system stabilized. Membrane fouling analyses was performed by firstly passing distilled water over the clean membrane surface, and after passing microalgae inoculation with a concentration of 2 g/L passed through the membrane,

the distilled water was passed again to observe the clogging in the membrane.

2.5. Automation of cross-flow and submerged membrane systems in raceway ponds

Two raceway ponds with 1,000 L working volume were operated with the cross flow and submerged membrane photobioreactor system. The pH was increased from 9 to 10.5 where the temperature was 25°C ± 3°C and turbidity was 850 NTU in both reactors. Submerged membrane had 4.5 m^2 area, meanwhile cross-flow membrane had 2.4 m^2 area. These two photobioreactors were operated by two different automation systems. The outline of the automation system is shown in Fig. 1.

The cross-flow membrane was activated by the automation system when sufficient microalgae biomass was formed and the turbidity meter reached the specified value. The filtered clean water and concentrated microalgae biomass were transferred to the tanks by two different lines under the membrane, where membrane pressure was adjusted by controlling the flow rate on the membrane surface by the valve. Also, for the submerged membrane (Fig. 2), it was activated by the automation system when sufficient microalgae biomass was formed. Membrane air pump completed the cycle with 2 min rest and 3 min working principle.

3. Results and discussion

3.1. Preliminary studies for determination of membranes

In order to observe the relationship between microalgae and particle size distribution of wastewater, particle distribution analysis of raw anaerobic digestate and microalgae inoculation were performed, and the expected fluctuation was observed at the desired intervals (Fig. 2). According to the particle size distribution of microalgae inoculation (Fig. 2a), 40% of mixed algal culture's size was ranging between 2 and 10 μm and the 60% of them was between 20 and 100 μm. Meanwhile, the raw wastewater's particle size (Fig. 2b) was ranging between 0.1 and 0.8 μm 90% of the time. Since the purpose of this study was harvesting microalgae without particles, choosing the pore size bigger than 0.8 μm would be preferable to let the particles pass through into the permeate water and help to have pure concentrated microalgae. At the end of the cultivation (Fig. 2c), it can be observed that the dominancy of first peak culture ranging between 2 and 10 μm was decreased and the second peak culture 20–100 μm was increased which demonstrated abundancy change in mixed culture by cultivation time.

Microscobic observations (Fig. 3) and particular size distribution (Fig. 2) analyses clearly demonstrated that there were two dominant species. According to the microbial community analysis in a previous study by authors (data not shown), *Synechocystis PCC-6803* and *Chlorella sorokiniana* were found as mostly detected species which correlates with the particle size of the species. *Chlorella* sorokiniana's the size found to be within a range of 2–10 μm in diameter [15], where as the size of *Synechocystis PCC-6803* found to be within a range of 1.67–2.46 μm [16]. Those two dominant species were in the same range with the same peak of the particle distribution (Fig. 2a), whereas the second peak could be explained by the agglomeration of microalgal cells.

Polypropylene membranes are positively charged while nylon (NY) membranes are negatively charged. To observe this difference, the permeability values for NY, PES, and PVDF membranes were calculated. When the results were examined, the flux increase was observed in all membranes with increasing pressure.

The measurements were continued until the system stabilized and the results were recorded (Table 1). As expected, clogging increased, and flux decreased with decreasing pore size.

The difference between the clean membrane flux and the membrane flux at which the blockage takes place, gives the clogging. Clogging results for all membranes are given in Table 2. As the pore size of both NY and PVDF membranes decrease, clogging percentage increases, and flux

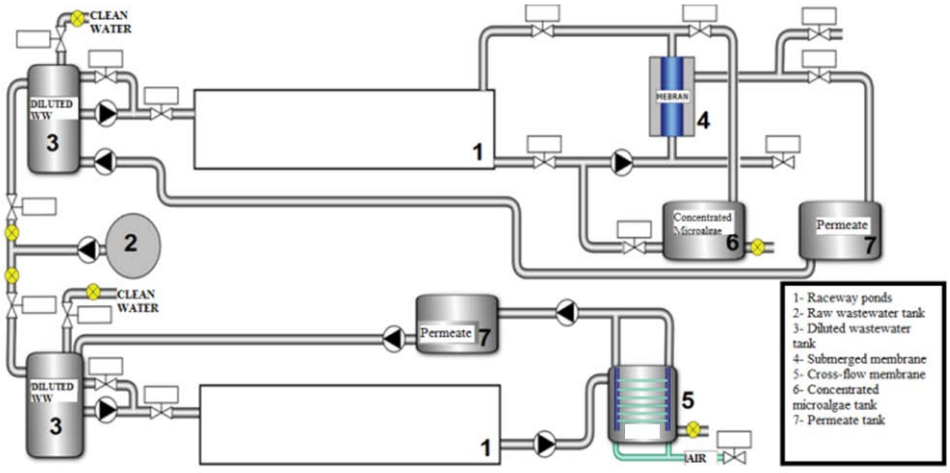

Fig. 1. Automation system of membrane systems (1) raceway pond, (2) raw wastewater tank, (3) diluted wastewater tank, (4) submerged membrane, (5) cross-flow membrane, (6) concentrated microalgae tank, and (7) permeate tank.

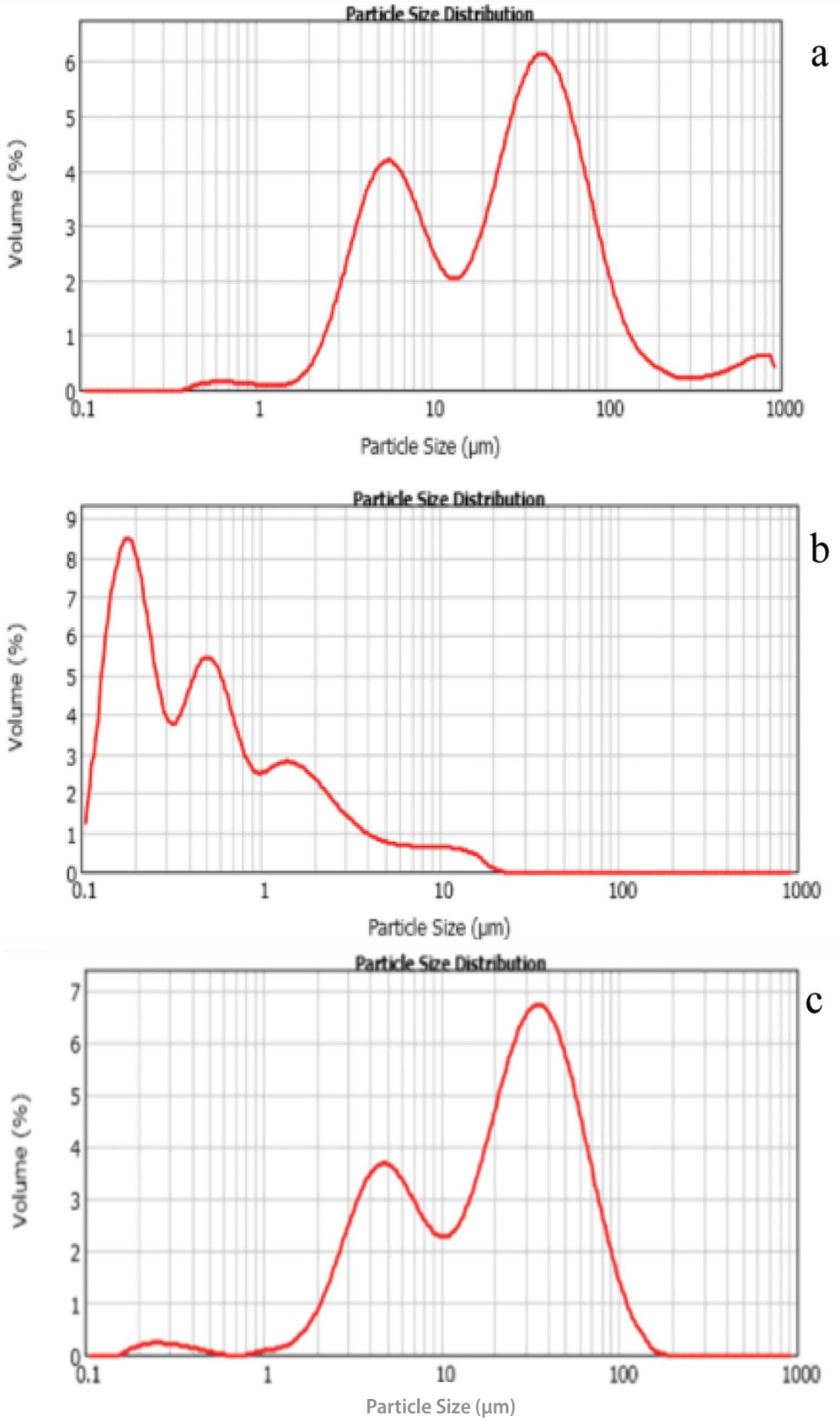

Fig. 2. Particle size distribution of microalgae inoculum (a), anaerobic digestate (b), and mixed microalgae (c) grown in 10% anaerobic digestate (last day of cultivation).

Table 1
Fluxes of all membranes at different pore sizes

Pore size (μm)	Mean stable flux* (LMH)			Mean flux** (LMH)			Maximum flux*** (LMH)		
	NY	PES	PVDF	NY	PES	PVDF	NY	PES	PVDF
1.2	2.34	2.77	2.30	9.67	11.87	10.17	82.33	85.42	88.09
0.8	2.27	1.91	3.43	9.18	10.18	10.01	77.55	68.93	72.47
0.65	2.12	1.43	2.75	7.89	8.80	9.16	72.93	61.54	74.92
0.45	2.06	1.39	1.23	8.19	6.10	6.78	70.16	41.85	39.64

*Mean stable flux values refer to the mean flux values after clogging in the system.
**Mean flux values refer to the average flux in the process until the blockage occurs in the system.
***Maximum flux values refer to flux values measured in the tenth second.

Table 2
Clogging results for all membranes

Pressure (bar)	NY membrane			PES membrane			PVDF membrane		
	Flux (LMH)		% Clogging	Flux (LMH)		% Clogging	Flux (LMH)		% Clogging
1	3,468		47.74	8,647		5.53	3,375		46.54
2	6,436	00.45 μm	35.14	13,432	0.45 μm	8.02	6,163	0.45 μm	33.07
3	9,018		33.62	17,897		3.62	8,636		30.67
1	5,693		39.76	7,897		24.07	5,056		37.35
2	11,440	00.65 μm	18.58	10,901	0.65 μm	32.05	9,206	0.65 μm	26.57
3	14,996		16.53	13,301		33.08	13,605		14.68
1	7,490		19.94	10,426		13.60	7,398		20.94
2	11,582	00.8 μm	20.62	15,940	0.8 μm	9.09	11,236	0.8 μm	19.42
3	16,064		14.92	19,251		10.94	14,888		14.23
1	9,676		6.90	2,495		60.58	9,296		10.68
2	14,829	10.2 μm	5.83	4,398	1.2 μm	74.71	14,027	1.2 μm	10.03
3	18,392		7.37	8,371		60.58	17,481		7.98

decreases. On the other hand, in the PES membrane, it was determined that the blockage was reduced by decreasing the pore size. This is thought to be due to the fact that these two membranes have different electrical charges. Theoretically, polypropylene membranes retain negatively-charged-algae cells better than nylon membranes. Riedl et al. [17] was also observed the flux behavior of NY, PVDF, and PES membranes with dead-end microfiltration and concluded that NY membrane produces a dense surface fouling layer, therefore the fluxes of PES and PVDF membranes were less affected by fouling formation. According to the recent studies on submerged membrane filtration systems for microalgae dewatering, *Chlorella pyrenoidosa* was harvested in Zhao et al. [18]'s study by 0.1 μm PVDF submerged membrane with 20 LMH flux and in Bilad et al. [3]'s study, *C. vulgaris* was harvested by 0.22 μm PVDF submerged membrane with 32.5 LMH flux. In this study, the maximum PVDF flux examined was 88, 72.4, 72.9, and 39.6 LMH for 1.2, 0.8, 0.65, and 0.45 μm pore size, respectively. In addition, as the pore size decreases in PES membranes, higher fluxes were observed compared to PVDF membranes which are important to not lose smaller algal cells. Considering the maximum flux, the highest flux was observed in the 1.2 μm PVDF membrane. However, when the mean flux is examined, it is seen that the most efficient membrane is the PES membrane with 0.8 μm pore size.

The aim of the study was to select the membrane with the lowest clogging and the highest flux. Considering the results, the best pore size was chosen as 0.8 for all membranes. According to microscopic observations and the particular size distribution analyses, it was supported that the selected pore size (0.8 μm) was suitable. Therefore, the PES membrane with the highest flux was selected as the most suitable membrane for further studies.

3.2. Operation of pilot-scale membrane photobioreactors

The mixed algal culture was harvested by cross-flow and submerged membranes when they were at the stationary phase. When the mean SS-VSS-TS and TDS values were analyzed, the raceway pond with cross-flow membrane system reached to 1.65 g/L SS, 1.19 g/L VSS, and 2.98 g/L TS. The concentrated microalgae reached after filtration to 6.99 g/L SS, 4.93 g/L VSS, and 8.65 g/L TS; where permeate was calculated as 0.015 g/L SS, 0.014 g/L VSS, and 0.022 g/L TS; which 4.23-fold concentration with 83.3 LMH was achieved with the cross-flow membrane. When TDS results were considered, according to Environmental

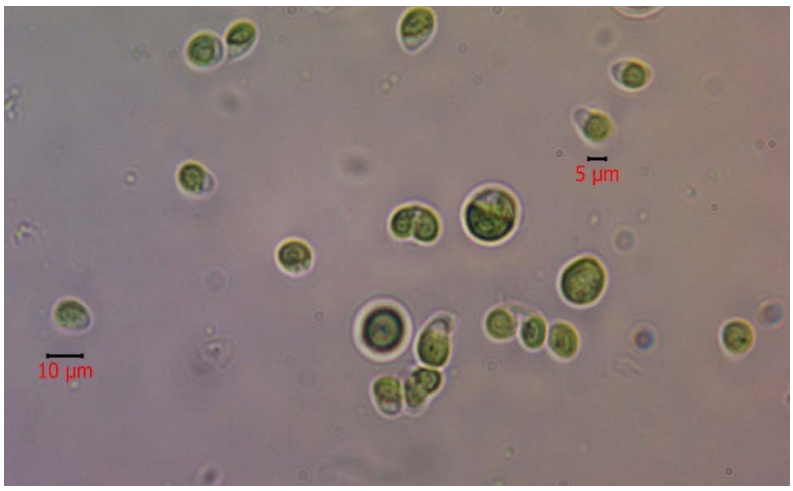

Fig. 3. Microscopic observation image of mixed culture.

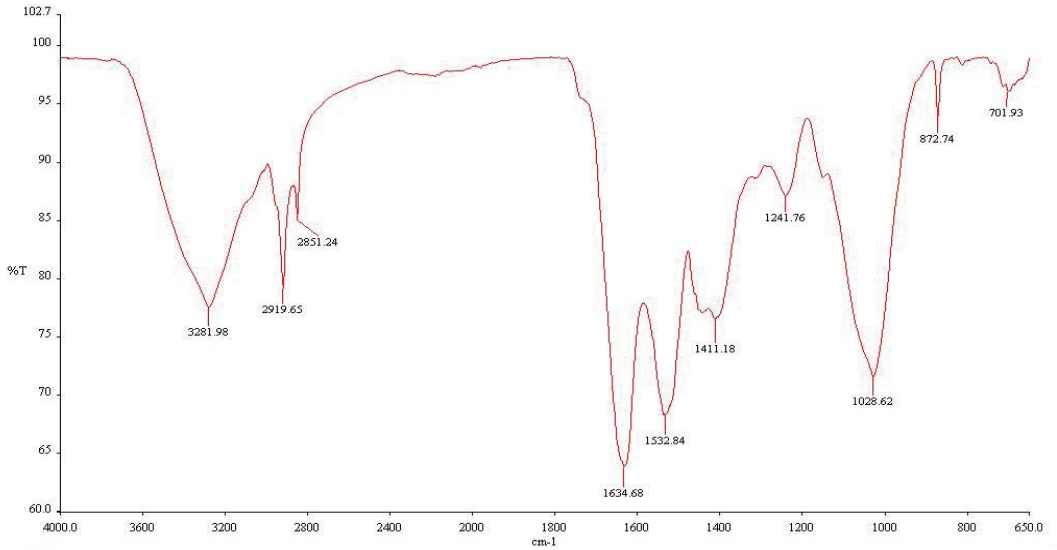

Fig. 4. FT-IR spectra of the mixed culture in digestate.

Protection Agency's [19] drinking water standards, water below 500 mg/L TDS is suitable as drinking water; where the TDS of the raceway pond with cross-flow membrane system was calculated as 1,800 mg/L. When the raceway pond with submerged membrane system was examined; SS was calculated as 1.74 g/L, VSS was 1.41 g/L, and TS was 3.11 g/L. After filtration, the concentrated algal biomass calculated as 10.44 g/L SS, 7.88 g/L VSS, and 13.1 g/L TS; whereas the permeate calculated as 0.038 g/L SS, 0.036 g/L VSS, and 0.056 g/L TS. Submerged membrane system provided 6-fold concentration with 88.9 LMH flux. In conclusion, the highest algal concentration reached by submerged membrane system which was 10.44 g/L; and by cross flowed membrane system maximum biomass reached was 6.99 g/L, which corresponds to concentrate 6 and 4.2 fold, respectively (Table 3) with almost 100% recovery for both membrane systems. According to the recent studies on membrane filtration systems for microalgae dewatering, Susanto et al. [20] observed performance of membrane filtration by crossflow membrane systems with *Chlamydomonas* sp. with PES material with 100 kDa pore size and 86.8%–91.1% recovery was observed. Elcik and Cakmakci [21] was observed the performance and the fouling effect of cross-flow membrane filtration with *C. vulgaris* with 0.8 µm PES membrane and examined 100% recovery efficiency. Moreover, in Monte et al. [22]'s study, *Dunaliela salina* was harvested by 0.1 µm PES crossflow membrane with 88.9%–89.9% recovery. Moreover, Petrusevski et al. [23] used a cross filtration with a membrane pore size of 0.45 µm and achieved a algal biomass recovery efficiency of 70%–89%. Zhang et al. [24] used a cross-flow ultrafiltration membrane with a cross-flow and examined an increase in algae concentration from 0.104% to 92.5%.

Wang et al. [25] operated a pilot-scale submerged membrane bioreactor (MBR) for real municipal wastewater treatment in order to investigate EPS properties and

Table 3
Comparison of cross-flow and submerged membrane

Parameters	%10 digestate	Cross-flow membrane			Submerged membrane		
		Reactor	Permeate water	Concentrated algae	Reactor	Permeate water	Concentrated algae
SS (g/L)	0.7	1.65	0.015	6.99	1.74	0.038	10.44
VSS (g/L)	0.47	1.19	0.014	4.93	1.41	0.036	7.88
TS (g/g)	2.1	2.98	0.022	8.65	3.11	0.056	13.1

their role in membrane fouling and he concluded that there were positive correlations with membrane fouling and EPS. The use of membrane technology in most applications, including microalgae processing, is limited by the membrane fouling problem where fouling can be categorized into four categories: pore blocking, adsorption, cake layer formation, and gel layer formation [26]. Moreover, surface charge and hydrophobicity are also a consideration when selecting membrane material, as these characteristics can affect membrane fouling [11]. Zhang et al. [24] concentrated *Scenedesmus quadricauda* up to 15% in batch filtration by applying air bubble scouring and backwashing to control membrane fouling. In this study for both membrane systems, backwash was applied when the flux started to decrease by time. In Ahmad et al. [27] study, *Chlorella* sp. was harvested by CA membranes (1.2 μm with 70–120 L/m^{2}h flux) with cross-flow system and it was observed that fouling was dominated by pore blocking at an early stage of filtration. Babel and Takizawa [28] observed the cake layer formation on PVDF membranes using *Chorella* sp. and concluded that the development of cake layer resistance with algal deposition could be divided roughly into three stages, which are low cake resistance, the exponential progression of resistance, and maximum resistance. The flux loss of membranes during cell fouling was also studied by Qu et al. [29] and Zhang et al. [24] also observed flux loss of membranes during cell fouling using *S. quadricauda* and *M. aeruginosa* cells and examined that the accumulation of algal cells led to an extreme decline in the flux; the decline rate increased with increase in the cell concentration. Zhang and Fu [30] concluded that MF and UF fouling could worsen during the filtration of algal cells and their organic material together, that algal cells in the cake layer are impacted in a matrix of organic material as the glue keeping the cells together on the membrane surface. Therefore, Qu et al. [29] concluded that under certain operational conditions, the characteristics and contents of algal organic material have a great influence on the structure of the cake layer during the filtration of algal suspension. Cho et al. [31] found out that the specific cake resistance became higher as the amount of bound EPS increased. To observe the possible EPS formation, FT-IR was performed to characterize the major functional groups of organic matters and to predict the major components in the system (Fig. 4). The transmittance of the spectra showed fluctuation due to the differences in sample quantity. The spectra show a broad region of adsorption around a peak at 3,281 cm^{-1}, which was attributed to stretching of the O–H bond in hydroxyl functional groups, and peaks at 2,919 and 2,851 cm^{-1} which might be due to stretching of C–H bonds [32]. Two peaks at 1,634 and 1,532 cm^{-1} were also observed in the spectra, which are unique to the protein secondary structure, namely amides I and II [33]. It indicated that proteins were one of the components of the bound EPS. In addition, a broad peak at 1,028 cm^{-1} exhibited the character of carbohydrates or carbohydrates-like substances, which indicated that carbohydrates were present in the EPS [34].

4. Conclusion

Membrane technology selection can be made according to the purpose of operating systems. In this study, according to the dead-end filtration studies, the PES membrane with 0.8 μm pore size was selected as the most suitable membrane for mixed algal culture harvesting grown in anaerobic liquid digestate. In the pilot-scale operation, the highest microalgae concentration was achieved by submerged membrane system (10.44 g/L), while the lowest suspended solid of permeate was obtained by cross-flow systems (0.015 g/L). Since the aim of this study was to reach the highest algal biomass, submerged membrane systems with PES material having 0.8 μmoL pore size had been selected as the most efficient system to be used in open raceway pond systems. It was advocated that coupling of microalgae growth with liquid digestate treatment and harvesting with membrane systems was an economical and ecologically friendly technology for cheap biomass production for further valuable applications.

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