PILOT SCALE DEWATERING OF *CHLORELLA SOROKINIANA* AND *DUNALIELLA TERTIOLECTA* BY SEDIMENTATION FOLLOWED BY DYNAMIC FILTRATION

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ABSTRACT: The present work focuses on the application of pH-induced sedimentation combined with dynamic filtration for microalgae culture concentration at pilot scale. Concentrations were performed on cultures of two microalgae species: *Dunaliella tertiolecta* and *Chlorella sorokiniana*. The objective of the combined process was to reduce microalgae dewatering costs. It is true that sedimentation reduces operation costs considerably, but the results of membrane filtration offer a total rejection and high final concentrations, at even a cheaper cost than centrifugation . When using the two technologies in series, high concentration factors with values up to 207.4 for *Dunaliella tertiolecta* and 245.3 for *Chlorella sorokiniana* were achieved. The final concentration of *Dunaliella tertiolecta* was 184.58 g/L with 81.5% of water content in the sludge. The concentrations obtained were high enough to dispense with further operations for the sludge to be ready for a cell disruption step using steam explosion. Analytic techniques used were dry weight and optical density. For the filtration, experiments were performed using both commercially available and self-prepared membranes, manufactured from Acrylonitrile Butadiene Styrene: a novel polymer in membrane technology, selected to reduce costs. Each of them could perform in a similar way to commercial membranes in a pilot scale high-shear stress membrane module.

Keywords: pilot demonstration; dewatering; sedimentation; ABS; dynamic membrane filtration.

List of abbreviations

ABS – acrylonitrile butadiene styrene

CA – contact angle

DMA – N,N-dimethylacetamid

MF – microfiltration

MWCO – molecular weight cut-off

NMP – 1-methyl-2-pyrrolidinone

OD – optical density

ODCF – optical density concentration factor

PAN – polyacrylonitrile

PBR - photobioreactor

PE – polyethersulfone

SEM – scanning electron microscope

TCF – total concentration factor

UF – ultrafiltration

VCF – volumetric concentration factor

VSEP – vibratory shear enhanced process

1 INTRODUCTION

Microalgae are the scope of wide research studies concerning the culture and the final composition, harvesting techniques as well as biorefinery [1]. Being a source of lipids, proteins and carbohydrates microalgae can be processed into food supplements, fodder, colorants, enzymes, biofuels and pharmaceuticals [2-4]. In the general production process, they are primarily cultivated either in an open pond or in a closed photobioreactor (PBR), reaching a biomass concentration between 0.02–0.5wt% [5]. However, for most of the applications, microalgae need to be harvested after cultivation. From the culture medium, the biomass can be concentrated to 15–22% in a single step or in a sequence of concentration steps, before further treatment via drying, extraction or other downstream processing steps [6]. Nevertheless, as the costs of this single step as high as 20–30% of the total cost of microalgal biomass production, harvesting optimization is strongly recommended [7].

The cheapest and most conventional method available is flocculation/sedimentation, which allows to discard at least 90% of the liquid for further processing. This technique is commonly being used at wastewater treatment plants for sludge treatment. Sedimentation enables liquid or solid particles to separate from suspensions with different densities, producing effluents of mostly clear liquid. In order to decrease the sedimentation time, the aeration of microalgae cultures can be stopped, which causes the cells to flocculate on their own. This technique, called auto-flocculation, occurs as a result of the precipitation of carbonate salts with algal cells at higher pH, arising from algae's photosynthetic CO_2 consumption [8]. Moreover, auto-flocculation can be improved by adding NaOH to achieve optimal pH values [9, 10]. In many cases the average dry solids concentration of microalgal biomass to be achieved is around 0.5–3%. However, if the density difference is small, the process can result in being slow and ineffective [11, 12].

A quick dewatering of algae using centrifugation can be obtained with 84% removal efficiency (0.2 g/L algal culture at a flow of 379 L/min and under a rotational velocity of 3000 rpm) although, at the same time, it is high energy demanding. To harvest algae cultures with the same technique from 0.04% to 4% dry weight costs 1.3 kW h/m^3 of pond water. To increase the efficiency of the drying process, the algal biomass concentration has to be increased to at least 20% dry weight in the dewatering stage. The energy demand for increasing the microalgae culture concentration to 22% of

dry biomass via centrifugation is of 8 kWh/m^3 [13, 14]. This could be applicable in processes to obtain high-value products, whereas for other applications, e.g. a biodiesel production process, this would be too expensive.

Other techniques such as membrane filtration, which is capable of consuming as little as 0.25 kWh/m^3 at 70% harvest efficiency, appears to be more suitable for this purpose [13, 14]. However, as biological feeds are a mixture of organic matter of different size and shape, they are usually difficult to filter because the cake is very compressible. Also, the surface charge of the cells may result in concentration polarization phenomena, affecting the interaction between the membrane surface and the biomass [15]. The filtration ability depends also on the cell viability and the harvesting time [16]. The fouling issue is the main disadvantage when working with the conventional cross-flow filtration and can result in up to 99% permeability reduction [17-19]. Vibratory shear enhanced process (VSEP) also called dynamic filtration can overcome this issue by increasing the turbulence and raising the shear stress over the membrane surface [20, 21]. Moreover, in the case of dynamic filtration it was proved that in spite of the permeability decrement, when the initial biomass concentration increases, an asymptotic behavior occurs. Therefore, the filtration performance may continue to be satisfactory with sludge concentration increment [22]. For the purpose of microalgae dewatering, membrane micro/ultrafiltration (MF/UF) can be applied by using ceramic as well as polymeric membranes. However, as the cost of the overall process is the key parameter, polymeric materials are much more suitable as their price is considerably lower compared to the ceramic ones [17].

In order to reach the highest concentration of microalgae with the lowest dewatering cost, two techniques should be combined resulting in an effective and economic harvesting process [23]. The more efficient and cheaper the methods chosen are, the lower the final cost of the process will be. The main hypothesis of the work is that the combination of sedimentation and dynamic membrane filtration reduces the harvesting cost. This work describes the combination of pH-induced sedimentation of two different microalgae species, *Dunaliella tertiolecta*, and *Chlorella sorokiniana*, with dynamic membrane filtration. Novel cheap polymeric membrane material was compared with commercially available ones and tested for the dewatering of microalgae *Dunaliella tertiolecta* with both conventional and dynamic filtration setups.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Microalgae biomass

Sedimentation and filtration experiments were performed with the green microalgae *Chlorella sorokiniana* (strain CCAP 211/8k) and *Dunaliella tertiolecta* (strain CCAP19/6B).

Cultures of *Dunaliella tertiolecta* for experiments designed to compare the performance of commercial membranes and self-made membranes in cross flow and dynamic filtration were grown in 5 L flasks. Culture medium consisted of 4 L natural seawater (37‰) enriched with $NaNO_3$ (4.4 mM), $Na_2HPO_4.2H_2O$ (0.04 mM) and the same micronutrient concentrations as in Guillard's f/2 medium described in Andersen (2005). The cultures were aerated with air enriched with 0.5% CO_2 and illuminated with OSRAM L30W/865 Lumilux, Cold Daylight fluorescents giving an irradiance at the flask surface of 200 µmol photon m^{-2} s^{-1} in a L: D cycle of 16:8.

The cultures of *Chlorella sorokiniana* and *Dunaliella tertiolecta* used in the sedimentation experiment and the culture of *Dunaliella tertiolecta* used in the experiment for the determination of the maximum concentration attained by VSEP were grown in column photobioreactors (50 cm diam., 300 L or 150 L for the maximum concentration experiment). They were aerated with air and illuminated with Philips MASTER TLD 58W/865 fluorescents giving an irradiance at the photobioreactor surface of 300 µmol photon m^{-2} s^{-1} in a L: D cycle of 16:8. *Chlorella sorokiniana* was grown in tap water enriched with $NaNO_3$ (2 mM) Na2$HPO_4.2H_2O$ (3 µM) and the micronutrients of BBM (Andersen 2005) at 1/8 strength. *Dunaliella tertiolecta* was cultured in artificial seawater prepared with tap water and 37 g·L-1 of Aquaforest Reef Salt® enriched with $NaNO_3$ (2 mM), $Na_2HPO_4.2H_2O$ (3 µM) and the same micronutrient concentrations as in Guillard's f/2 medium. In the cultures prepared with tap water, phosphate was daily fed-batch to increase 3 µM the concentration in the medium, in order to avoid precipitation, presumably operated by magnesium and calcium ions. Temperature during culture was 20 ±2 °C.

2.1.2 Membranes

Experiments were performed with both commercially available polymeric membranes and synthesized ones. The filtration area was 0.0139 m^2 for conventional cross-flow filtration module and

0.0446 m^2 for dynamic filtration module. The properties of the commercial membranes are listed in Table 1.

Table 1: Commercial polymeric ultrafiltration membranes used for the dewatering of microalgae

Membrane commercial names	**Producer**	**Supplier**	**Material**	**MWCO**
PE5	Sepro	Nanostone	Polyethersulfone	5,000 Da
PAN50	Sepro	New Logic	Polyacrylonitrile	50,000 Da

DMA (N,N-Dimethylacetamide, ≥99.5%) was purchased from Sigma-Aldrich. ABS copolymer Novodur P2H-AT NR, kindly provided by Styrolution, was employed with a density of 1.05 g/cm^3, processing temperature between 230 and 260 °C and tensile stress at yield of 44 MPa. DMA was used as a solvent to dissolve the polymer for the synthesis of non-commercial membranes.

2.2 Methods

2.2.1 Membrane synthesis

Polymeric membrane synthesis was performed via phase inversion precipitation with a polymer concentration of 30 wt % and water used as a non-solvent in a coagulation bath.

The polymer and the solvent were mixed and stirred for 72 h to obtain homogenous polymeric solution. Afterwards, the solution was left for at least 24 h to remove all the bubbles from the bulk. The solution was deposited onto a glass plate using a casting knife with adjustable thickness gap regulated by an incorporated micrometer [24]. The casting knife gap was adjusted to 300 µm and set in motion by an automatic film applicator with a constant traverse speed of 50 mm/sec (BYK – Gardner Automatic Film Applicator). The immersion of casted polymeric solution into a coagulation bath caused a phase inversion precipitation, which resulted in the formation of a thin film. The temperature of the coagulation bath was fixed to 50 °C, ± 5 °C, to produce a membrane applicable for use with dynamic filtration module.

2.2.2 Sedimentation combined with dynamic filtration

In order to determine the optimum pH value for sedimentation in 300L photobioreactors, a preliminary study of sedimentation experiments was performed with both microalgae species in 2 L

graduated cylinders. 2M NaOH solution was added into the cylinders and mixed with a magnetic stirrer until flocculation occurred. Once the formation of aggregates was observed the stirring was stopped and the suspension was allowed to settle. pH was constantly monitored during those experiments.

1200 L of *Dunaliella tertiolecta* and 900 L of *Chlorella sorokiniana* cultures were treated with pH induced sedimentation by adding 2M NaOH solution into the vertical photobioreactor containing microalgae culture. To obtain a uniform pH distribution, aeration was kept for 2 minutes after the addition of the alkali solution. Then, the air-flow was stopped and the culture was left to settle for 60 minutes. Every pH adjustment was performed in each one of the 300 L reactor and after sedimentation the four concentrated bottom volumes were mixed to proceed to membrane filtration. The samples of the clarified liquid were collected from three different levels of the PBR for the pH measurement.

The filtration was performed with the dynamic filtration setup (VSEP, serie L, New Logic Research, Inc., detailed description: Section 2.3) and PE5 commercial membrane (MWCO=5,000 Da). The filtration was continued until the maximum volume of permeate was reached (3.4L of the dead volume of the equipment). The total microalgae rejection was confirmed by absorbance measurements of the permeate samples.

The dry weight of the samples was measured to calculate the concentration of microalgae. Samples were rinsed with NaCl 0.5M. This allowed for the elimination of the organic matter to be dissolved. Samples were then dried for 24 hours at 100 ºC and weighted afterwards.

2.2.3 Contact Angle

A sessile drop technique with an automatic video-based analysis system OCA 35 (Dataphysics) was used to measure the membranes' contact angles (CA). Demineralized water was used as a liquid. Usually, the droplet reached a steady state on a membrane surface around 30 s after dispensing. At least five measurements were performed for each membrane.

2.2.4 Permeability

The initial permeability of virgin membranes was determined by water flux measurements. After that, the filtration of microalgae biomass was performed. At the end of the experiment with the microalgae sludge, the membrane permeability with water was measured again after cleaning the

system. The last step allowed for the determination of the irreversible fouling resistance of membranes.

2.2.5 Optical density

To confirm total microalgae rejection by a membrane during the filtration, the turbidity of permeate was estimated by measuring its absorbance at 750 nm. For each sample, four measurements were performed. Absorbance was measured in 96 well plates using a microplate reader (INFINITE M200 PRO, Tecan). Values were converted to optical density (OD750 nm) by dividing them by the path-length. The OD750nm of filtered (0.45 µm) seawater was used as a reference.

2.2.6 pH measurements

For the sedimentation experiments, flocculation was induced by modifying the pH with a NaOH solution (2N) whereas pH change during the experiments was measured using a GLP 21 pH-Meter (CRISON Instruments, S.A.).

2.3 Equipment

Experiments were carried out using two filtration setups, as shown in the scheme in Figure 1. In the cross-flow filtration, the microalgae culture was placed in the temperature controlled recirculation tank (cooled by using Refrigerated Heating Bath with air-cooled refrigerating unit, Huber, K6-cc-NR) and pumped by a screw pump towards a membrane cell system (SEPA CFII, GE Osmonics). The transmembrane pressure was regulated with a compact back pressure regulator and a volumetric flow meter. The retentate was returned from the membrane module to the recirculation tank, while the permeate was collected in the permeation tank placed over a scale. The scale was connected to a computer to read the actual mass of permeate during the experiment and to calculate the actual mass flow rate and permeability in a five second frequency.

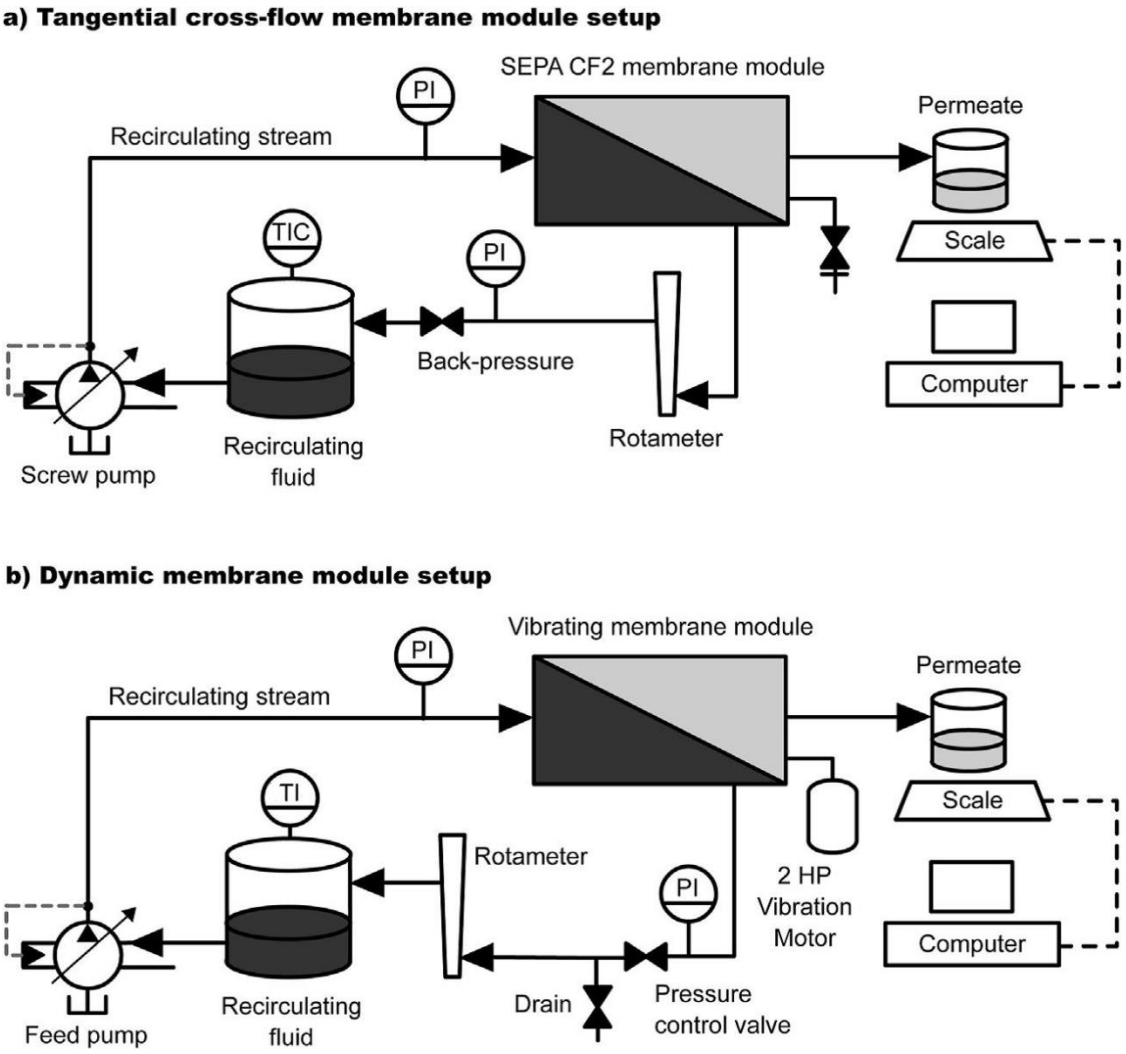

Figure 1: Filtration setups: a) cross-flow filtration; b) dynamic filtration (PI: Pressure Indicator, TIC: Temperature Indicator Controller, TI: Temperature Indicator, HP: Horsepower, SEPA CF2: commercial name for the tangential cross-flow membrane module). [22]

The transmembrane pressure was fixed at 3.5 bar and the recirculating flow rate at 50 L/h. The volume of microalgae culture used as the feed was 1.5 L. Two repetitions of each experiment were performed.

The dynamic membrane filtration of microalgae culture was performed by using a Vibratory Shear Enhanced Processing (VSEP, serie L, New Logic Research, Inc.) system. A detailed description of this setup can be found elsewhere [25].

The vibrational frequency applied was 55.4 ± 0.1 Hz. The commercial setup includes a motor that provides vibration to the membrane module (BALDOR VM3555, 2HP 3450RPM, 208-240 VAC 3

phase), which is managed by an electronic control system that permits the user to set the frequency.

The recirculating flow rate was equal to 570 ± 5 L/h and the transmembrane pressure was fixed at 3.5 bars. With these conditions three experiments were performed:

a) The dewatering of *Dunaliella tertiolecta*, using a volume of 38 L of the original culture as a feed, two replicas of the experiment were performed;
b) The dewatering of sedimented *Dunaliella tertiolecta*, using a volume of 47 L of the floc (the concentrated part of the sedimentation) as a feed. Experiment performed once.
c) The dewatering of sedimented *Chlorella sorokiniana*, using a volume of 28 L of the floc as a feed. Experiment performed once.

3 RESULTS

3.1 Membrane surface characterization via contact angle measurements

The surface of the materials was characterized by water contact angle measurements with all the membranes tested. In all the results, the ± values report a standard deviation between measurements. The contact angle value gives information as to whether the surface is either hydrophilic (CA < 90º) or hydrophobic (CA > 90º). The smaller the contact angles, the better the hydrophilicity of the membrane is. Both the commercial and the self-prepared membranes resulted in a CA < 90º, thus revealing hydrophilic properties of the surface. The more hydrophilic the membrane, the better the water permeability, therefore this property is strongly anticipated for the dewatering experiments. Similar CA were obtained for ABS and PE5 membranes, with values of 69.9 ± 1.1, n = 5, and 64.2 ± 4, n = 6, respectively. The lowest CA value, 55.1 ± 0.5, n = 5, was measured for PAN50 membrane, indicating the best performance in terms of water permeability, as confirmed by the filtration experiments. Despite its high hydrophilicity, PAN is one of the most expensive materials available in the membrane industry. Therefore, as cost reduction is the goal, PAN membrane should be used only as a reference, but not as potential candidate for this purpose.

3.2 Filtration experiments

3.2.1 Cross-flow versus dynamic filtration of *Dunaliella tertiolecta*

Figure 2 shows the permeability results obtained for experiments with *Dunaliella tertiolecta* using conventional cross-flow filtration technique. The permeability with microalgae suspension as well as with water before and after microalgae dewatering for all the membranes tested was measured.

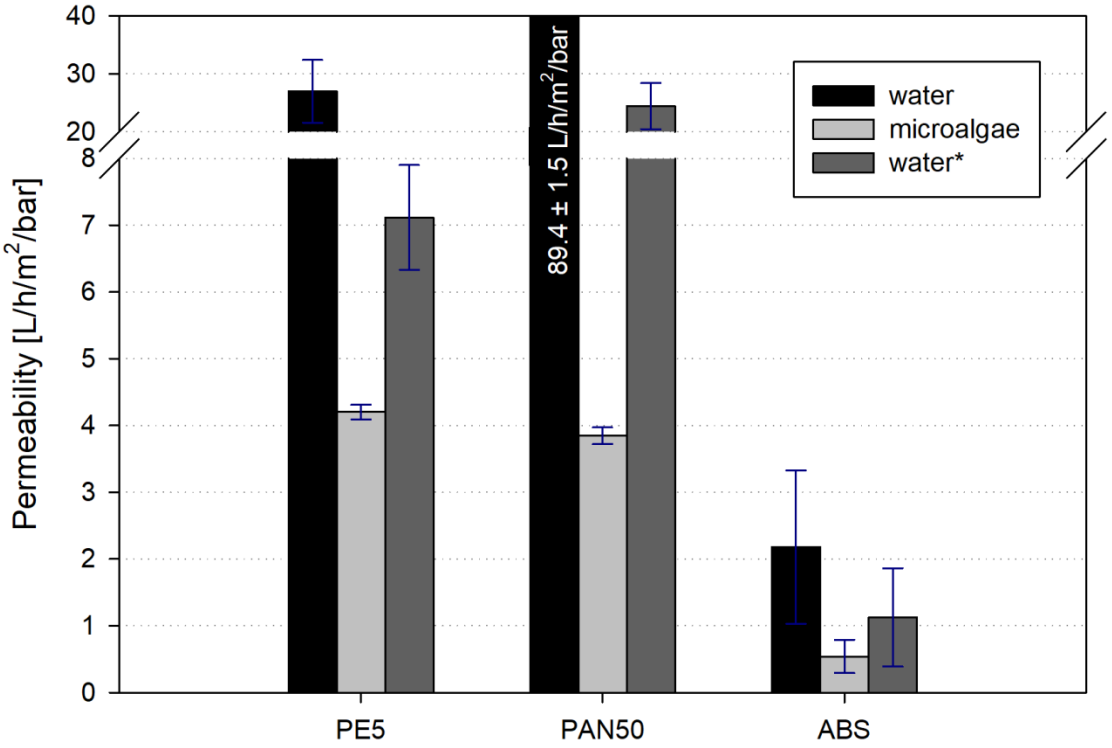

Figure2: Permeability results for the cross-flow filtration of Dunaliella tertiolecta: water permeability with the virgin membrane, microalgae culture permeability and water permeability after the experiment and with the cleaning procedure performed (n = 2). The error bars report standard deviation between measurements. PE5- commercial polyethersulfone membrane, PAN50 – commercial polyacrylonitrile membrane, ABS – own-made acrylonitrile butadiene styrene membrane.*

The highest water permeability was obtained when working with PAN50 virgin membrane, giving the value of 89.4 ± 1.5 L h^{-1} m^{-2} bar^{-1}, n = 2. This result confirms that PAN50 is the most hydrophilic commercial membrane considered in this study. The lower value given by PE5 membrane (27 ± 5 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$, n = 2) might be explained by being ten times lower MWCO compared to PAN50. Regarding the ABS membrane, permeability with water before the experiment was the lowest, giving

the value of 2.2 ± 1.2 L h^{-1} m^{-2} bar^{-1}, n = 2, but considering that those were membranes prepared in laboratory conditions, it is very likely that an industrial scale optimization will significantly improve this value.

For the microalgae filtration the best results were obtained when testing PE5 membrane, resulting in the permeability of 4.2 ± 0.1 L h^{-1} m^{-2} bar^{-1}, n = 2. A similar, but slightly lower value was obtained with PAN50 (3.9 ± 0.1 L h^{-1} m^{-2} bar^{-1}, n = 2), while the ABS membrane gave a value of 0.5 ± 0.3 L h^{-1} m^{-2} bar^{-1}, n = 2. Again, in the case of self-made membrane there is room for improvement in terms of permeability and although the microalgae permeability with non- optimized ABS membranes is around seven times lower than with commercial membranes, the cost of the ABS polymer is three orders of magnitude cheaper than other polymers like PAN [22]. Therefore, considering the differences between the membrane cost and the final cost reduction target, the permeability results make the ABS membrane become very competitive.

To calculate the total and the irreversible fouling, water permeability with membranes after microalgae filtration and system cleaning was measured. In terms of total fouling, PAN50 membrane resulted in the highest volumetric flow reduction (VFR, ratio between the microalgae and water permeability), followed by PE5 and ABS membranes (95.6% PAN50, 84.0% PE5 and 63.8% ABS). This means that the self-made material had the most resistant surface for the fouling formation. Moreover, the ratio between water permeability before and after the experiment was measured to get the information regarding the irreversible fouling (IF) of the membranes. The results obtained show a similar performance of PAN50 (72.7%) and PE5 (73.6%). Once more, the ABS membrane gave the lowest value, 48.4%. This means that the fouling over the surfaces of all the membranes tested can be reduced after cleaning, which makes the use of ABS very viable.

Figure 3 shows the permeability results obtained for experiments with *Dunaliella tertiolecta* filtration using a dynamic filtration setup. The permeability with the microalgae culture as well as with water before and after the experiment for all the materials was measured.

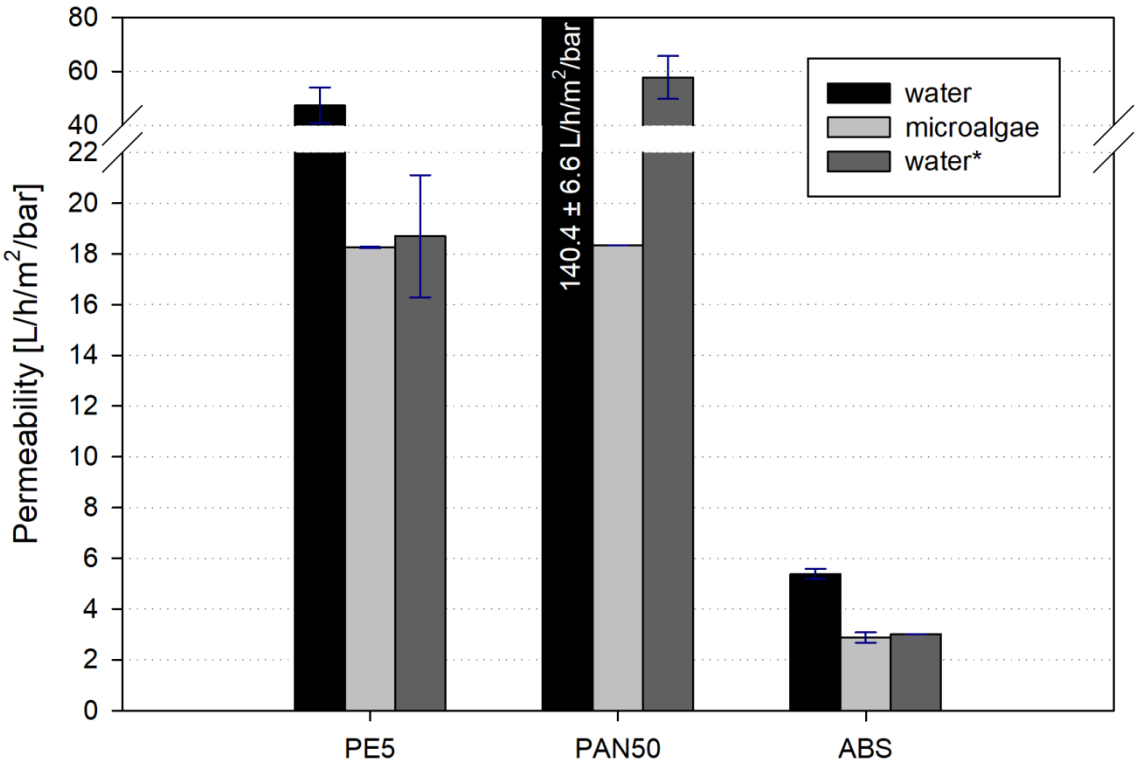

Figure 3: Permeability results for the dynamic filtration of Dunaliella tertiolecta: water permeability with the virgin membrane, microalgae culture permeability and water permeability after the experiment and with the cleaning procedure performed (n = 2). The error bars report standard deviation between measurements. PE5- commercial polyethersulfone membrane, PAN50 – commercial polyacrylonitrile membrane, ABS – own-made acrylonitrile butadiene styrene membrane.*

For the permeability of water, the tendency was similar to one of the experiments with the conventional technique. The highest water permeability was obtained with the PAN50 membrane before microalgae dewatering, reaching a value of 140 ± 20 L h^{-1} m^{-2} bar^{-1}, n = 2. PE5 resulted in a water permeability of 47 ± 7 L h^{-1} m^{-2} bar^{-1}, n = 2, and ABS performed with the result of 5.4 ± 0.2 L h^{-1} m^{-2} bar^{-1}, n = 2. All the results obtained are higher than with cross-flow filtration, which can be explained by the reduction of the primary membrane fouling thanks to the vibrational movement of the module. Again, water permeability differences between commercial membranes were those expected according to their MWCO, as explained above / as previously explained. For the synthesized membrane, water permeability was lower when compared to the commercially available materials for the same reasons which have been explained in the case of conventional cross-flow filtration.

Concerning microalgae permeability, the performance for all membranes was much greater with dynamic filtration than with the conventional technique. The ratio between permeability results (dynamic/crossflow) within all the materials tested ranged from 4.3 for PE5 membrane, 4.8 for PAN50 membrane and up to 5.3 for ABS membrane.

These results indicate that, in terms of total and irreversible fouling, a technical and an economic improvement of the process was achieved, considering that the additional energy demand in the system for vibration is less than 10% of the system energy requirement [26]. Comparing the performance of the commercial membranes with this technology, results showed that independently of the differences in the MWCO, a similar permeability of microalgae sludge was obtained in both cases (18.3 L h^{-1} m^{-2} bar^{-1}). This is a great improvement compared with the results reached with the cross-flow filtration setup. Moreover, for PE5 and ABS membranes, the results indicated that permeability with microalgae sludge was close to permeability with water, which means low volumetric flow reductions.

3.2.2 Pilot scale experiments with dynamic filtration focused to maximize final sludge concentration

Pilot scale experiments with dynamic filtration were performed to substantially increase the final microalgae sludge concentration and to check the performance of the operation as the concentration of biomass increases. The initial volume of *Dunaliella tertiolecta* was of 38 L with the culture concentration of 1.1 g/L. Figure 4 presents the permeability results obtained for experiments of maximum concentration of *Dunaliella tertiolecta* culture using PE5 and PAN50 commercial membranes and dynamic filtration setup. The permeability with microalgae culture as well as with water before and after the experiment was measured.

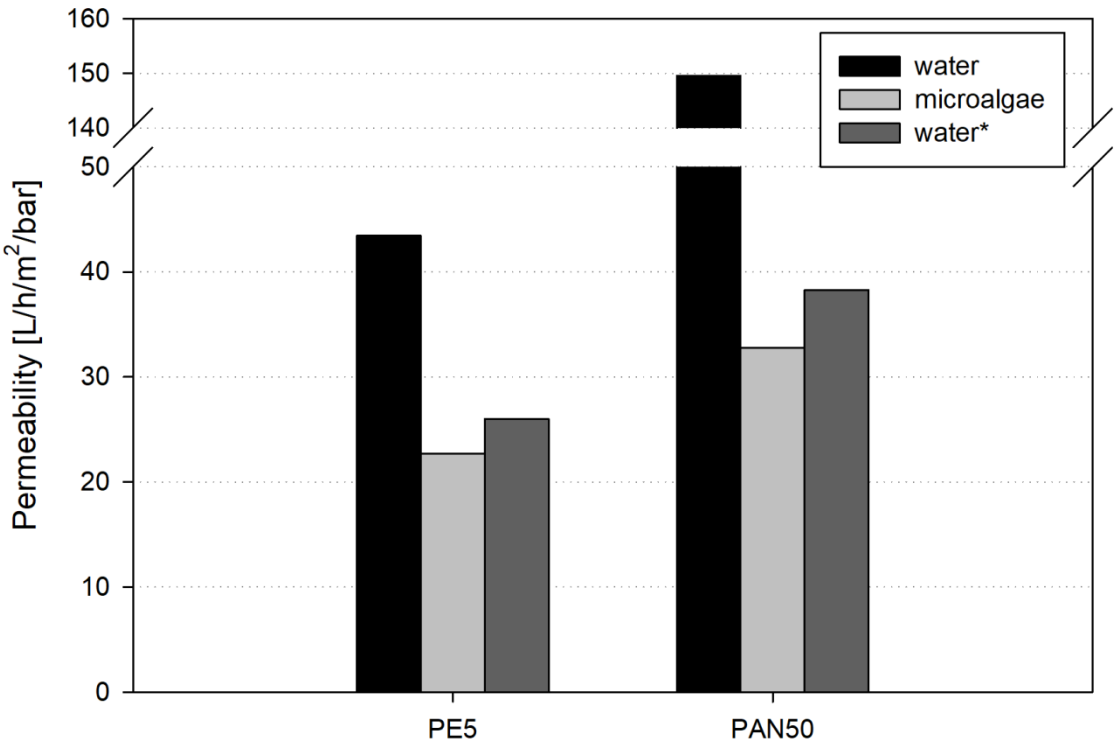

Figure 4: Permeability results for experiments of maximum concentration of Dunaliella tertiolecta: water permeability with the virgin membrane, microalgae culture permeability and water permeability after the experiment and with the cleaning procedure performed (n = 1). PE5 - commercial polyethersulfone membrane, PAN50 – commercial polyacrylonitrile membrane.*

For the permeability of water before microalgae concentration experiments, the results for both materials were similar as in the previous study (section 3.2.1), thereby giving values of 43.4 L h^{-1} m^{-2} bar^{-1}, n = 1, with PE5 and 149.5 L h^{-1} m^{-2} bar^{-1}, n = 1, with PAN50. With the microalgae sludge, although much larger volumes were filtered, in terms of permeability, both membranes maintained a similar performance as previously noted, resulting in values of 22.7 L h^{-1} m^{-2} bar^{-1}, n = 1, for PE5 and 32.7 L h^{-1} m^{-2} bar^{-1}, n = 1, for PAN50. Moreover, when comparing permeability with microalgae sludge to permeability with water after concentration, similar results were obtained. In terms of fouling, PAN50 membrane resulted in the VFR of 78.1% and the IF value of 74.4%. In the case of PE5 membrane, the VFR was of 47.7% and the IF of 40.1%. This means that in dynamic filtration the volumetric flow reduction does not depend on either the volume of the filtrated sludge or the duration of the experiment.

The volumetric concentration factor (VCF) for those experiments was calculated and based on the initial and final volume of the microalgae sludge. The final volume of the concentrate after the filtration was of 3.4L, which was equal to the dead volume of the equipment. Considering that total microalgae rejection was obtained, which was confirmed by optical density measurements of the permeate samples, a final VCF of 11.2 was obtained which resulted in a sludge concentration of 12.3 g/L.

3.3 Sedimentation combined with dynamic filtration

Figure 5 describes the procedure followed in the experiments of sedimentation combined with dynamic filtration. To cause the sedimentation of microalgae, the pH change for *Dunaliella tertiolecta* and *Chlorella sorokiniana* was induced by adding the NaOH solution to the microalgae cultures. The sedimentation of *Dunaliella tertiolecta* was obtained with a lower pH value than in the case of *Chlorella sorokiniana*, however the initial value for both cultures also varied. For *Dunaliella tertiolecta* the pH required an increase from 8.7 to 9.5 to obtain a good flocculation, while for *Chlorella sorokiniana* the required final pH value was 11.7, starting from 9.5.

Figure 5: Scheme of the steps in sedimentation combined with dynamic filtration experiments

Table 2: Concentrations of the initial culture of microalgae, the clarified, sedimentate/feed, permeate and concentrate in the experiments of sedimentation combined with dynamic filtration

Microalgae specie	**Concentration [g/L]** (i*n all results n = 2)*				
	Initial culture	**Clarified**	**Sedimentate/ Filtration Feed**	**Permeate**	**Concentrate**
Dunaliella tertiolecta	0.89 ± 0.01	0.38 ± 0.01	13.26 ± 0.04	0.00 ± 0.00	184.58 ± 0.04
Chlorella sorokiniana	0.12 ± 0.01	0.01 ± 0.00	3.52 ± 0.02	0.00 ± 0.00	29.43 ± 0.03

Table 2 shows the concentrations of the microalgae during the different stages of the sedimentation/filtration experiments. The final concentration of *Dunaliella tertiolecta* was 184.58 ± 0.04, n = 2 g/L with 81.5% of water content in a suspension. This concentration is high enough for further treatments, such as steam explosion cell disruption without any intermediate operation. This means there is no need for centrifugation or any other concentration technique, thereby resulting in a significant cost reduction of the harvesting step.

To obtain the total concentration factor (TCF) for those experiments, the ratio between the initial culture concentration and the concentration of the final sludge was calculated. With *Dunaliella tertiolecta* the TCF reached the value of 207.4. For *Chlorella sorokiniana* the TCF obtained was 245.3. Even though those results are already fully satisfying, they are not the highest to be obtained. If some limitation of the laboratory equipment could be overcome, the resulting TCF could be even higher. For instance, the initial concentration of *Dunaliella tertiolecta* was over 7.4 times higher than in case of *Chlorella sorokiniana*. The ratio between the final concentrations of both species was maintained considering that the concentrate of *Chlorella sorokiniana* was 6.9 times more diluted than the *Dunaliella tertiolecta* one. However, because of the low initial concentration of *Chlorella sorokiniana* and the equipment limitations a higher concentration of the final concentrate was impossible to reach. Another limitation was the volume to be used in the laboratory scale equipment. If in considering that there was no such limitation in terms of the initial volume and the internal volume of the equipment used, a much higher TCF could be obtained and achieve the limitation of high microalgal sludge viscosity.

In order to calculate the concentration factor after sedimentation, absorbance measurements were the chosen technique, since a certain amount of microalgae cells was still present in the liquid phase after the flocculation. Optical density concentration factor (ODCF) after sedimentation was calculated based on the absorbance measurements of the initial culture and collected sedimentate (Table 3). In the case of *Dunaliella tertiolecta* the ODCF was 14.9, while for *Chlorella sorokiniana* the ODCF reached the value of 29.2.

Table 3: Optical density of microalgae before and after pH-induced sedimentation

Microalgae specie	**Optical density** (i*n all results n = 4)*		
	Initial	**Clarified**	**Floc**
Dunaliella tertiolecta	0.08 ± 0.01	0.05 ± 0.02	1.19 ± 0.41
Chlorella sorokiniana	0.25 ± 0.02	0.02 ± 0.01	7.30 ± 0.63

After the sedimentation was completed, the clarified liquid phase was separated and the floc was collected for further filtration. The filtration was performed with the dynamic filtration setup and PE5 commercial membrane. The filtration was continued until the maximum volume of permeate was reached (3.4L of the dead volume of the equipment). Total microalgae rejection (no microalgae detected in the permeate) was confirmed by the absorbance measurements of the permeate samples.

Table 4: Volumes of the initial culture of microalgae, the clarified, sedimentate/feed, permeate and concentrate in the experiments of sedimentation combined with dynamic filtration

Microalgae specie	**Volume [L]** *(in all results n = 5 with interval of confidence < 0.0)*				
	Initial culture	**Clarified**	**Sedimentate/Feed**	**Permeate**	**Concentrate**
Dunaliella tertiolecta	1200	1152.7	47.3	43.9	3.4
Chlorella sorokiniana	900	871.6	28.4	25.0	3.4

After the filtration of sedimented microalgae, the volumetric concentration factor was calculated, as the total microalgae rejection was confirmed (***Table 2*** and Table 4). In the case of *Chlorella sorokiniana* the VCF reached a value of 8.4, while for *Dunaliella tertiolecta* the VCF was 13.9. The value obtained with *Dunaliella tertiolecta* in this experiment was similar to the one reached in the maximum concentration study (VCF = 11.4). As mentioned above, higher VCF could be obtained if working with bigger initial volume of microalgae culture.

4 CONCLUSIONS

The results presented in this work show how the use of dynamic membrane filtration is recommended for *Dunaliella tertiolecta* dewatering over conventional tangential cross-flow filtration. The undesired issues of cake formation and pore blocking were overcome by using dynamic filtration, which led to much higher membrane permeability.

When performing membrane filtration for this application, the use of ABS membranes is also recommended as total microalgae rejection and membrane stability are achieved. ABS material is three orders of magnitude cheaper than the commercially available membranes. Thus, a reduction of operational cost can be achieved in industrial operation if this type of membrane is used instead of traditional membranes manufactured with high-grade polymers such as polysulfone and polyacrylonitrile. The next step in this direction is to optimize the industrial production of such types of membranes to achieve proper permeability.

In the microalgae harvesting step, significant energy and cost reductions can be achieved by combining flocculation with membrane filtration. This is because pH induced sedimentation combined with dynamic filtration for *Dunaliella tertiolecta* and *Chlorella sorokiniana* permits reaching a high concentration without using centrifugation. This could lead to concentrations high enough to proceed to cell disruption without the need of further operations. In the pilot scale experiments described in this work, the concentration factors reached were 205 and 245 for the studied strains. They can still be increased, since the limitation in this case was the availability of initial volume (due to equipment sizing) but not to technical issues like the viscosity.

The conclusions obtained in this work are especially transcendent since pilot scale experiments were successfully completed, reaching a high concentration by combining sedimentation + membrane filtration and thereby avoiding the use of centrifugation. This proof-of-concept can set the basis for pre-industrial tests of such a harvesting procedure.

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Statement of Informed Consent, Human/Animal Rights. No conflicts, informed consent, human or animal rights applicable.

Contributions

Ms. Hapońska, Dr. Clavero, Prof. Salvadó, Prof. Farriol and Dr. Torras substantially contributed to the conception and design of the study, acquisition of data and analysis and interpretation of it. Ms. Hapońska, Dr. Clavero, Prof. Salvadó, Prof. Farriol and Dr. Torras substantially contributed drafting the article and revising it critically for important intellectual content. Ms. Hapońska, Dr. Clavero, Prof. Salvadó, Prof. Farriol and Dr. Torras approved the version submitted.

All authors agree to authorship and to the submission of the manuscript for peer review.

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