BIOREFINING OF MICROALGAE: FROM HARVESTING TO BIOFUEL PRODUCTION

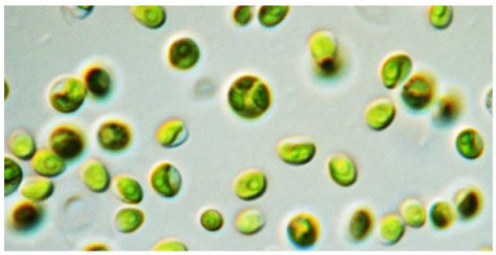
Monika Haponska

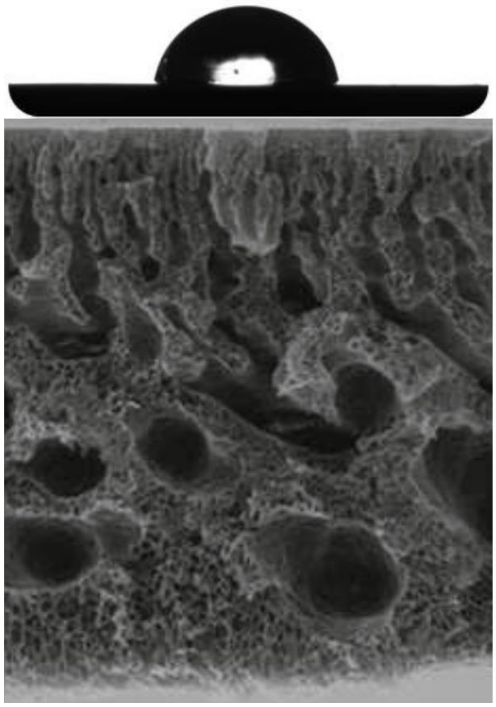
UNIVERSITAT ROVIRA i VIRGILI

BIOREFINING OF MICROALGAE:

FROM HARVESTING TO BIOFUEL PRODUCTION

Monika Hapońska





DOCTORAL THESIS

TARRAGONA 2018

UNIVERSITAT ROVIRA I VIRGILI
BIOREFINING OF MICROALGAE: FROM HARVESTING TO BIOFUEL PRODUCTION
Monika Haponska

Monika Hapońska

BIOREFINING OF MICROALGAE:
FROM HARVESTING TO BIOFUEL PRODUCTION

DOCTORAL THESIS

Supervised by

Prof. Joan Salvadó Rovira

Dr. Carles Torras Font

Department of Chemical Engineering

Tarragona, 2018

UNIVERSITAT
ROVIRA i VIRGILI

UNIVERSITAT ROVIRA I VIRGILI
BIOREFINING OF MICROALGAE: FROM HARVESTING TO BIOFUEL PRODUCTION
Monika Haponska

UNIVERSITAT ROVIRA i VIRGILI

Departament d'Enginyeria Química

Av. Països Catalans, 26

43007 Tarragona (Spain)

I STATE that the present study, entitled "Biorefining of microalgae: from harvesting to biofuel production", presented by Monika Hapońska for the award of the degree of Doctor, has been carried out under my supervision at the Department of Chemical Engineering of this university.

Tarragona, 11 May 2018

Doctoral Thesis Supervisors

Prof. Joan Salvadó Rovira

Dr. Carles Torras Font

UNIVERSITAT ROVIRA I VIRGILI
BIOREFINING OF MICROALGAE: FROM HARVESTING TO BIOFUEL PRODUCTION
Monika Haponska

ACKNOWLEDGEMENTS

And the day has come! You cannot even imagine how happy I am to write these words. After a few years of work, I can finally give thanks to people who make all this possible.

Firstly, I would like to thank my supervisors, Dr. Carles Torras and Prof. Joan Salvadó, for their support, patience and constant motivation. At the very beginning of my PhD study I was told that in research you can plan the path to reach the objectives, but you cannot be sure if at the end you reach your goals or not. Although there were some moments of doubts, thanks to your guidance, encouragement and the knowledge you decided to share with me I was able to successfully accomplish the planned work and write this thesis.

I would also like to thank my colleagues, Ester Clavero, Esther Lorente, Francesc Valls, Sònia Abelló, César Berrueco, Jordi Plana, Javier Recari, Claudia Nurra, Lídia Arce, Francisco Belio, Elena Fuentes, David Waddicor and all the people with whom I had a pleasure to work with. I can say that with your help there is no problem that cannot be solved! It means a lot to me that you were always so supportive, in professional as well as in a personal stuff.

I want to thank my family and friends. Thank you for believing in me even when it was hard for me to believe in myself. Thank you for supporting me even when I was thousands of kilometers away from home. You are always there for me, no matter what happens I know I can count on you. Thank you for allowing me to grow up into the person I am today. I know I would not be who I am now without you!

Monika

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ABS – acrylonitrile butadiene styrene

CA – contact angle

CMR – catalytic membrane reactor

DMA – N,N-dimethylacetamid

IMRCF - inert membrane reactor with catalyst on a feed side

IPA - isopropanol

MF – microfiltration

MWCO – molecular weight cut-off

NMP – 1-methyl-2-pyrrolidinone

OD – optical density

ODCF – optical density concentration factor

PAN – polyacrylonitrile

PBR – photobioreactor

PES – polyethersulfone

SEM – scanning electron microscope

SrO – strontium oxide

TCF – total concentration factor

UF – ultrafiltration

VCF – volumetric concentration factor

VFR – volumetric flux reduction

VSEP – vibratory shear enhanced process

SUMMARY

This thesis focuses on the modernization of the downstream process of microalgae biorefining by membrane technology. In particular, the project concerns the optimization of the following: harvesting, cell disruption, carbohydrates, proteins and lipids fractionation and development of catalytic membrane reactor for transesterification in order to obtain biodiesel. Cost reduction of the overall process can be achieved by finding cheaper and better solutions for each step.

In order to reach the objectives, the following studies have been performed:

(I) Preparation and application of new cheap polymeric membranes for the microalgae dewatering using conventional cross-flow filtration and novel dynamic filtration technique

(II) Combination of sedimentation and dynamic filtration for microalgae harvesting

(III) Steam explosion cell disruption combined with membrane filtration as a novel technique for the microalgae fractionation

(IV) Application of water-free technologies for the transesterification combined with membrane separation for biodiesel production.

In the first stage the filtration using own-made ABS polymeric membranes as well as the commercially available ones was carried out in order to check their performance for microalgae dewatering. This study included ABS membranes preparation and characterization using different techniques. Also, the comparison of two filtration methods, cross-flow and dynamic was performed to compare the viability of membranes affected by a fouling and a cake formation.

In a second stage, the pilot scale dewatering of two microalgae specie, *Chlorella sorokiniana* and *Dunaliella tertiolecta* by sedimentation followed by dynamic filtration was performed. The objective of the combined process was to reduce microalgae dewatering costs since sedimentation offers a very cheap operation and membrane filtration offers total rejection with high final concentrations at a lower cost than centrifugation.

In a third stage cell disruption and fractionation for lipids, sugars and proteins recovery was studied. Acid-catalysed steam explosion, cross-flow and dynamic membrane filtration were used as unit operations. Several microalgae species with different cell wall characteristics were tested. The aim of this work was to improve microalgae biorefining downstream process.

In the fourth stage the comparison of novel catalytic and inert membrane reactors for biodiesel production with strontium oxide as a heterogeneous catalyst was performed. The main objectives were to identify a proper catalyst, to choose the proper immobilization technique, to establish the membrane with the adequate pore size and to control the reaction and separation process.

1

INTRODUCTION

This chapter aims to introduce a research on membrane filtration for microalgae biorefinery. Recently this technology is developing fast and opening new possibilities on many industrial fields, including dewatering and separation of microalgal biomass. The main goal of this work was to improve the overall process of microalgae treatment from the harvesting step through cell disruption to transesterification for biodiesel production. This chapter is addressed to the motivations, scope and objectives of this investigation.

1.1.Motivation

This thesis was inspired by the growing need to find the alternative food and energy sources. The increasing demand for energy consumption leading to the end of an era of fossil fuels requires extensive study in order to create unconventional solutions. Combination of novel methods for microalgae processing and membrane technology is very promising and interesting field to be studied.

1.2. Thesis scope

The scope of this thesis was to improve the microalgae biorefining process using novel technologies and applying ones that are not so common in this domain. Multidimensional improvement was expected to be achieved in the dewatering, fractionation, separation as well as in the transesterification step in biodiesel production.

1.3. Hypothesis

Modernization of the following steps in the downstream process of microalgae biorefining will provide optimization of biodiesel production:

- Dewatering of microalgae
- Lipid extraction from microalgal cells
- Transesterification of lipids for biodiesel production

Regarding the dewatering step:

- Vibrating membrane filtration is a technique that reduces fouling, which is the main problem in this field. Modification of ABS membranes will provide a cheap material with high performance.

Regarding the lipid extraction step:

- Cell disruption by steam explosion makes the lipids accessible for extraction and due to prehydrolysis of carbohydrates can also be used as pretreatment for biogas production
- Separation of another products reached in this step leads to improvement in economic viability of the overall process

Regarding the transesterification process:

- Novel membrane reactor with heterogeneous catalyst will improve the homogeneous transesterification process

1.4. Objectives

- To optimize dewatering step with dynamic concentration using cheap membrane materials
    - Preparation and characterization of acrylonitrile butadiene styrene membranes
    - Comparative studies of microalgae dewatering with commercial and synthesized membranes
    - Comparison of membrane performance with conventional cross-flow filtration technique and with novel dynamic filtration
    - Combination of pH-induced sedimentation with dynamic filtration of two microalgae specie
- To evaluate the fractionation method of products obtained via steam explosion process used for cell disruption.
    - Direct separation of disrupted microalgae cells with different membranes in cross-flow and dynamic filtration process
    - Separation of disrupted microalgae cells after lipid extraction
- To optimize the transesterification step
    - To evaluate the proper technique for catalyst immobilization into the membrane structure and apart with commercially available materials
    - To provide the tests with commercially available membranes and self-prepared ones
    - To study and compare the conventional CMR and the novel IMRCF

- To characterize the membrane materials and study their interaction with catalyst
- To characterize the composition of fatty acid methyl esters produced

1.5. Document description

Chapter 1 contains of an overall introduction and the state of the art of microalgae treatment and biodiesel production. This chapter plays a significant role for a reader since gives a general idea about the current situation in the biorefinery and how important and needed the R&D is in this field. It also explains the fundamental background of presented work. **Chapter 2** describes the use of dynamic filtration with membranes manufactured from acrylonitrile butadiene styrene polymer for dewatering of *Chlorella sorokiniana*. **Chapter 3** focuses on the application of pH-induced sedimentation and dynamic filtration for microalgae *Chlorella sorokiniana* and *Dunaliella tertiolecta* concentration at pilot scale. **Chapter 4** deals with microalgae *Nannochloropsis gaditana* fractionation using combined steam explosion, vibratory and tangential cross-flow membrane filtration. **Chapter 5** includes the description of study regarding three microalgae specie *Nannochloropsis gaditana*, *Chlorella sorokiniana*, and *Dunaliella tertiolecta* treated with steam explosion and dynamic filtration in order to improve the processing cost of cell disruption and fractionation. In **Chapter 6** the comparison of novel catalytic and inert membrane reactors for biodiesel production with strontium oxide as a heterogeneous catalyst is described. As a final point, general conclusions and possible future work of this thesis are presented. All the chapters have been written as separate publications and can be read independently.

1.6.Background

The vision of decreasing amount of fossil fuels on the Earth leading to the inevitable end of an era forced researchers to look for the alternative energy sources. (1) Although the sustainable substitutes such as hydroelectricity, solar and wind energy, wave and tidal power or geothermal energy are able to produce some clean electricity, the biomass and renewable fuels are those alternative energy sources with the sufficient potential to fulfill all the energy needs. (2)

The major benefit of considering microalgae as a bio-based crop is their capability of converting solar energy into biomass. The quantum efficiency of this process ranges from 2% to 10%, while that of terrestrial plants is lower than 1%. Moreover, the microalgae growth rate can reach up to 1–3 times per day and because of their ability of accumulating lipid levels higher than 50% of their dry cell weight, microalgae are considered as a proper feedstock for biodiesel production. (3,4)

Nonetheless in the terms of industrial scale production the microalgae processing still require modernization and cost reduction. Figure 1.1 presents the scheme of microalgae biorefinery from the cultivation step to the biodiesel production. To improve the economic aspect of large-scale microalgae processes, the advantage has to be taken of all the possible components, thus needing a multiproduct biorefinery. (5) In this case the downstream processing of microalgae is too expensive, generating the costs of 50-60% of the total production costs, while the cost of products from other bulk industrial biotechnology downstream processes accounts for 20-40%. (6) The cost reduction can be reached when simplifying the main steps of the process (Figure 1.1) and finding proper mild technologies to access different fractions (proteins, carbohydrates, lipids).

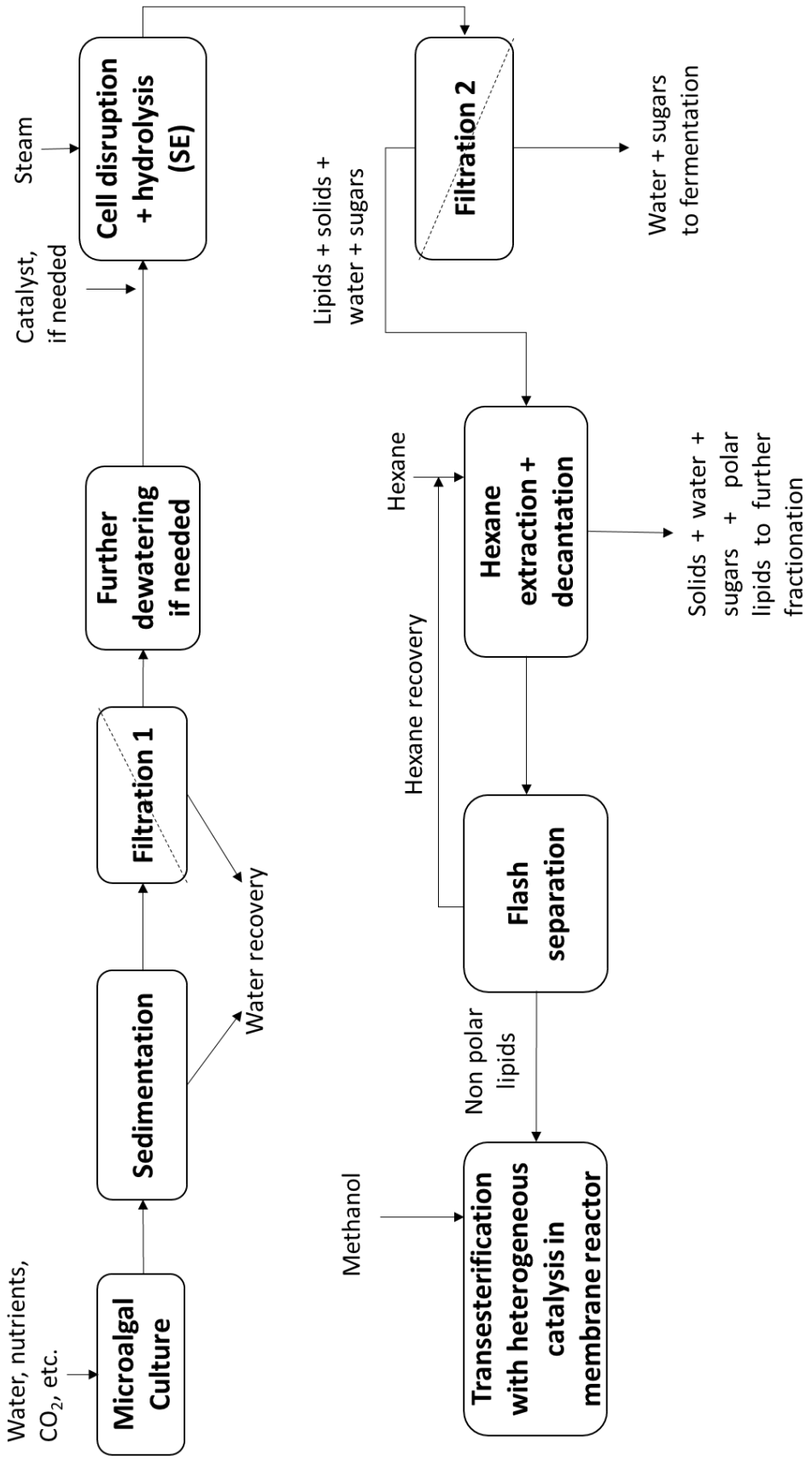


Figure 1.1: **Proposed scheme of microalgae biorefinery.**

Due to the high water content, the first step after microalgae cultivation is harvesting. There are several techniques used in order to reduce processing of large volume of suspension and may include one or combine more stages of physical, biological or chemical methods in order to reach the desired concentration. (7) The common practice for microalgae harvesting is a two-step separation, which contains of the thickening phase (the culture is concentrated to 2–7% of entire suspended solids) and the dewatering phase (the concentration reaches 15–25% of total suspended solids). The thickening can be performed using the following methods: coagulation/flocculation, gravity sedimentation, flotation and electrical based techniques. (8,9) Among the others, the cheapest is flocculation followed by gravity sedimentation. It can remove the majority of the water volume from the suspension. This technique, although well developed in the water treatment field, is not so well-established for the harvesting of microalgae.(10) The possibility of autoflocculation as a natural formation of flocs arises as an effect of the precipitation of carbonate salts together with algal cells at high pH. The pH value may change as a consequence of photosynthetic CO_2 consumption, but it can also be increased by adding an alkali to the suspension. (11) Once the thickening phase is finished, the concentration of microalgae is still too low to continue with the downstream processing. The second phase, the dewatering, can be performed using either centrifugation or filtration. The use of centrifuges offers many advantages: the biomass doesn’t contain flocculants or chemicals and high concentration can be achieved fast and easily. However, at a pilot scale, the use of centrifuges affects significantly the capital costs, which increase with a scale. Moreover, high gravitational and shear forces may damage the cell structure causing the loss of the valuable materials. In general, the maintenance of the centrifuges is related with high

expenses and the process itself is considered energy intensive (at 8 kWh/m^3 of microalgae suspension with a feed rate of 1 L per minute). All these parameters make the separation by centrifugation expensive. (12) The promising alternative is dewatering of microalgae by filtration. Filter presses working under pressure or vacuum can operate with several types of filters. The use of conventional materials is not suitable for all the microalgae specie due to the variety of cells dimension. (13) The novel solution is to apply the membrane technology, which already meets the demand for a variety of commodities including water, food and energy as well as in wastewater treatment. Membrane separation selectively permits for the mass transfer from one phase to another, typically forced by pressure, concentration, electrical or chemical potential gradient. (14) Membranes can be classified due to the pore size: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO). They can also be categorized by the fabrication material (zeolite, organic, inorganic) or configuration (spiral-wound, fiber, tubular). The diversity in the membranes properties leads to the list of advantages which includes the ease of scaling up, no chemical additives, low costs of the operation and maintenance, compact and modular design, automated and continuous operation allowing at the same time for a selective separations. (15) In order to determine the membrane performance two key parameters should be considered: permeability and rejection. The permeability quantifies the ability of membrane to let the permeate pass and the rejection gives the quantitative value of the capability to reject certain compounds/particles. Certain factors affect the transport of a solute across the membrane: the solution temperature, viscosity, mixing rate as well as particles shape, charge and size. (16) All of those parameters can be a reason of fouling – the phenomenon caused by pore blocking and cake formation over the membrane surface. The conventional filtration methods, such as cross-flow filtration, result in a significant permeability reduction with time,

mainly due to the fouling problem. To overcome this issue the membrane shear stress and turbulence can be increased when working with dynamic filtration. (17) There are several types of dynamic filtration: with vibrating or rotating membranes as well as with rotors between fixed membranes. Although some research has been done using dynamic filtration for microalgae dewatering, a lot of parameters still need to be checked. (18) More detailed description regarding the harvesting step can be found in Chapters 2 and 3.

The next step of microalgae downstream process is cell disruption followed by lipid extraction. The cell wall of some microalgae specie is too thick to allow the direct extraction of all the lipids using organic solvents hence cell disruption needs to be performed. The methods of cell disruption include high-pressure homogenization, bead milling, hydrodynamic cavitation, microwave/ultrasonic/ pulsed electronic field treatment, steam explosion, as well as solvent, osmotic shock, ionic liquid, surfactant, algicidal and hydrolytic enzyme treatment. (19) Previous study showed that sonication, microwave radiation and steam explosion are suitable for large-scale operations while other mechanical methods as well as freeze-drying, autoclave and enzymatic pretreatment are not effective because of high cost and longtime operation, high maintenance costs and the difficulties with a scale up. It was also showed that steam explosion being environmentally friendly results in good cell wall breaking and high content release with relatively low operational cost. (20,21) Once the cell content is released, further separation of different fractions is necessary. After the cell disruption the following phases are obtained: solid phase, aqueous phase with sugar dissolved and another liquid phase containing lipids. Normally, an emulsion can also be found in the mixture. (22) Depending on the final product wanted, different recovery paths can be applied: an extraction

with supercritical CO_2 at high pressure for the high value products, an extraction with non-polar organic solvent for upper scale extraction procedures or a fractionation by mechanical separation. (23) For the last position mentioned, membrane filtration can be considered as a novel and interesting solution for this application since can lead to cost reduction and simplification of the process. Comprehensive description of this topic can be found in the introduction to the Chapters 4 and 5.

After fractionation of microalgal cells content the scope of interest of biorefinery downstream industry is the lipid phase for biodiesel production. There are several processes that can be used for biodiesel production such as micro-emulsion, blending, catalytic cracking or transesterification. The most commonly used technique, the transesterification, involves the methanolysis of triglycerides using catalyst in order to produce methyl esters and glycerol. To improve the reaction rate and the conversion of the products, the interfacial surface area has to be increased since the triglycerides and methanol phases are immiscible. (24) The catalysts used for the transesterification can be categorized in three groups: acids, alkalis and enzymes. Although using the enzymes can avoid the soap formation, the long reaction time and high cost discard them from commercial application. The acid and alkali catalysts, more common in the biodiesel production, can be subcategorized into homogeneous and heterogeneous. (25) Sodium or potassium hydroxides, being favorable economically (high conversion under low temperature and pressure), are the most popular catalyst in the industry. However, due to the soap formation, they generate additional costs and energy demand related to the washing step. The alternative is to use a heterogeneous catalyst instead of homogeneous one, which can be easily separated from the product, allows skipping the washing as well as the pre-

esterification steps. (25) The residual triglycerides, glycerol and free fatty acids should be removed during the process of biodiesel production. One of the possible approaches is the use of a membrane reactor for a products separation allowing the continuity of the operation. (26) A membrane with a proper molecular weight cut-off can separate the large oil droplets, which are unable to cross the barrier, from the FAME and methanol overcoming at the same time the equilibrium limitations. More insight into the transesterification subject is presented in the Chapter 6 of this thesis.

2

MICROALGAE DEWATERING BY MEMBRANE FILTRATION[1]

This chapter describes the use of dynamic membrane filtration with cheap membranes made of acrylonitrile butadiene styrene polymer for dewatering of Chlorella sorokiniana microalgae strain.

Dynamic and cross-flow filtration techniques were compared to study the membrane performance in terms of fouling and cake formation. Experiments were carried-out with different types of commercial membranes from different pore sizes and materials.

Synthesized membranes production methods and material characterization (scanning electron microscopy, contact angle and porosity measurements) as well as results from filtration experiments are presented in this chapter.

[1] This chapter is based on the following publication:
M. Hapońska, E. Clavero, J. Salvadó, C. Torras, *Application of ABS membranes in dynamic filtration for Chlorella sorokiniana dewatering,* Biomass and Bioenergy, Volume 111, 2018, Pages 224-231, ISSN 0961-9534, http://doi.org/10.1016/j.biombioe.2017.03.013

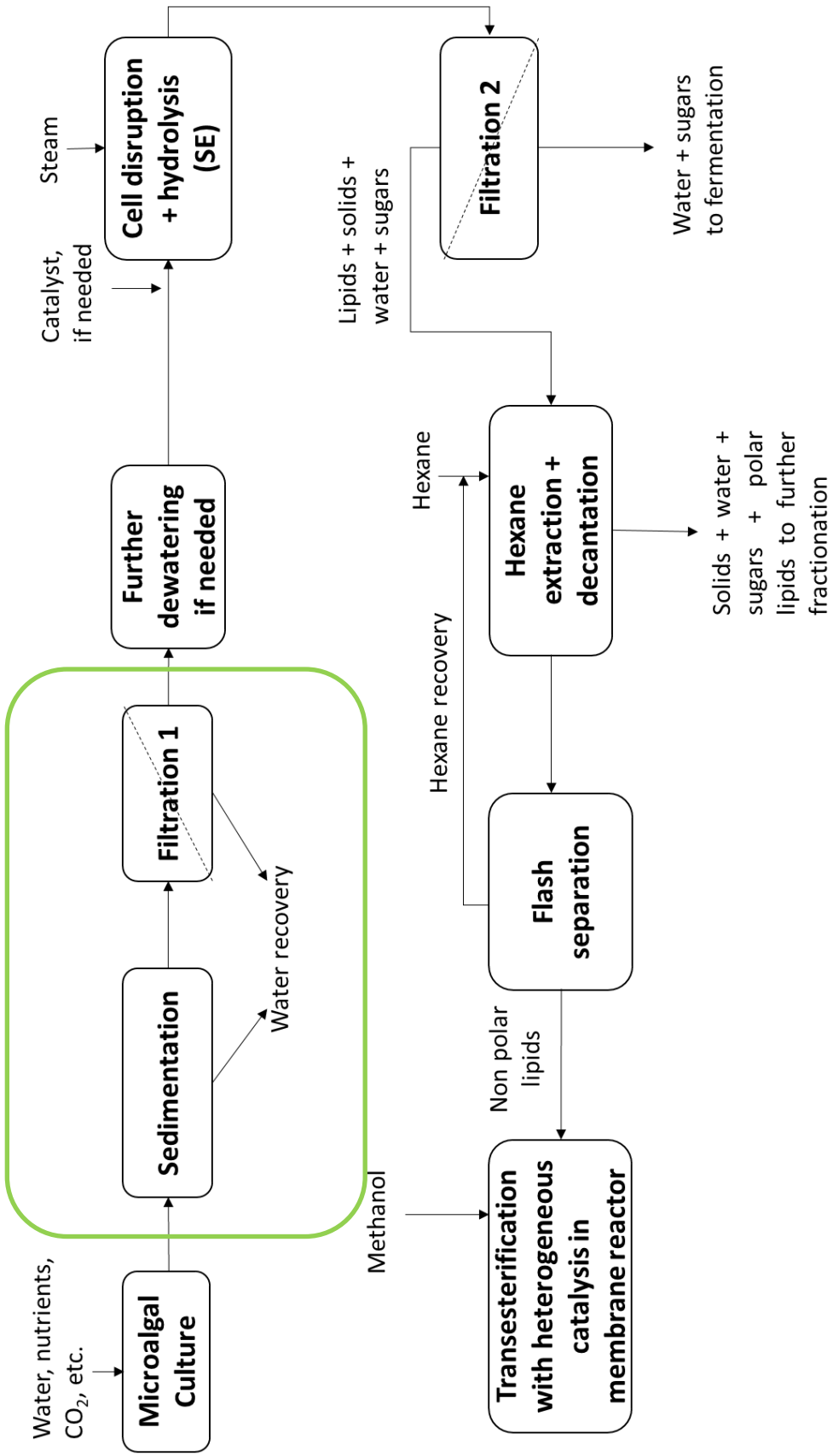


Figure 2.1: **Proposed scheme of microalgae biorefinery – dewatering.**

2.1.Introduction

Finding an alternative for nonrenewable energy sources became the objective of extensive studies. Because of its advantages over conventional fuels, its sustainability, biodegradability and suitability to use in existing diesel engines, biodiesel seems to be a proper substitute for petroleum diesel(27,28) (27,28). Microalgae with their unicellular structure can efficiently turn solar into chemical energy. Due to their ability to capture carbon dioxide, fast growth rate and high content of lipids, carbohydrates and proteins are considered as a competitive material for various industrial purposes (29,30).

Microalgae cell size allows for the application of membrane micro/ultrafiltration (MF/UF) for the dewatering purpose. The list of benefits in using membranes includes no chemical additives, simplicity in operation and low energy consumption (31). For the dewatering purpose, both polymeric and ceramic membranes can be used. Although ceramic membranes offer good performances in terms of flow and reproducibility, they are much more expensive than polymeric ones (32). Recent studies showed that membranes produced from cheap polymers, such as ABS, are promising materials which could be applied in the dewatering step for microalgae biorefining (33). Therefore, when using those cheaper membranes, a significant reduction in the costs of the overall process can be obtained.

The main disadvantage in microalgae MF/UF is fouling (32). Filtration of biological feeds results in additional difficulties due to the compressibility of the mass formed. Another factor that has a significant influence on the membrane performance is the increase in the feed concentration. In conventional cross-flow filtration, cake formation over the membrane surface and pore-blocking can result in up to 99% permeability reduction. Previous studies showed that fouling issues can be

minimized by using dynamic filtration, which increases turbulence and raises shear stress over the membrane surface (34). There are several types of commercially available dynamic filtration systems, like rotating cylindrical membranes, rotating disk systems and vibrating systems (35). Vibratory shear enhanced process (VSEP) was already successfully applied for the purification of drinking water, skim milk ultrafiltration, pervaporation as well as for baker's yeast microfiltration (36). It was also found to be a proper technique for microalgae dewatering (18,37). However, so far only commercial membranes have been used in the microalgae filtration experiments with VSEP.

When compared to other polymers, ABS is up to three orders of magnitude cheaper. Depending on the market, PES costs vary between 432 \$ kg^{-1} (GoodFellow) and 480 \$ kg^{-1} (Sigma Aldrich), PAN 375 \$ kg^{-1} (GoodFellow) and 1,850 \$ kg^{-1} (Sigma Aldrich), and ABS price is only 2.4 \$ kg^{-1} (Plasticker) (33). ABS polymers are highly resistant, have good thermal stability and durability (38). Due to their properties and low price, they are being commonly used in packaging industry, for toy production as well as for 3D printing (39–41). Although this material is so ubiquitous in everyday life, it is not so common in membrane industry. Some research with ABS membranes can be found in gas permeation studies (42–44). Preliminary studies with filtration of *Phaeodactylum tricornutum* were performed for ABS synthesized membranes, however only conventional cross-flow technique was used for this purpose (33).

The main aspect considered in this work was to combine vibrating filtration method with new cheap membrane materials for the dewatering of microalgae. *Chlorella sorokiniana* was used in dewatering with both conventional and dynamic filtration modules.

2.2.Microalgae biomass

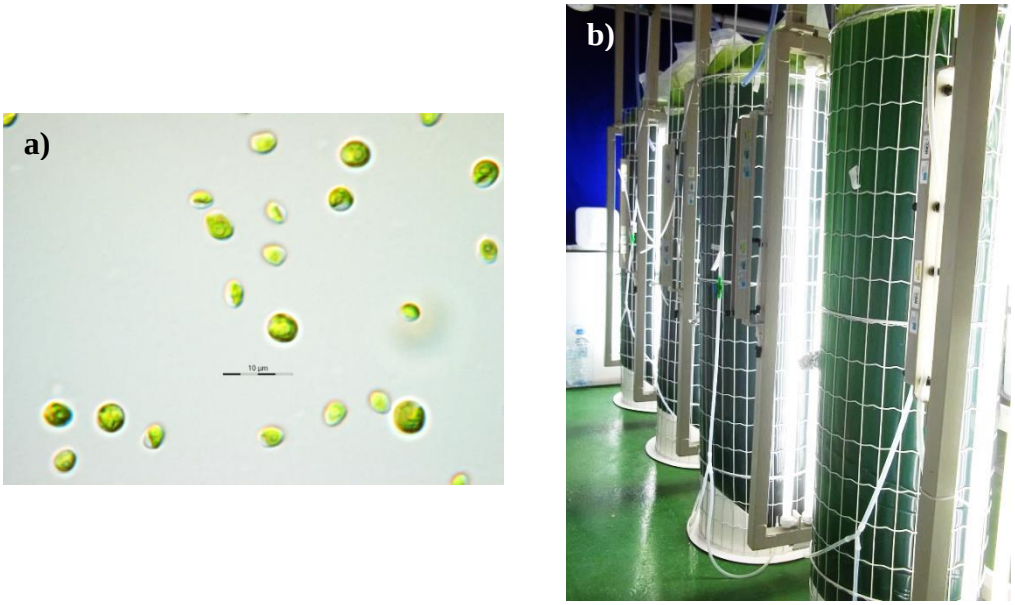


Figure 2.2: ***Chlorella sorokiniana*: a) optical microscope image; b) vertical photobioreactors cultivation.**

Experiments were carried out with the freshwater microalgae *Chlorella sorokiniana* Shihira & R.W.Krauss (strain CCAP 211/8K), a 2-5 µm spherical to ellipsoidal freshwater green unicellular alga. Dynamic filtration was performed with 300 L cultures whereas cross flow filtration was conducted with material from either 300 L cultures or 4 L cultures (Figure 2.2). Cultures were illuminated (16:8 light: dark cycle) with cool daylight fluorescents and kept at 24±2.5 °C. Four litre cultures were grown in five litre flasks (18 cm in diameter) with BBM3N3S medium (45) and aerated with air with 0.5% CO_2. They were illuminated with OSRAM L30W/865 fluorescents, which gave irradiance on the flask's surface of 200 µmol photon m^{-2} s^{-1}. 300 L cultures were grown in column photobioreactors (50 cm diam.) with tap water enriched with the following nutrients (in g m^{-3}): $NaNO_3$ ($5.00\cdot 10^{-4}$), $K_2HPO_4.3H_2O$ ($2.10\cdot 10^{-5}$), KH_2PO_4 ($3.75\cdot 10^{-5}$), Na_2EDTA ($1.67\cdot 10^{-5}$), $FeCl_3.6H_2O$ ($4.84\cdot 10^{-6}$), $ZnSO_4\cdot 7H_2O$ ($4.85\cdot 10^{-7}$), $MnCl_2\cdot 4H_2O$ ($8.87\cdot 10^{-7}$), $Na_2MoO_4.2H_2O$ ($2.46\cdot 10^{-8}$), $CuSO_4\cdot 5H_2O$ ($4.31\cdot 10^{-8}$) and $CoCl_2\cdot 6H_2O$ ($1.37\cdot 10^{-8}$).

Cultures were aerated with air and illuminated with Philips MASTER TLD 58W/865 giving irradiance on the photobioreactor surface of 300 µmol photon m^{-2} s^{-1}.

For the tests with concentrated microalgae biomass, retentate obtained from the vibratory dewatering of original culture was collected and used as a feed for further experiments.

2.3.Membranes

Experiments were performed with commercially available polymeric membranes and synthesized ones. The filtration area was 139 cm2 for conventional cross-flow filtration module and 446 cm2 for dynamic filtration module. In order to ensure total microalgae rejection, the main criterion for membrane selection was the molecular weight cut-off (MWCO), chosen according to Chlorella sorokiniana cell size. Commercial membranes PES5, PAN50 and PES20, listed in Table 2.1, were purchased from New Logic (United States).

Table 2.1: **Commercial polymeric ultrafiltration membranes used for the dewatering of microalgae.**

Membrane commercial names	Producer	Supplier	Material	Molecular weight cut-off
PES5	Sepro	New Logic	Polyethersulfone	7,000 Da
PAN50	Sepro	New Logic	Polyacrylonitrile	50,000 Da
PES20	Sepro	New Logic	Polyethersulfone	200,000 Da

For the synthesis of non-commercial membranes N,N Dimethylacetamid, DMA (≥99.5%, CAS 127-19-5), 2-Propanol, IPA (≥99.8%) and 1 Methyl 2 pyrrolidinone, NMP (anhydrous, 99.5%, CAS 872-50-4) were purchased from Sigma-Aldrich

(Spain). Acetone, for synthesis (BP, USP) was purchased from LABKEM (Spain). ABS copolymer Novodur P2H AT NR, kindly delivered by Styrolution (Spain), was employed with a density of 1050 kg m-3, processing temperature between 230 and 260oC and tensile stress at yield of 44 MPa.

2.4.Methods

2.4.1. Membrane synthesis

Polymeric membrane synthesis was performed via phase inversion precipitation with several polymer/solvent systems and different non-solvents in coagulation bath (Table 2.2).

Table 2.2: **Composition of synthesized polymeric membranes.**

Membrane	Polymer	Solvent	Concentration of polymeric solution [%]	Non-solvent	Temperature of coagulation bath [^{o}C, ± 5^{o}C]
M1	ABS	DMA	15	water	20
M2	ABS	DMA	20	water	20
M3	ABS	DMA	25	water	20
M4	ABS	DMA	30	water	20
M5	ABS	DMA	30	water	50
M6	ABS	DMA	15	IPA/water	20
M7	ABS	DMA	20	IPA/water	20
M8	ABS	DMA	25	IPA/water	20
M9	ABS	DMA	30	IPA/water	20
M10	ABS	NMP	15	water	20
M11	ABS	NMP	20	water	20
M12	ABS	NMP	25	water	20
M13	ABS	acetone	30	water	20

The polymer and the solvent were mixed and stirred using magnetic stirrer at room temperature for 72 h to obtain a homogenous polymeric solution. Afterwards, the solution was left for at least 24 h in order to remove all the bubbles from the bulk. The solution was deposited onto a glass plate using a casting knife with an adjustable thickness gap regulated by an incorporated micrometer. In all cases, the casting knife gap was adjusted to 200 µm, except for M5, where the gap thickness applied was 300 µm. It was necessary to obtain the membrane with good mechanical properties for the incorporation in the vibratory system. The casting knife was set in motion by an automatic film applicator with a constant traverse speed of 50 mm s-1 (BYK – Gardner Automatic Film Applicator, Figure 2.3).

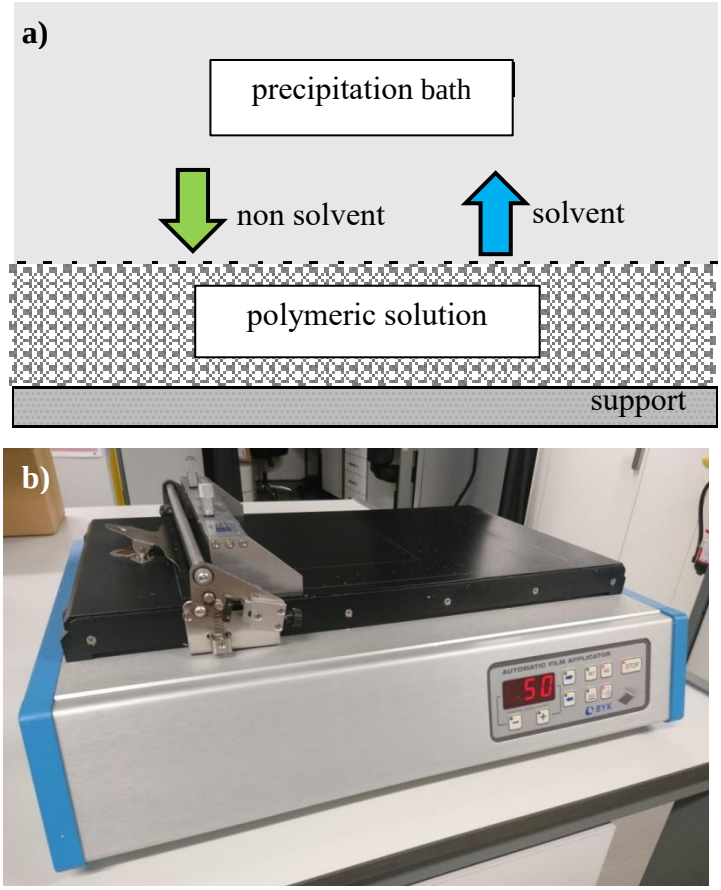


Figure 2.3: **Membrane preparation: a) phase inversion precipitation scheme; b) BYK - Gardner automatic film applicator.**

Immersion of the cast polymeric solution into a coagulation bath caused phase inversion precipitation, which resulted in the formation of a thin film. The temperature of the coagulation bath was fixed to 20 oC, ± 5 oC, except for M5, where the temperature was fixed to 50 oC, ± 5 oC, in order to produce a membrane applicable for use with dynamic filtration module.

2.4.2. Membrane morphology

Scanning electron microscope (SEM, JEOL JSM-6400 Scanning Microscopy Series with working voltage of 20kV) was used to study the cross-section and the surface of membranes. Samples were immersed first into ethanol, and afterwards into liquid nitrogen for freezing. This procedure allowed the membrane to be broken preserving the internal porous structure. Next, deposition of gold layer over the samples was performed using sputtering in order to induce conductive properties (46).

Porosity of materials was analyzed based on SEM images using membrane SEM micrographs interpretation software IFME (47).

2.4.3. Contact Angle

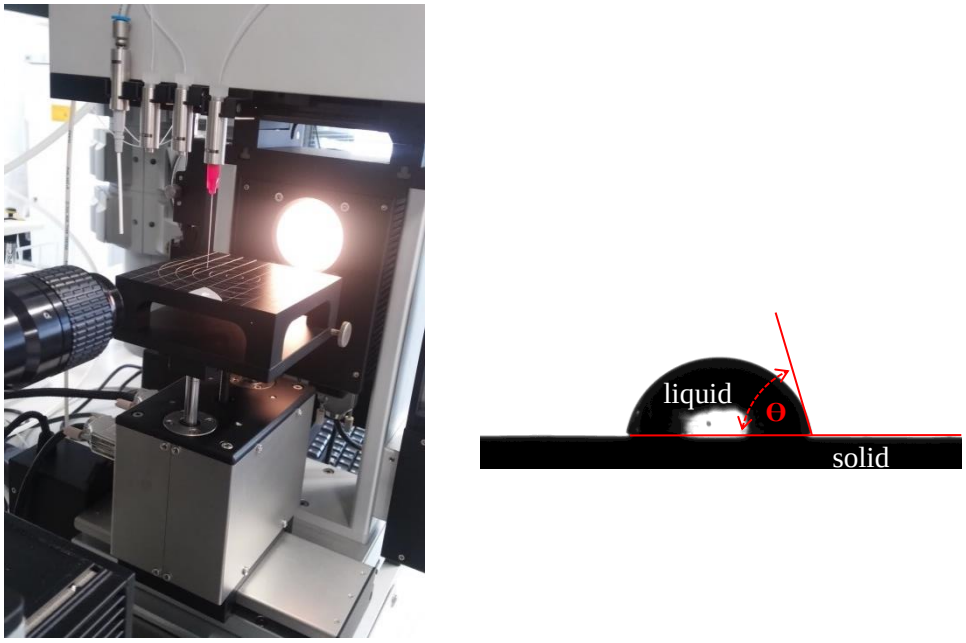


Figure 2.4: **Contact angle measurement equipment.**

Sessile drop technique with automatic video-based analysis system OCA 35 (Dataphysics, Figure 2.4) was used to measure membranes contact angles (CA, Θ). 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.

2.4.4. Permeability

The initial permeability of membranes was determined by water flux measurements. After that the filtration of microalgae biomass was performed. At the end, permeability for water was measured after cleaning the system. The last step allowed us to determine the irreversible fouling resistance of membranes. In the case of conventional cross-flow filtration distilled water was always used and for the experiments with vibrating set-up tap water instead of distilled water was used. This procedure in terms of water usage needed to be adjusted to the size of equipment and to the volume of liquid processed.

2.4.5. Optical density

Optical density (OD) was calculated from the results of absorbance measurements for feed, permeate and concentrate of microalgae dewatering. Absorbance was measured using a microplate reader (INFINITE M200 PRO, Tecan).

Absorbance was always read at concentrations in which the relation between absorbance and concentration maintained linearity. Therefore, if necessary, samples were adjusted to an absorbance below 0.4 and the resulting absorbance of the diluted sample was multiplied by the dilution factor. Finally, the absorbance data obtained from 96 well plates (path length of 0.5052 cm) were converted to OD values.

2.5. Equipment

Experiments were carried out using two filtration setups presented in the Figure 2.5.

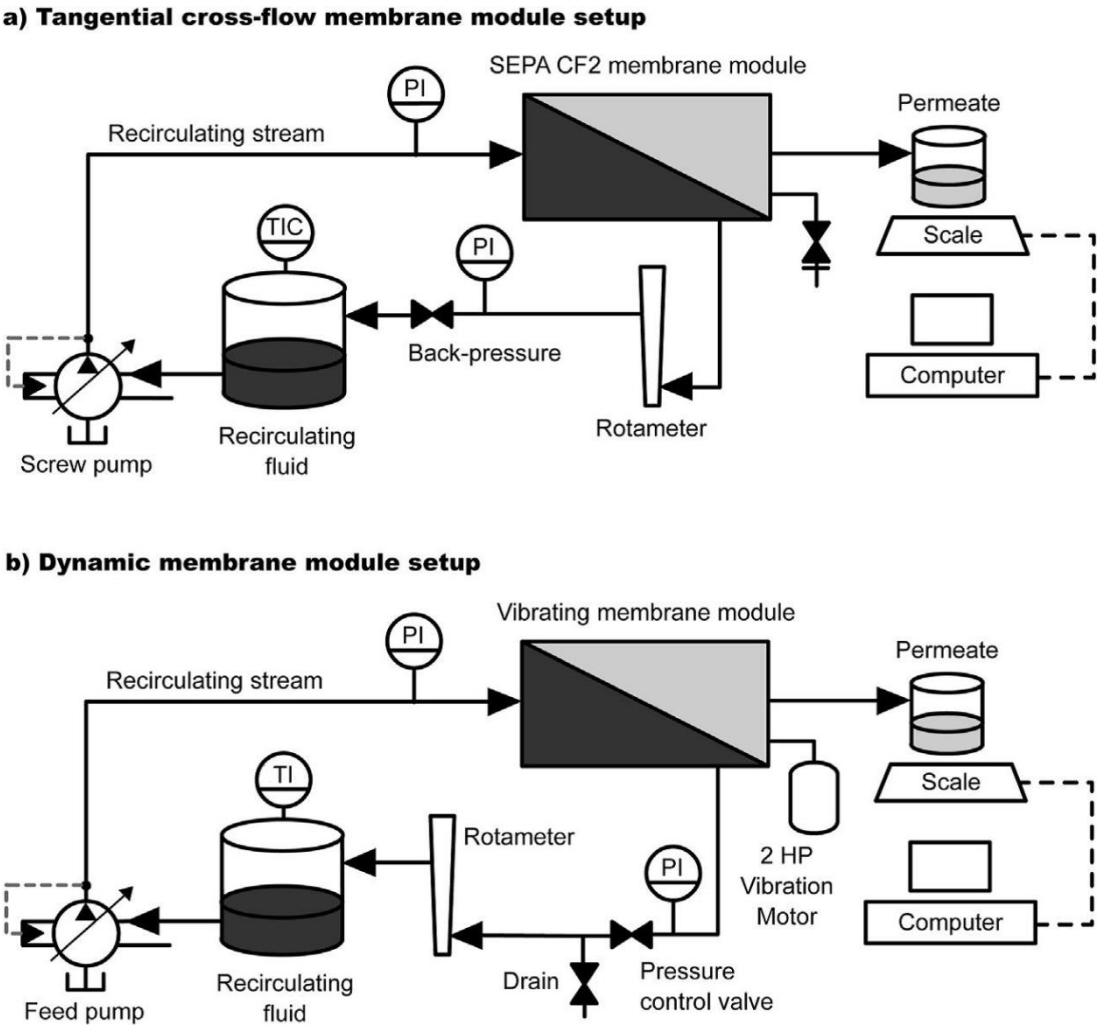


Figure 2.5: **Scheme of experimental equipment for microalgae dewatering: (a) cross-flow membrane module setup, (b) dynamic membrane module setup.**

In the cross-flow filtration, microalgae culture was placed in the temperature-controlled recirculation tank (cooled 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). A 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 permeate was collected in the permeation tank placed over the scale. The scale was connected to a computer in order to read the actual mass of permeate during the experiment and to calculate the actual mass flow rate in a five- second frequency.

Transmembrane pressure was fixed at 350 kPa and recirculating flow rate at 50 L h^{-1}. The volume of microalgae culture used as the feed was 2 L.

Dynamic membrane filtration of microalgae culture was performed using Vibratory Shear Enhanced Processing (VSEP, series L, New Logic Research, Inc.) system. Detailed description of this setup can be found elsewhere (48).

Vibrational frequency applied was 55.4 ± 0.1 Hz, recirculating flow rate was equal to 570 ± 5 L h-1 and the transmembrane pressure was fixed at 350 kPa. The microalgae volume used with the VSEP was 38 L when original culture was filtered and 15 L for the dewatering of concentrated biomass.

2.6.Results

2.6.1. Membrane characterization

2.6.1.1. Morphology – scanning electron microscopy micrographs

Cross-section micrographs of commercial and synthesized materials provided information about membranes morphology (Figure 2.6). All commercially available membranes showed a similar structure with big macrovoids. PES5 and PAN50 membranes had several types of macrovoids throughout the membrane thickness. Big vertical macrovoids were found in the whole membrane matrix, while smaller macrovoids were also present near the membrane top side (the selective). PES20 membrane did not exhibit the latter near the selective surface.

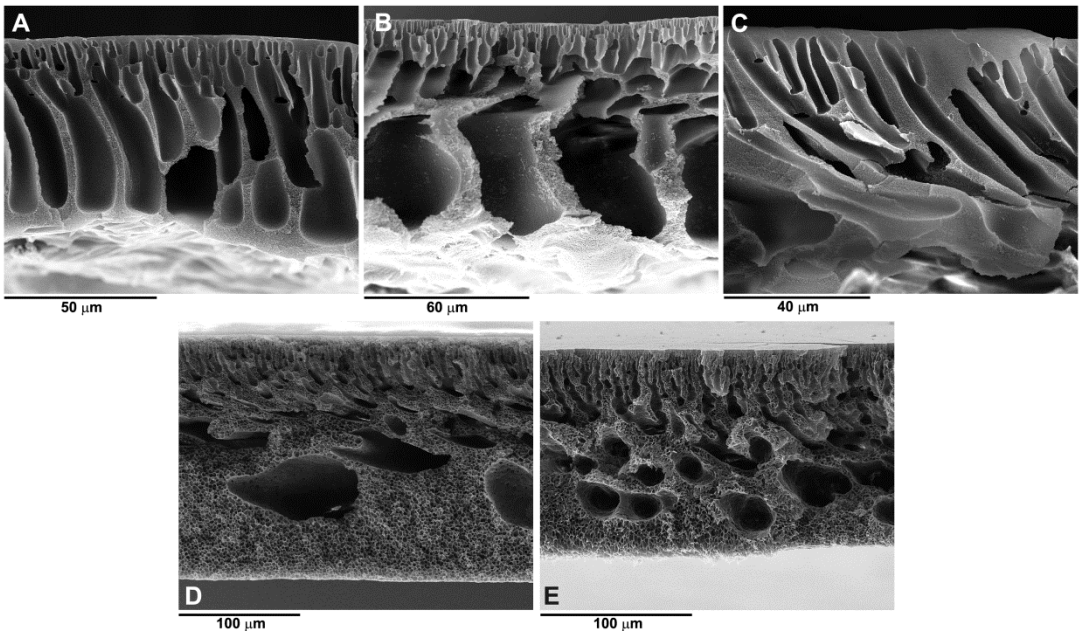


Figure 2.6: **SEM cross-section micrographs of commercial and synthesized membranes: a) PES5, b) PAN50, c) PES20, d) M4, e) M5.**

On the contrary, synthesized ABS membranes had sponge-like morphology with smaller and enclosed macrovoids inside the structure compared with the commercial membranes. M4 contained bigger pores than M5 as a consequence of different temperatures of the coagulation bath applied. A higher temperature of the coagulation bath resulted in a slower phase inversion precipitation and in the formation of a denser structure.

In all cases, a dense top layer was observed. It ensured total microalgae rejection in the dewatering experiments.

The results of porosity measurements of all membranes are presented in Table 2.3. Because of the presence of macrovoids commented above, commercial membranes were more porous than ABS synthesized ones. PES5 was the membrane with the greatest value of porosity within all tested materials. Thus we expected that commercial membranes would exhibit greater permeability than the synthesized ones, which were not optimized.

As regards of synthesized membranes, an important factor that was also considered was its mechanical behavior. Membranes not only need to separate desired compounds with the highest possible flow rate, but also need to be mechanically stable. A main non-desirable behavior encountered when producing ABS membranes was its brittle performance. It was found that coagulation bath temperature influenced significantly this property. By increasing the temperature, significantly less brittle membranes were obtained. Therefore, M5 membrane produced in a coagulation bath with a temperature of 50 ºC was mechanically better than that obtained with a temperature of 20 ºC.

Mechanical properties were not measured in this study but references can be found elsewhere (33).

Table 2.3: **Porosity and water contact angle values of membranes.**

Membranes	Porosity [%]	Contact Angle [º]
Commercial		
PES5	66.6	86.9 ± 1.1
PAN50	63.8	55.1 ± 0.5
PES20	63.2	89.4 ± 1.1
Synthesized		
M4	37.1	80.7 ± 2.0
M5	41.3	69.9 ± 1.1

2.6.1.2. Contact angle

Contact angle values measured for commercial and synthesized membranes are summarized in the Table 2.3. It can be observed that all the materials gave values

lower than 90º, which indicated hydrophilic properties of the surface, strongly desired for the dewatering purpose. The smaller the contact angles, the better the hydrophilicity of the membrane is (49). Nevertheless, PES membranes offered values very close to the theoretical limit.

Concerning commercial membranes, polyethersulfone materials, PES5 and PES20, with CA values greater than 85º were more hydrophobic when compared to polyacrylonitrile one (PAN50) with CA lower than 60º. This result indicated that PAN50 was offering the best properties of permeability with water, which was confirmed by tests performed before microalgae sludge filtration (Table 2.4 and Table 2.5). In fact, one of the main advantages of PAN material is its hydrophilic property although it is one of the most expensive materials within the common polymeric membrane materials family. It should be considered that, in this case, cost reduction is one of the main targets, so PAN material is useful for technical reference but not for this industrial application.

For synthesized ABS membranes, M4 had greater values of contact angle than M5. It means that a higher temperature of coagulation bath results in better hydrophilicity of the surface. Moreover, the contact angle value of the M5 membrane was the closest one to that of the most hydrophilic commercial membrane, PAN50. Therefore, another advantage of ABS material is its clear hydrophilic behavior, closer to PAN material than others like polysulfone or polyethersulfone but much cheaper than all of them.

2.6.2. Filtration experiments

2.6.2.1. Conventional cross-flow filtration

Figure 2.7 shows the permeability values obtained with all the membranes tested in the conventional setup. The results include permeability measurement with water of the virgin membrane and after the experiment. It allows comparing initial membrane performance as well as irreversible fouling. Also, permeability with the microalgae sludge is presented. Numerical values can be found in the Table 2.4.

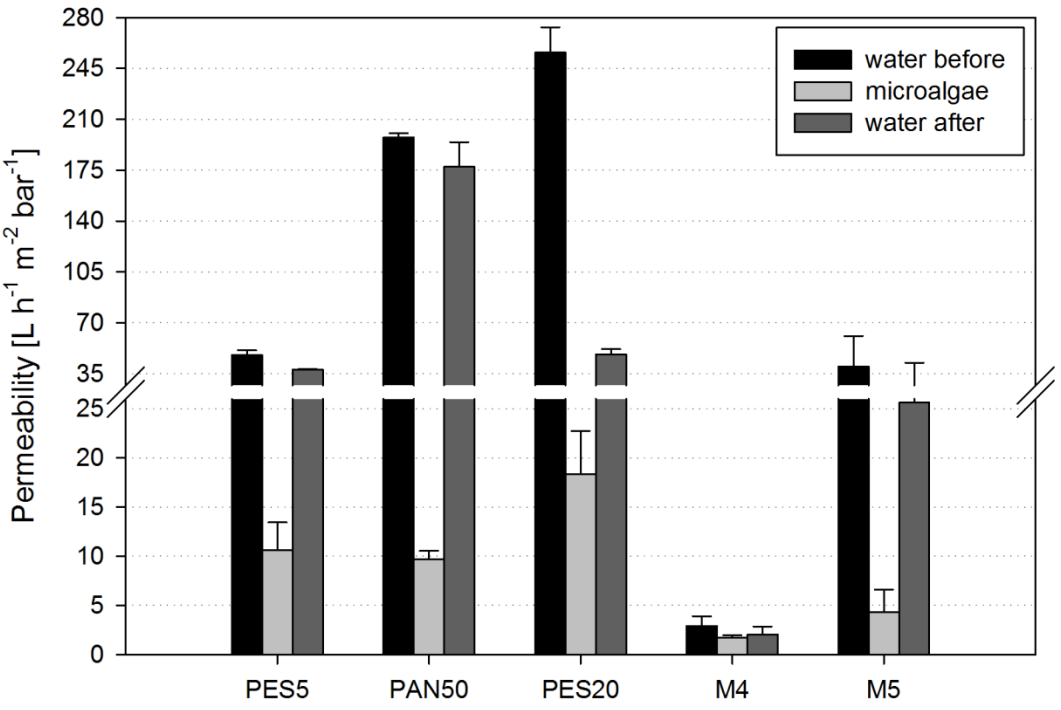


Figure 2.7: **Permeability results of cross-flow filtration experiments**.

The membrane that exhibited larger water permeability was PES20, followed by PAN50 and PES5. A large difference between the last one and others is according to their MWCO. Synthesized membranes (M4, M5) offered less water permeability due to their non-optimized synthesis (i.e. less porosity than commercial membranes) as explained above.

Table 2.4: **Permeability results for conventional cross-flow filtration.**

Membranes	Permeability [L h^{-1} m^{-2} bar^{-1}]		
	Water before experiment	Microalgae dewatering	Water after experiment
Commercial			
PES5	47.8 ± 3.3	10.6 ± 2.8	38.0 ± 0.4
PAN50	197.4 ± 3.0	9.7 ± 0.9	177.4 ± 16.9
PES20	255.7 ± 17.5	18.4 ± 4.4	48.3 ± 3.5
Synthesized			
M4	2.9 ± 1.0	1.7 ± 0.2	2.0 ± 0.8
M5	39.8 ± 21.0	4.3 ± 2.3	25.6 ± 17.0

Concerning the microalgae sludge permeability, results showed a severe fouling when using commercial membranes, especially with PAN50 and PES20 membranes. The PES5 ultrafiltration membrane, with the lowest MWCO, exhibited a permeability value between those obtained for the other two membranes. This implied that the volumetric flow reduction (ratio between the microalgae and water permeability) was much less in this membrane than in the others and therefore, it corresponded to the membrane with less fouling (78% for PES5, 95% for PAN50 and 93% for PES20). Although the microfiltration range would be enough to reject microalgae, ultrafiltration membrane offered better performance due to the less fouling. Nurra (18), Zhang (50) and Tansel (51) in their studies reported fouling formation due to the different pore size of membranes thereby pointing in the same direction. Considering our own synthesized membranes, results showed that despite their water permeabilities being much lower than for commercial membranes, microalgae permeabilities were closer. Volumetric flow reduction was 41% for M4

and 89% for M5. Therefore, in both cases, this value was lower than for commercial membranes. In absolute terms, although microalgae permeability was higher for commercial membranes, M5 membrane offered a microalgae permeability that was only half of the PAN50 one (best case). This result is promising considering that the synthesized membranes were not optimized and that the price of ABS material is three orders of magnitude lower than PAN material.

The measurement of water permeability after performing the experiment and cleaning the system (including the membrane) allowed determining the irreversible fouling. The membrane with higher irreversible fouling was PES20. The ratio between water permeability before and after the experiment was 81%. The other membranes exhibited similar behavior, including synthesized membranes, with ratios lower than 36%.

From among the synthesized membranes tested, a better permeability for water as well as for microalgae filtration was obtained with M5 membrane. For this reason and because of better mechanical resistance, it was chosen in order to be tested in dynamic filtration experiments.

2.6.2.2. Dynamic filtration

Figure 2.8 shows the permeabilities obtained with all the membranes tested in the vibrational setup. The results include water permeability measurement with the virgin membrane and then after the experiment. Also, permeability with the microalgae sludge is presented. Numerical values are presented in Table 2.5.

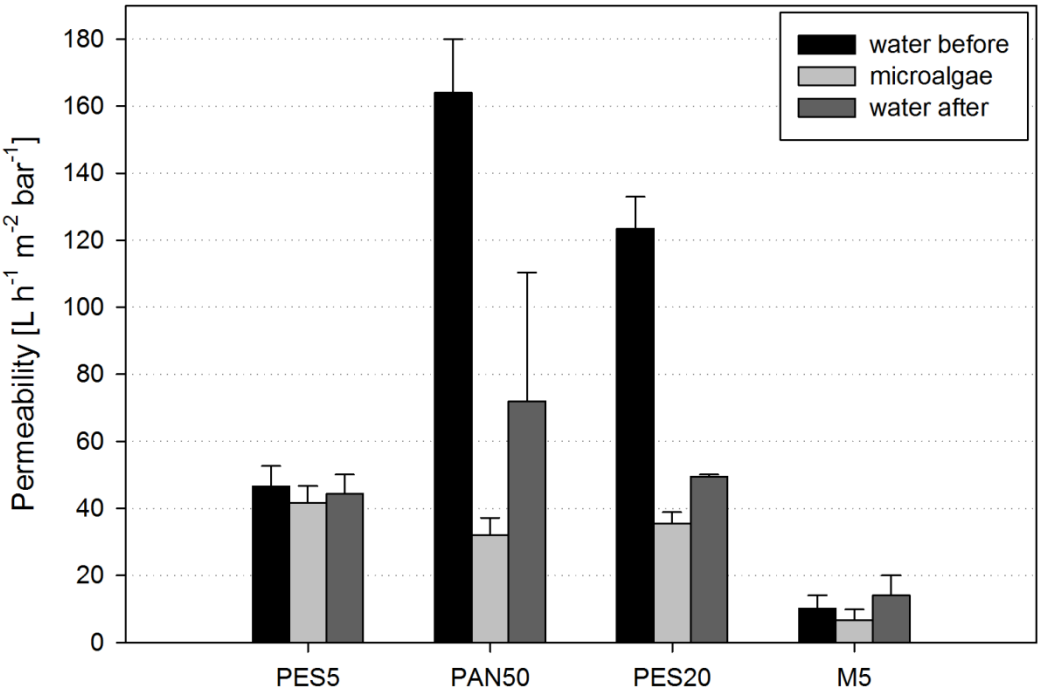


Figure 2.8: **Permeability results of dynamic filtration experiments**.

In terms of water permeability for vibrating filtration, the highest values were obtained with PAN50 membrane. Water permeability for PES20 decreased when compared to the results obtained with cross-flow filtration (Figure 2.7 and Figure 2.8), likely due to dis-homogeneities of the membrane. Again, water permeability differences between commercial membranes were those expected due to their MWCO and porosity. For the synthesized membrane, water permeability was also lower for the same reasons explained in the case of conventional filtration.

Table 2.5: **Permeability results for dynamic filtration.**

Membranes	Permeability [L h^{-1} m^{-2} bar^{-1}]		
	Water before experiment	Microalgae dewatering	Water after experiment
Commercial			
PES5	46.6 ± 6.0	41.7 ± 5.0	44.3 ± 5.8
PAN50	163.9 ± 16.0	32.0 ± 5.2	72.0 ± 38.3
PES20	123.5 ± 9.4	35.5 ± 3.4	49.5 ± 0.6
Synthesized			
M5	10.2 ± 3.8	6.6 ± 3.2	14.0 ± 6.0

Regarding microalgae permeability, the most noticeable result was that performance was in all cases much higher in dynamic filtration than in conventional. The ratio between permeabilities ranged from 1.5 for M5 membrane up to 4 for PES5 membrane. A ratio of 4 not only indicated a technical improvement of the process but also an economic one considering that the plus of energy added in the system for vibration represents approximately only 10% of the pumping cost. Comparing the performance of the commercial membranes with this technology, results showed that the membrane with less MWCO (PES5) still improved the operation, as it was the one with the highest permeability ($4.2 \cdot 10^{-7}$ m h^{-1} Pa^{-1}). For PES5 and M5 membranes, results showed that permeability with microalgae sludge was close to permeability with water (low volumetric flow reductions).

To assess irreversible fouling, permeability with water before and after the experiment was considered (the system was cleaned before measuring permeability with water after the experiment). Results showed that, also in this aspect, dynamic

filtration enabled a decrease on irreversible fouling by reducing the cake formation over the membrane and pore blocking. In case of PES5 membrane, the value of permeability with water after the experiment was only 5% less than permeability with water before the experiment (Table 2.5). This means that the vibration prevented fouling and membranes used for this purpose might expect a longer lifetime. Even though PES5 gave the lowest value of permeability with water within all commercial membranes, it resulted in offering the most similar results for microalgae filtration as well as for water after experiment. Membrane performance was steady during all the time.

Permeability with water after the experiment for M5 membrane was higher than the one obtained with the virgin membrane. The explanation for this phenomenon can be the influence of membrane swelling on the pore size, resulting in increasing porosity and improvement of performance in terms of permeation.

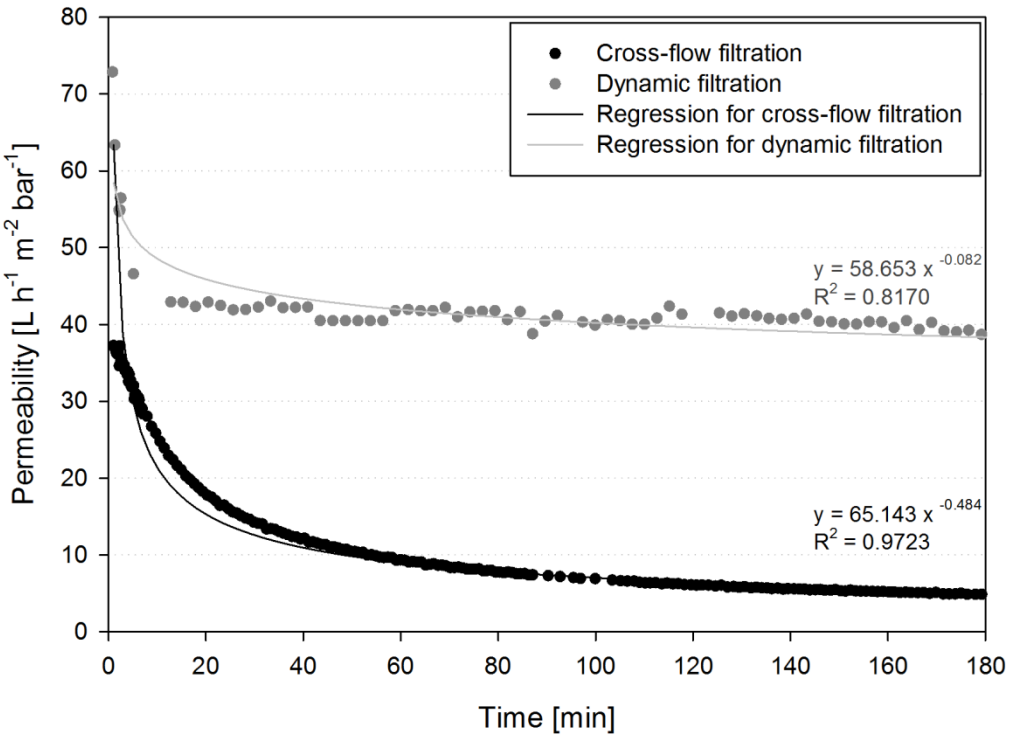


Figure 2.9: **Permeability profiles with time for PES5 membrane in cross-flow and dynamic filtration experiments.**

Figure 2.9 presents the permeability change with time during experiments for both microalgae filtration techniques using PES5 membrane. In the first minutes of the experiments, permeability was decreasing significantly due to primary fouling effect. However, after around 15 minutes the system was becoming stabilized and, in the case of dynamic filtration, after 20 minutes the steady state was reached. In the cross-flow filtration much more time was required to attain the plateau. Again, the cause was cake formation over the membrane surface and pore blocking, which were significantly reduced by using the vibrating set-up (Figure 2.10). Another advantage is that steady state with dynamic filtration was reached at the permeability value around 3 times higher than with the conventional method.

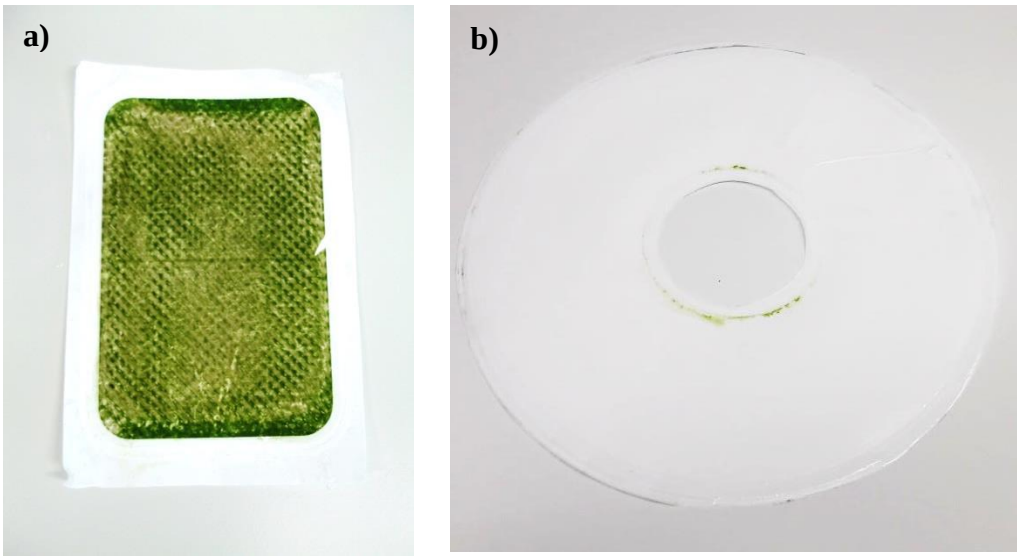


Figure 2.10: **Cake formation over the membrane surface after microalgae dewatering: a) cross-flow filtration; b) dynamic filtration**.

2.6.2.3. Initial biomass concentration effect

Another variable checked was the influence of the initial biomass concentration on dynamic filtration experiments. To assess this parameter, experiments with VSEP were performed with PES5 membrane, which corresponded to the commercial membrane giving the best performance.

Figure 2.11 shows permeability results of three different experiments performed with three different initial biomass concentrations. For each experiment, permeability with water before and after the experiment was measured as well as the microalgae one.

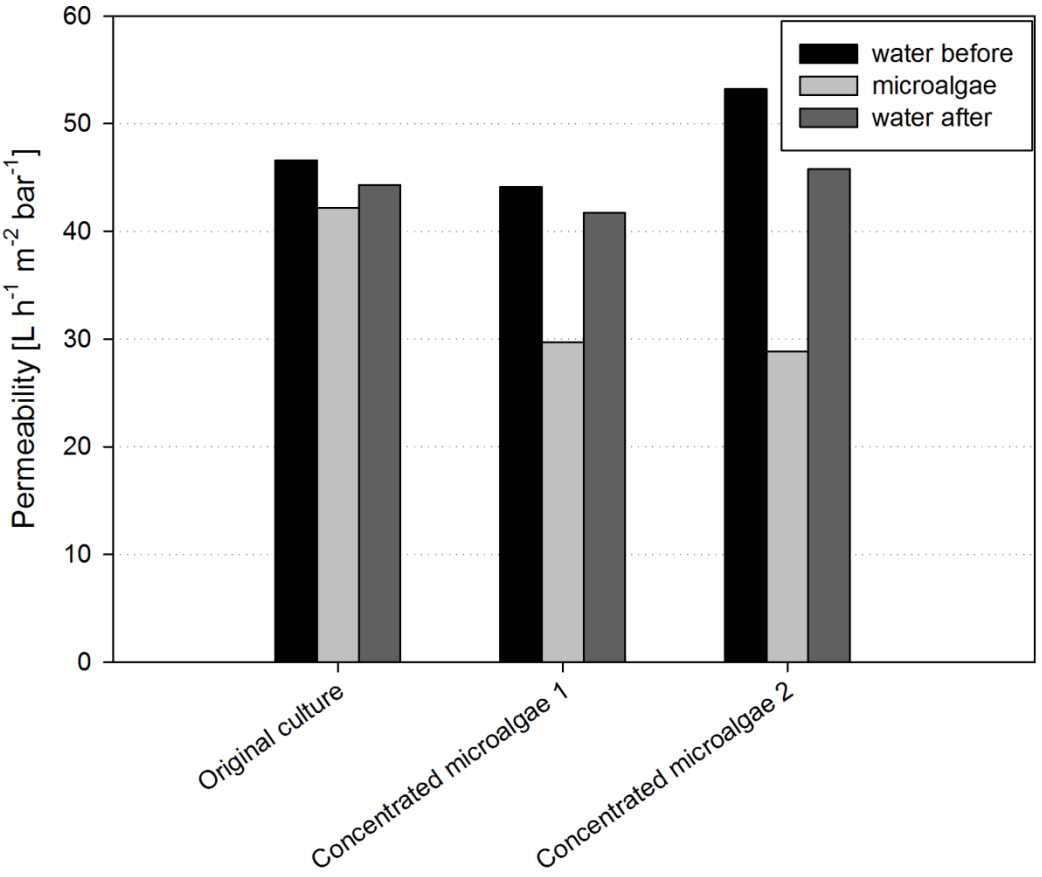


Figure 2.11: **Permeability results for the dewatering experiments with different concentrations of *Chlorella sorokiniana* culture.**

Figure 2.11 shows that the concentration of microalgae had a clear influence on permeability. An initial tendency was that when the initial concentration increased, permeability decreased. This can be observed comparing the first and the second experiment, with initial optical densities of 0.2 and 0.8 respectively. For these two experiments, permeability with microalgae sludge decreased 25%. Nevertheless, an interesting result was that when the initial concentration was further increased, the permeability with microalgae sludge did not significantly decrease any further. If experiments 2 and 3 are compared, permeability with microalgae sludge was around $3.0 \cdot 10^{-7}$ m h^{-1} Pa^{-1} while initial optical density of the sludge was 0.8 and 1.5

respectively. This means that in terms of dynamic filtration a higher concentration of feed did not contribute to more fouling generation on the membrane.

Although reaching the highest concentration was not the objective of this study, the experiments resulted in obtaining a noticeable concentration factor of 18 using the dynamic system. From an optical density of 0.2, a final one of 3.6 was achieved. As a reference, the measure was that an optical density of 0.413 is related to a microalgae ash free concentration of 0.26 g/L.

2.6.2.4. Biomass rejection

The concentrations of microalgae culture used as a feed for filtration experiments as well as concentrations of permeate and retentate were characterized by using results of optical density measurements.

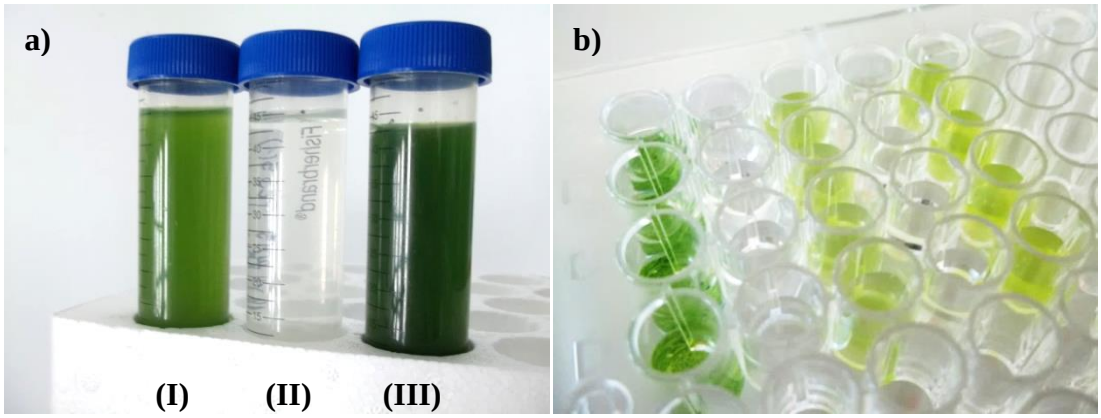


Figure 2.12: **Microalgae samples before and after filtration: a) samples of (I) feed, (II) permeate and (III) concentrate; b) samples prepared for absorbance measurements.**

The total rejection of microalgae was obtained and confirmed by absorbance measurements within all the filtration experiments performed (Figure 2.12). For example, the results of the optical density measurements of *Chlorella sorokiniana* culture in dynamic filtration experiments using PES5 membrane with different

concentrations of microalgae are presented in the Figure 2.13. As was mentioned before, in this particular experiment retentate obtained from vibratory dewatering of original culture was collected and used as a feed for further experiments (Concentrated culture 1 and Concentrated culture 2). It can be observed that permeate in all cases had a similar value of OD as fresh water, which means that it was free of microalgae cells and total rejection was achieved.

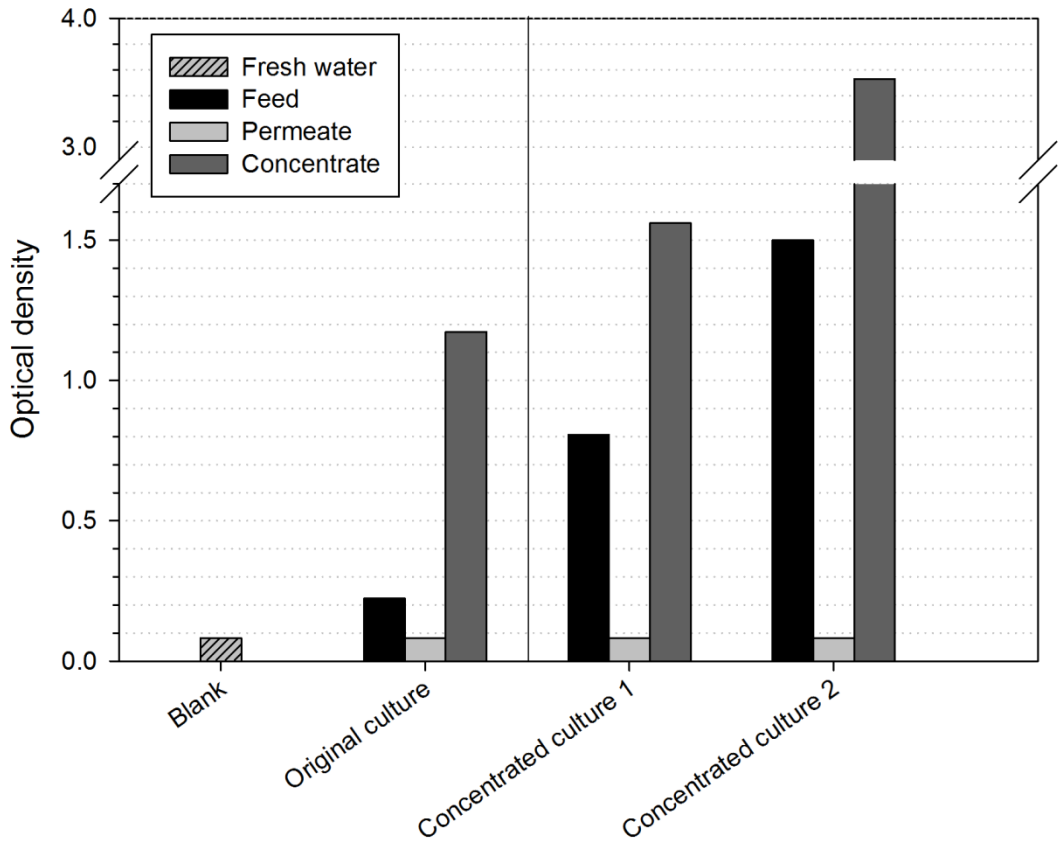


Figure 2.13: **Optical density of *Chlorella sorokiniana* culture in dynamic filtration experiments with different concentrations of microalgae.**

2.7.Conclusions

Chlorella sorokiniana dewatering by means of vibrational membrane filtration substantially improves performance compared to conventional membrane cross-flow filtration. Permeability is more than doubled.

A reduction of operational cost in membrane dewatering was demonstrated after producing and using three order of magnitude cheaper membranes than commercial ones from acrylonitrile butadiene styrene. ABS membranes worked successfully in the dynamic module setup and completely rejected microalgae, which make them suitable for this application. ABS membrane production should consider polymeric composition and the temperature of the coagulation bath as key parameters in order to obtain a membrane with proper mechanical characteristics.

A first positive scale-up indicator obtained in this study is that, although there exists an initial permeability decrement when the initial biomass concentration increases, an asymptotic behavior occurs. Therefore, filtration performance may continue to be satisfactory with sludge concentration increment.

Acknowledgements

This work was supported by the project CTQ2014-56285-R “Cultivo, concentración, fraccionamiento y obtención de producto en refinería de microalgas” funded by the Spanish Ministry of Economy and Competitiveness.

The research was also supported by the European Regional Development Funds (ERDF, FEDER Programa Competitividad de Catalunya 2007-2013).

Special thanks to Styrolution Europe GmbH (Frankfurt am Main, Germany) for providing the ABS material and to Mr. Stephan Gschwind and Mr. Udo Köster (Styrolution) for the management.

3

MICROALGAE DEWATERING BY SEDIMENTATION COMBINED WITH MEMBRANE FILTRATION[2]

This chapter describes the experiments of pH-induced sedimentation combined with dynamic filtration of two microalgae species, Dunaliella tertiolecta and Chlorella sorokiniana. The concentration factors were calculated based on dry weight and optical density measurements as well as on the volumes processed. Novel acrylonitrile butadiene styrene (ABS) membrane materials were characterized by contact angle measurements and tested for Dunaliella tertiolecta dewatering with cross-flow and dynamic filtration. The experiments were performed using self-prepared and commercially available membranes. Total microalgae rejection was confirmed by optical density measurements.

[2] This chapter is based on the following publication:
M. Hapońska, E. Clavero, J. Salvadó, X. Farriol, C. Torras, *Pilot scale dewatering of Chlorella sorokiniana and Dunaliella tertiolecta by sedimentation followed by dynamic filtration*, Algal Research, Volume 33, 2018, Pages 118-124, ISSN 2211-9264, https://doi.org/10.1016/j.algal.2018.05.007

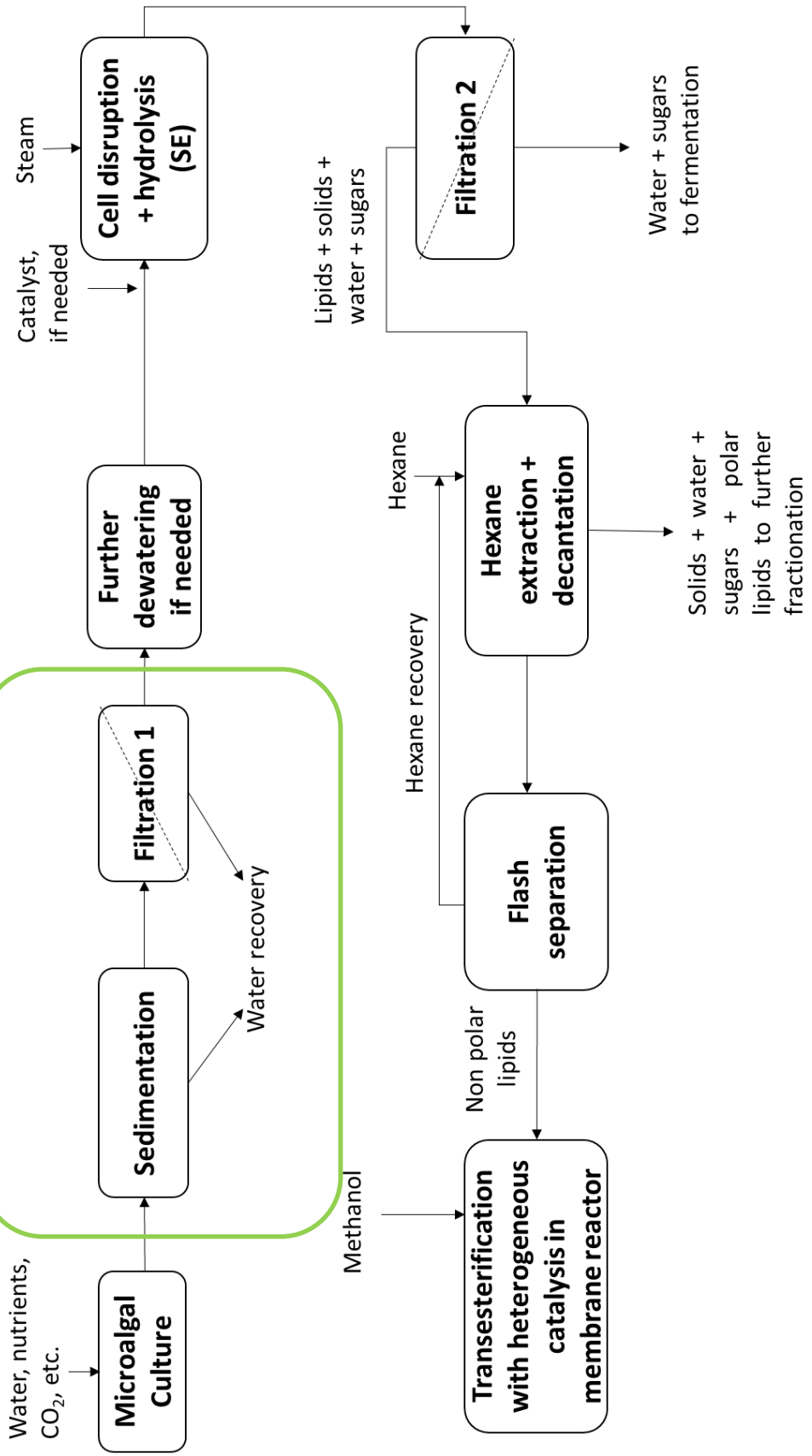


Figure 3.1: **Proposed scheme of microalgae biorefinery – sedimentation + membrane filtration.**

3.1.Introduction

Microalgae are the scope of wide research studies concerning the culture and the final composition, harvesting techniques as well as biorefinery (52). Being a source of lipids, proteins and carbohydrates microalgae can be processed into food supplements, fodder, colorants, enzymes, biofuels and pharmaceuticals (29,30,53). 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% (9). 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 treating via drying, extraction or other downstream processing steps (16). Nevertheless, as the costs of this single step reach up to 20–30% of the total cost of microalgal biomass production, harvesting optimization is strongly recommended (54).

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 being commonly 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 (55). Moreover, auto-flocculation can be improved by adding NaOH to achieve optimal pH values (56,57). 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 (10,58).

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) being at the same time high energy demanding. To harvest algae cultures with the same technique from 0.04% to 4% dry weight costs 1.3 kW h/m3 of pond water. In order 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 (59). It could be applicable in processes to obtain high-value products, while 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, appear to be more suitable for this purpose (60). However, being biological feeds a mix of organic matter of different size and shape, they are usually difficult to filter as the cake is very compressible. Also, surface charge of the cells may result in concentration polarization phenomena, affecting the interaction between the membrane surface and the biomass (61). The filtration ability depends also on the cell viability and the harvesting time (62). The fouling issue is the main disadvantage when working with the conventional cross-flow filtration and can result in up to 99% permeability reduction (32,63,64). Vibratory shear enhanced process (VSEP) also called dynamic filtration can overcome this issue by increasing turbulence and raising shear stress over the membrane surface (18,65). Moreover, in the case of dynamic filtration it was proved that despite 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 (66). For microalgae dewatering purpose, membrane micro/ultrafiltration (MF/UF) can be applied 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 much lower compared to the ceramic ones (32).

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 (67). The more efficient and cheap the methods chosen the lower the final cost of the process. 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.

3.2.Materials and methods

3.2.1. Materials

3.2.1.1. 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.

3.2.1.2. 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 3.1.

Table 3.1: **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 $^{\circ}$C and tensile stress at yield of 44 MPa. DMA was used as solvent to dissolve the polymer for the synthesis of non-commercial membranes.

3.2.2. Methods

3.2.2.1. 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 incorporated micrometer. 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). Immersion of casted polymeric solution into a coagulation bath caused 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.

3.2.2.2. Sedimentation combined with dynamic filtration

To determine the optimum pH value for sedimentation in 300L photobioreactors preliminary study of sedimentation experiments was performed with both microalgae specie in 2 L graduated cylinders. 2M NaOH solution was added into the cylinders and mixed with magnetic stirrer until flocculation occurred. Once aggregates formation was observed the stirring was stopped and the suspension was let 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 each 300 L vertical photobioreactor containing microalgae culture. To obtain a uniform pH distribution, aeration was kept for 2 minutes after addition of the alkali solution. Then, the air flow was stopped and the culture was left to settle for 60 minutes. The samples of the clarified liquid were collected from three different levels of the PBR for the pH measurement. The clarified liquid was separated from the sedimentate and three samples of sedimentate were collected for the pH measurement. The sedimentate was collected for further filtration.

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 carried on until the maximum volume of permeate was reached (3.4L of the dead volume of the equipment). Total

microalgae rejection was confirmed by absorbance measurements of the permeate samples.

Dry weight of the samples was measured to calculate the concentration of microalgae. The samples were rinsed and dried for 24 hours in the temperature of 100^{o}C and weighted afterwards.

3.2.2.1. Contact angle

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

3.2.2.2. 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, membrane permeability with water was measured again after cleaning the system. The last step allowed determination of the irreversible fouling resistance of membranes.

3.2.2.3. 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 reference.

3.2.2.4. pH measurements

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

3.2.3. Equipment

Experiments were carried out using two filtration setups, as shown on the scheme in the Figure 2.5. In the cross-flow filtration, the microalgae culture was placed in the temperature controlled recirculation tank (cooled 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.

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.

Dynamic membrane filtration of microalgae culture was performed using Vibratory Shear Enhanced Processing (VSEP, serie L, New Logic Research, Inc.) system. Detailed description of this setup can be found elsewhere (48).

The vibrational frequency applied was 55.4 ± 0.1 Hz, 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) Dewatering of *Dunaliella tertiolecta*, using a volume of 38 L of the original culture as a feed, two replications of the experiment were performed;
b) Dewatering of sedimented *Dunaliella tertiolecta*, using a volume of 47 L of the floc (concentrated part of the sedimentation) as a feed;
c) Dewatering of sedimented *Chlorella sorokiniana*, using a volume of 28 L of the floc as a feed.

3.3. Results and discussion

3.3.1. Membrane surface characterization via contact angle measurements

The surface of the materials was characterized by water contact angle measurements with all the membranes tested. Within all the results, the ± values report standard deviation between measurements. The contact angle value gives the information if the surface is either hydrophilic (CA < 90^o) or hydrophobic (CA > 90^o). The smaller the contact angles, the better the hydrophilicity of the membrane is. Both, the commercial and self-prepared membranes resulted in a CA < 90^o, 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.

3.3.2. Filtration experiments

Cross-flow versus dynamic filtration of Dunaliella tertiolecta

Figure 3.2 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.

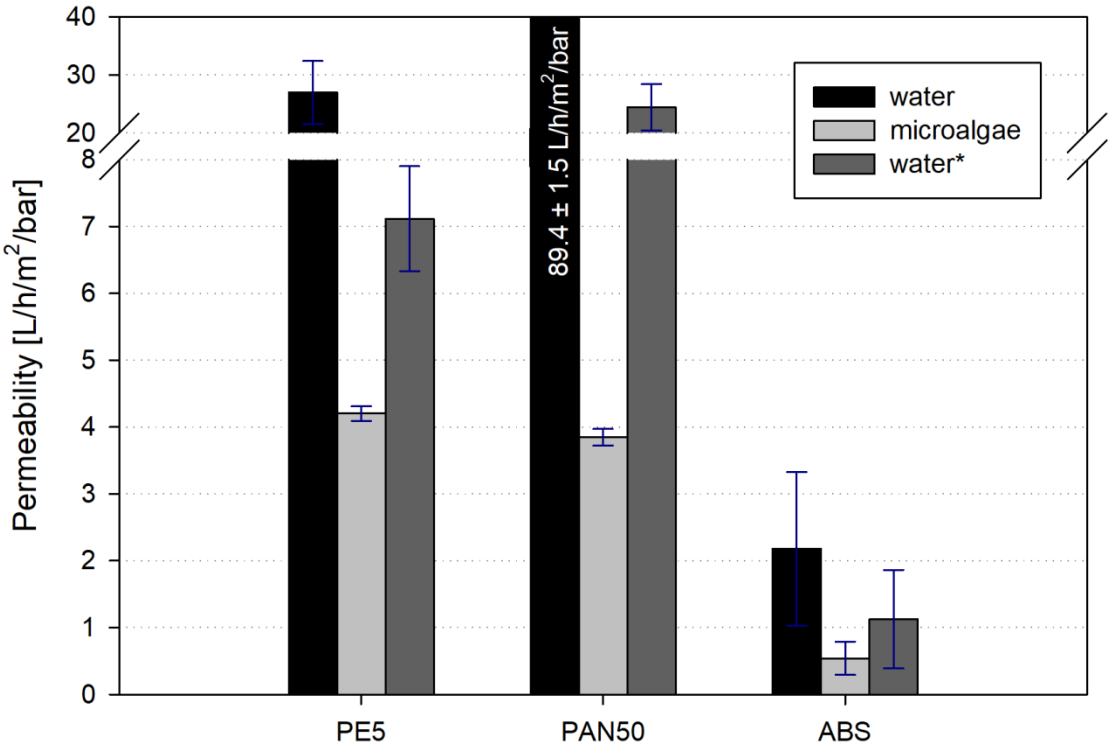


Figure 3.2: **Permeability results for the cross-flow filtration of Dunaliella tertiolecta: water permeability with the virgin membrane, microalgae culture permeability and water* permeability after the experiment and with the cleaning procedure performed (n = 2). The error bars report standard deviation between measurements.**

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 h^{-1} m^{-2} bar^{-1}, n = 2) might be explained by 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 the 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 case of self-made membrane there is a room for improvement in terms of permeability and although the microalgae permeability with not optimized ABS membranes is around seven times lower than with commercially available ones, the polymer is three orders of magnitude cheaper [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.

In order to calculate the total and 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), following 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 about irreversible fouling (IF) of the membranes. The results obtained show similar performance of PAN50 (72.7%) and PE5 (73.6%). The ABS membrane again gave the lowest value, 48.4%. It 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 3.3 shows the permeability results obtained for experiments with *Dunaliella tertiolecta* filtration using 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.

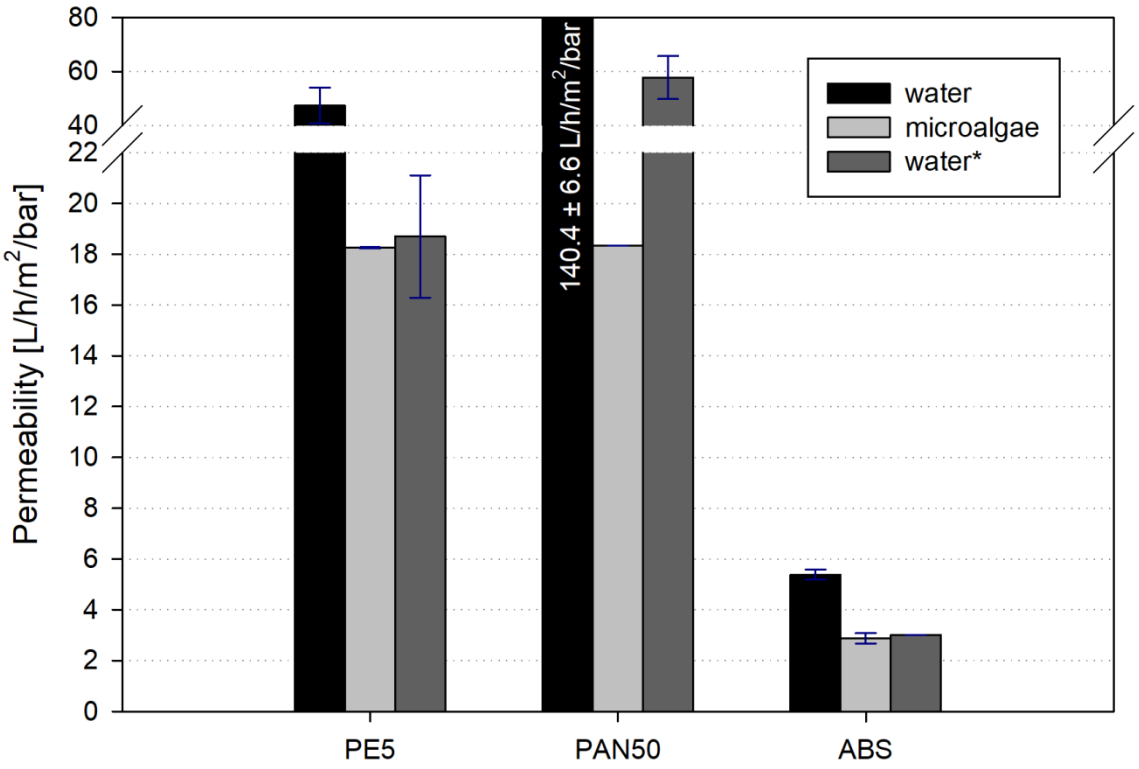


Figure 3.3: **Permeability results for the dynamic filtration of Dunaliella tertiolecta: water permeability with the virgin membrane, microalgae culture permeability and water* permeability after the experiment and with the cleaning procedure performed (n = 2). The error bars report standard deviation between measurements.**

For the permeability of water, the tendency was similar to the 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 vibrational movement of the module. Again, water permeability differences between commercial membranes were those expected according to their MWCO, as explained before. For the synthesized membrane, water permeability was lower as compared to the commercially available materials for the same reasons 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 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.

Those results indicated 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 only 10% of the pumping energy. Comparing the performance of the commercial membranes with this technology, results showed that independently to the differences in the MWCO, similar permeability of microalgae sludge was obtained in both cases (18.3 L h^{-1} m^{-2} bar^{-1}). It is a great improvement comparing to the results reached with the cross-flow filtration setup. Moreover, for PE5 and ABS membranes, results indicated that permeability with microalgae sludge was close to permeability with water, which means low volumetric flow reductions.

Pilot 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 the biomass increases. Initial volume of *Dunaliella tertiolecta* was of 38 L with the culture concentration of 1.1 g/L. Figure 3.4 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.

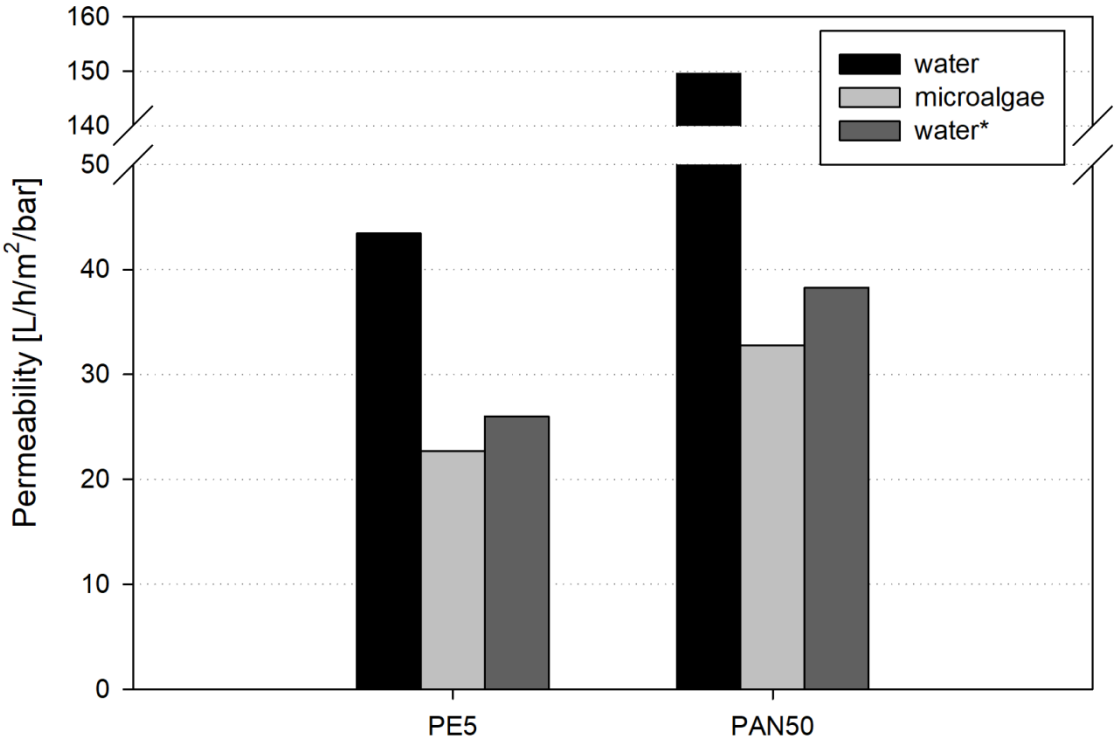


Figure 3.4: **Permeability results for experiments of maximum concentration of Dunaliella tertiolecta: water permeability with the virgin membrane, microalgae culture permeability and water* permeability after the experiment and with the cleaning procedure performed (n = 1).**

For the permeability of water before microalgae concentration experiments the results for both materials were similar as in the previous study, giving the 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 the permeability both membranes maintained 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. Also, 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%. It means that in dynamic filtration the volumetric flow reduction does not depend on neither the volume of the filtrated sludge or the duration of the experiment.

The volumetric concentration factor (VCF) for those experiments was calculated 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 resulting in a sludge concentration of 12.3 g/L.

Sedimentation combined with dynamic filtration

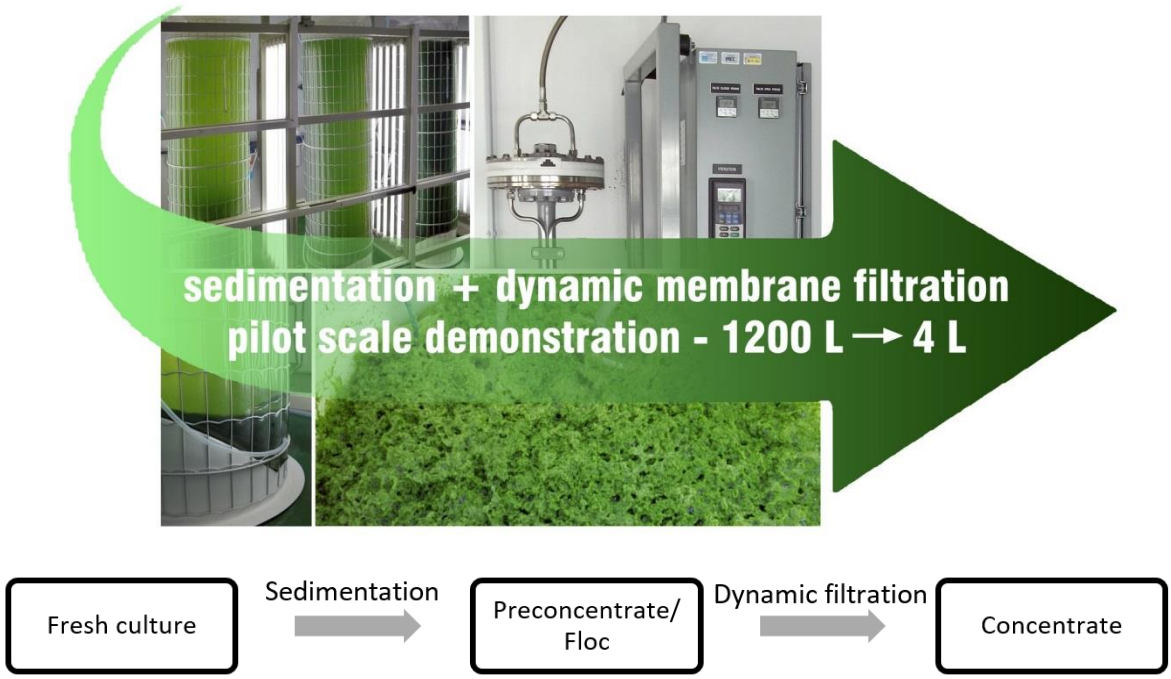


Figure 3.5: **Scheme of the steps in sedimentation combined with dynamic filtration experiments**

Figure 3.5describes 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. Sedimentation of *Dunaliella tertiolecta* was obtained with lower pH value than in case of *Chlorella sorokiniana*, but also the initial value for both cultures 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.

Table 3.2: **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 3.2 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. It means no need for centrifugation or any other concentration technique 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 low initial concentration of *Chlorella sorokiniana* and the equipment limitations higher concentration of the final concentrate was impossible to be reached. Another limitation was the volume to be used in the laboratory scale equipment. If considering that there was no such as limitation in terms of initial volume and internal volume of the equipment used, a much higher TCF could be obtained until reach the limitation of high microalgal sludge viscosity.

In order to calculate the concentration factor after sedimentation, the 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 initial culture and collected sedimentate (Table 3.3). In the case of *Dunaliella tertiolecta* the ODCF was 14.9, while for *Chlorella sorokiniana* the ODCF reached the value of 29.2.

Table 3.3: **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 the further filtration. The filtration was performed with the dynamic filtration setup and PE5 commercial membrane. The filtration was carried on 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 absorbance measurements of the permeate samples.

Table 3.4: **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 3.2 and Table 3.4). 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 before, higher VCF could be obtained if working with bigger initial volume of microalgae culture.

3.4. 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 using dynamic filtration, leading 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 is 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.

In the microalgae harvesting step, significant energy and cost reduction 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* allows reaching high concentration without using centrifugation. It 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 still can be increased, since the limitation in this case was the availability of initial volume (due to equipment sizing) but not technical issues like the viscosity.

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

Acknowledgements

This work was supported by the project CTQ2014-56285-R “Cultivo, concentración, fraccionamiento y obtención de producto en refinería de microalgas” funded by the Spanish Ministry of Economy and Competitiveness.

The research was also supported by the European Regional Development Funds (ERDF, FEDER Programa Competitividad de Catalunya 2007-2013).

M. Haponska is grateful to the Universitat Rovira i Virgili (URV) for her PhD scholarship.

Authors are thankful to Styrolution Europe GmbH (Frankfurt am Main, Germany) for providing the ABS material and especially to Mr. Stephan Gschwind and Mr. Udo Köster (Styrolution) for the management.

4

STEAM EXPLOSION CELL DISRUPTION OF *NANNOCHLOROPSIS GADITANA*[3]

This chapter describes microalga Nannochloropsis gaditana treatment with acid catalysed steam explosion and thefractionation of resulting exploded material in order to separate the different fractions (lipids, sugars and solids). A conventional and a vibrational membrane setups were used with several polymeric commercial membranes. Two different routes were followed: 1) filtration + lipid solvent extraction and 2) lipid solvent extraction + filtration.

[3] This chapter is based on the following publication:
E. Lorente, **M. Hapońska**, E. Clavero, C. Torras, J. Salvadó, *Microalgae fractionation using steam explosion, dynamic and tangential cross-flow membrane filtration,* Bioresource Technology, Volume 237, 2017, Pages 3-10, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2017.03.129

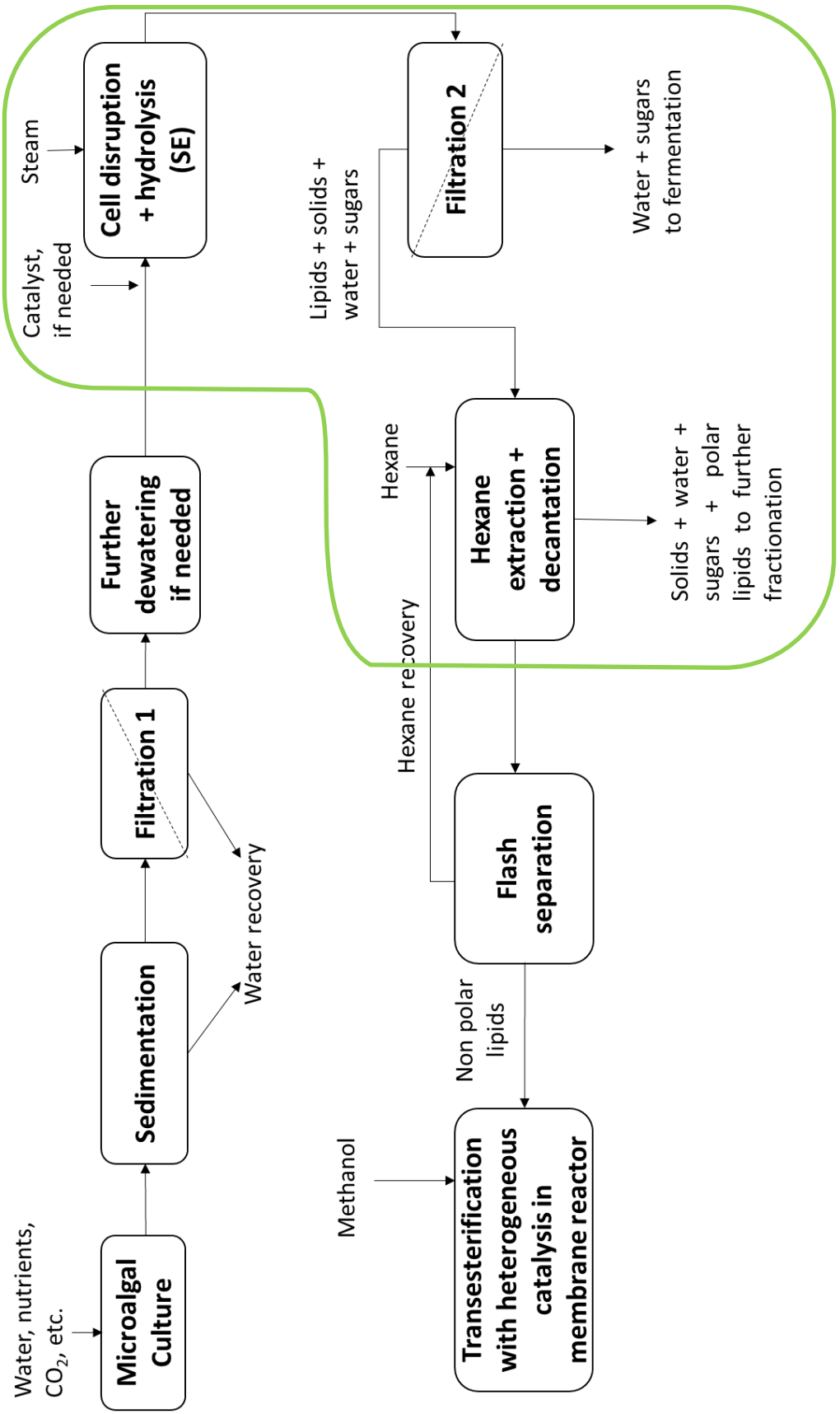


Figure 4.1: **Proposed scheme of microalgae biorefinery – steam explosion cell disruption and fractionation.**

4.1.Introduction

One of the ways to appreciate the importance of a research topic is to see the number of scientific publications that exist on a certain subject. On introducing the keyword “Microalgae” in the Scopus database (December, 2016), around 15,500 articles are listed. Many publications focus on aspects which concern the culture and final composition or focus on harvesting techniques (microalgae concentration). If the interest in research lies beyond the harvest, then the previous results can be refined. By crossing the keywords "Microalgae" and "Biorefinery" 240 publications (1.5 %) are listed and if the words "Microalgae" and "Fractionation" are introduced, the result is 182 (1.2 %). Moreover, if the game is continued by introducing "Microalgae" + "Biorefinery" + "Fractionation", the search will deliver 12 articles. It seems clear that the downstream steps of getting final valuable products from microalgae still require more attention regarding research and development, although there are some authors who are paying attention to this.

The first step to extract the different components of the microalgae is the disruption of the cell wall. To achieve this, several methods have been studied. Some of them are mechanical, such as bead milling, high pressure homogeneization, high speed homogeneization, ultrasonication, microwaves, Pulsed electric fields and other are non-mechanical methods such as enzymatic cell lysis and chemical methods (68).

Recently, Nurra et al. (2014a) used Steam Explosion (SE) to break the cell wall of algae (18). This technique is well known for treatment of lignocellulosic materials and provides mechanical and chemical disruption at the same time. Further, this procedure has already been compared to other cell disruption methods such as ultrasonication, microwave and autoclave, obtaining the best results in all cases under comparison (69). It is important to point out that steam explosion does not

require the sample to be previously dried. The main energetic consumption is calorific thus reducing process costs, particularly when a residual heat stream can be used. This process also causes the hydrolysis of carbohydrates which generate an aqueous phase containing monosaccharides that eventually can be fermented.

Once the cell wall is broken it is necessary to separate the different components of the obtained “jumble”. The mixture contains a solid phase and may contain two liquid phases: one aqueous with most of the sugars dissolved and another one with the lipids. It is also very common to find an emulsion in that mix (22). Therefore, what is needed is to define a recovery strategy which will recover each fraction from this heterogeneous mixture. This strategy will depend on the type of final product sought. For high value products for cosmetic, nutraceutical or pharmaceutical industry an extraction with supercritical CO2 at high pressure is used (23,70). When looking for higher scale lipid extraction procedures, like the production of biofuels, the extraction with organic solvents, such as hexane, is the usual industrial choice.

Another recovery approach is fractionation by using a mechanical separation. Membrane filtration is already widely used in the initial concentration of the culture. It is easy to scale up, has low energy requirements and there is no added chemical contamination (71). Some progress has already been made in expense reduction with new membrane materials that show a high reduction of cost for this application (33). The use of dynamic tangential filtration has also been introduced to avoid fouling caused when microalgae clog the pores of the membrane (17,18,32). Beyond the use of membranes in the harvesting, filtration can also be used in the separation of the fractions resulting after the cell disruption. Nanofiltration membranes can be helpful to concentrate sugars in the aqueous phase (14).

If membranes can be used to separate a complex mixture into fractions that are easier to handle then we will be nearer to achieving the goal of reducing the cost of downstream operations. Microalgae will then become a very interesting alternative for obtaining energy, food, pharmaceuticals and cosmetics. This article aims to shed a little more light on these strategies.

4.2.Microalgae sample

Nannochloropsis gaditana Lubián (strain CCMP1775, Provasoli- Guillard National Center for Marine Algae and Microbiota) was grown outdoors in a 3050 L semi-closed photobioreactor which consisted in a closed-loop build up by two collectors joined by six horizontal transparent plastic phototubes of 125 mm diameter (a detailed description is given in (21)). Microalgae were grown in filtered (1 µm) seawater enriched with 0.3 mL/L Codafol 14.6.5 (Coda Sustainable Agro Solution S.A.). Codafol 14.6.5 is a plant fertilizer that contains in w/w 14 % nitrogen, 6 % P2O5, 5 % K2O, 0.1 % Fe, 0.05 % Zn, 0.05 % Mn, 0.05 % Cu and 0.001 % Mo. The culture was CO_2 enriched during daylight via a solenoid valve activated by a pH controller set between 7.5 and 8.5.

The photobioreactor was operated as a semi-continuous culture in summer. During the period of culture the average of daily global solar irradiation was 20 MJ/m, and water temperature averaged 29.5 °C.

Microalgal biomass was concentrated with a continuous centrifuge (Clara 20 High Flow, Alfa-Laval) at 9060 rpm and a counter pressure of 4 bar. It was fed by a Seepex progressive cavity pump, BN series, with a nominal flow rate of 1000 L/h. The concentrated algal material was frozen in zip-lock plastic bags so that the sample were less than 2 cm thick to favour fast freezing and kept at -80°C until the

beginning of the experiment. Samples were slowly defrost at 4 °C for two days, immediately before the steam explosion procedure.

4.3.Methods

4.3.1. Fractionation strategies

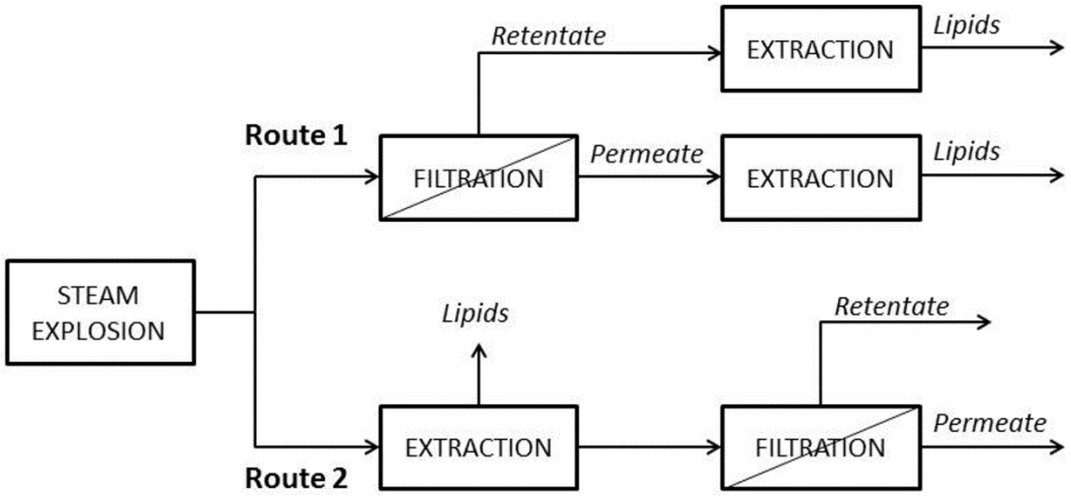


Figure 4.2: **Fractionation strategies for disrupted microalgae cells**

Figure 4.2 shows a schematic diagram of the fractionation strategies proposed in order to separate the different fractions (lipids, carbohydrates and residual solid) of a microalgal sample as independent streams. First the microalgal biomass was subjected to a steam explosion treatment and the resulting material followed one of two possible paths, which involved extraction and filtration processes applied in different order. A possible path (Route 1) was performing the filtration first and then extracting the permeate and/or retentate streams to get the lipid fraction. In the second route, the steam exploded sample was extracted with solvent to get the lipids separated in the first place, and then the remaining (raffinate) was filtered.

4.3.2. Steam explosion

Steam explosion of microalgae was carried out in a batch unit, equipped with a 16 L reactor and a collection vessel (Figure 4.3). An electric boiler (Boreal, 380 V/82 kW)

was used to generate steam, which was conducted to the reactor through high pressure pipes thermally isolated. The entrance of steam to the reactor was regulated by two valves placed in series. The sample was introduced through a valve (2" diameter) at the upper part. A flash valve at the bottom of the reactor allowed a sudden decompression to the atmospheric pressure of the collecting tank. The tank consisted in a cylinder with a diameter of 50 cm and a total volume of 100 L. It had a valve for steam release and another one at the bottom for liquid sample collection.

Figure 4.3: **Steam explosion equipment.**

Prior to the experiment, the microalgal sample was impregnated with sulphuric acid at a concentration of 5 % (w/w, wet sample basis) by mixing for 2 h at ambient temperature. The sample was then introduced into the reactor, which had been

preheated, and contacted with steam at 150 °C (corresponding to a saturated steam pressure of 4.7 bar) for 5 min. The experimental conditions, i.e. temperature, time and acid concentration were selected from a previous study (Lorente et al., 2015), in which the effect of these variables was investigated. A total of 10 kg of microalgae were treated (in two batches of 5 kg each) and the exploded material was collected together and neutralized (to pH 5) before using it in the fractionation experiments.

4.3.3. Filtration

Filtration experiments were carried out using two different setups (described in section 2.5). In the conventional cross-flow filtration, microalgal sample was placed in the recirculation tank and driven by a membrane 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 was measured. The retentate was returned from the membrane module to the recirculation tank, while permeate was collected in the permeation tank placed over the scale. The scale was connected to a computer in order to read the actual mass of permeate during the experiment and to calculate the actual mass flow rate in a five second frequency. Transmembrane pressure was fixed at 5 bar. The mass of pretreated microalgal sample used as the feed was 1.5 kg, approximately.

Dynamic membrane filtration of microalgae was performed using Vibratory Shear Enhanced Processing (VSEP, serie L, New Logic Research, Inc.) system. Detailed description of this setup can be found elsewhere (Nurra et al., 2014c). Vibrational frequency applied was 55.4 ± 0.1 Hz and the transmembrane pressure was fixed at 5 bar. The mass of the microalgal sample used with the VSEP was 6.0 kg, approximately.

Experiments were performed with commercial polymeric membranes purchased from Nanostone. Two membranes were used, one with a molecular weight cut-off (MWCO) of 5,000 Da manufactured from polyethersulfone (labeled PE5) and another of 100,000 Da from Polyvinylidene fluoride (labeled PV400). The filtration area was 0.0139 m2 for conventional cross-flow filtration and 0.0446 m2 for dynamic filtration.

The permeability of virgin membranes was determined by water flux measurements. After that, filtration of steam exploded microalgae biomass was performed and permeability vs time was measured during the experiment. The permeability with pretreated algae was measured at the fixed time of 60 minutes. Finally, water permeability was measured again after cleaning the system. The last step allowed determination of the irreversible fouling resistance of membranes. Irreversible fouling factor (IF, dimensionless, ratio between water permeability with the virgin membrane and water permeability after the experiment) and total fouling factor (TF, dimensionless, ratio between water permeability with the virgin membrane and microalgae sludge permeability) were calculated.

4.3.4. Lipid extraction

The extraction of lipids from microalgal samples was performed by mixing 20 mL of sample and 20 mL of n-hexane. The mixture was kept at 60 °C and 800 rpm for 2 h, and then centrifuged at 4000 rpm for 10 min. After centrifugation, the mixture partitioned into three fractions: organic phase, aqueous phase and residual solid. The top hexane phase was collected and then it was heated to dryness in the oven (at 70 °C) to enable gravimetric quantification of the lipid extract.

4.3.5. Analytical techniques

Light microscope

The effects of the steam explosion procedure on cell morphology were examined by light microscopy with a Zeiss Axio Scope A1 (Carl Zeiss Light Microscopy, Jena, Germany) microscope equipped with Nomarski interference contrast optics. Light micrographs were obtained with a digital camera JENOPTIK ProgRes Speed Xtcore 3.

Dry matter and ash content (TGA)

In order to check the mass balance of the steam explosion and membrane filtration processes, the dry ash free (DAF) weight of the samples was measured by means of a thermogravimetric analysis (TGA), using a LECO instrument (TGA701). For the dry matter content determination, the sample was dried in a nitrogen atmosphere at 105 °C to constant mass. The ash content was determined by increasing the temperature up to 550 °C under oxygen atmosphere.

Bligh and Dyer

Lipids were extracted from the fresh and steam exploded microalgal biomass using the Bligh and Dyer method which uses a ternary system of chloroform/methanol/water and is the most commonly used method for the quantitative extraction of lipids from microalgae at analytical level (Bligh and Dyer, 1959). In this method, 20 mL of the microalgal sample were mixed with 75 mL of a mixture chloroform-methanol (1:2 v/v) using a magnetic stirrer at 300 rpm for 10 min. Then 25 mL of chloroform and 25 mL of distilled water were added to form a two phase system. The phases were separated by 10 min centrifugation at 4000 rpm. The chloroform phase was then separated (after carefully transferring the mixture to a separatory funnel) and the solvent was evaporated using a rotary evaporator.

Finally, the amount of lipid obtained from each sample was measured after further drying overnight in an oven at 70 °C.

Analytical acid hydrolysis

The fresh microalgal samples were subjected to analytical acid hydrolysis in order to determine the total extractable sugars, following a standard procedure (ASTM D1106-84). This method is commonly used with lignocellulosic materials, but has been also previously applied for the analysis of microalgal biomass (72). In brief, 300 mg of freeze dried algal biomass was subjected to a two-stage sulphuric acid hydrolysis: 1 h at 30 °C in 72 % (w/w, wet basis) sulphuric acid in a water bath, followed by 45 min at 120 °C in 4 % (w/w, wet basis) sulphuric acid in an autoclave. After hydrolysis, the acid insoluble residues were separated from the hydrolysate using glass fiber filters (pore size <0.2 μm) and an aliquot of the hydrolysate was assayed quantitatively for component sugars by high performance liquid chromatography (HPLC).

Sugar analysis

The identification and quantification of the monosaccharides present in solution in the microalgal samples was achieved by HPLC analysis using a Biorad Aminex HPX-87H column (300 mm x 7.8 mm) and a refraction index detector. The temperature of the column was maintained at 50 °C and a solution of sulphuric acid 5 mM was used as mobile phase at a flow rate of 0.5 mL/min. Monomeric sugars were identified by comparing their retention times with those of standards and quantification was based on integration of individual peaks in the chromatograms together with the use of a calibration curve prepared with the standards. The determination of total sugar amount was achieved by integration of the sum of all identified peaks present in the HPLC chromatograms.

Particle size distribution

Particle size distribution was measured using a Malvern Mastersizer 2000 equipment with the Hydro 2000 MU module for liquid samples. 500 mL of demineralized water was used as medium and sludge sample drops without further treatment were added to that volume until getting an appropriate obscuration level stated by the equipment. Blue laser light was used.

In order to check if particles present in the sludge had been aggregated, measurements were also performed with same samples at two different levels of sonication: 3/20 and 12/20 (sonication levels following machine specifications).

Optical density

In order to confirm total particle or oil rejection after membrane filtration, the turbidity of permeate was estimated by measuring absorbance at 750 nm. Absorbance was measured in 96 well plates using a microplate reader (INFINITE M200 PRO, Tecan). Values were converted to optical density ($OD_{750\ nm}$) by dividing them by the pathlength. The OD_{750nm} of filtered (0.45 µm) seawater was used as reference.

4.4.Results and discussion

4.4.1. Steam explosion

In this study, microalgal biomass was subjected to acid catalysed steam explosion treatment and the resulting exploded material was subsequently fractionated to separate the different fractions. By measuring the dry ash free weight of the samples before and after the steam explosion treatment, a mass balance closure of 97 % was determined.

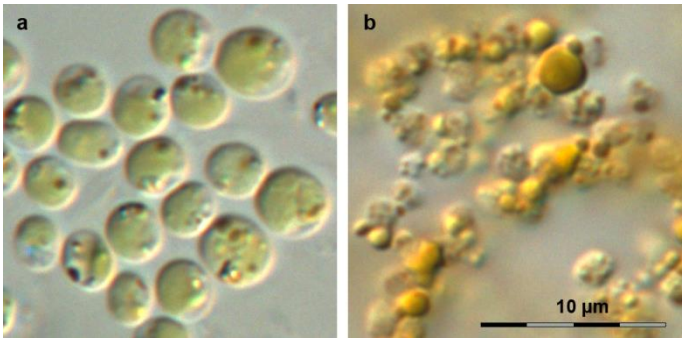


Figure 4.4: **Light micrographs of *Nannochloropsis gaditana*, before (a) and after (b) steam explosion.**

Light microscopy of defrost material (Figure 4.4a) showed intact cells, with yellow-green parietal chloroplasts. After steam explosion (Figure 4.4b) no algal cells were found. Instead, algal material was unevenly distributed in aggregates of particles of different sizes, some of which of a yellow-brown colour could correspond to chloroplast remains. Accordingly, particle size distribution results showed that aggregates are formed due to the steam explosion process (Figure 4.5). No particles with the original mean size (around 3 µm) were detected even after sonication was applied. This result indicated that microfiltration membranes would be enough to reject particles present in the solution.

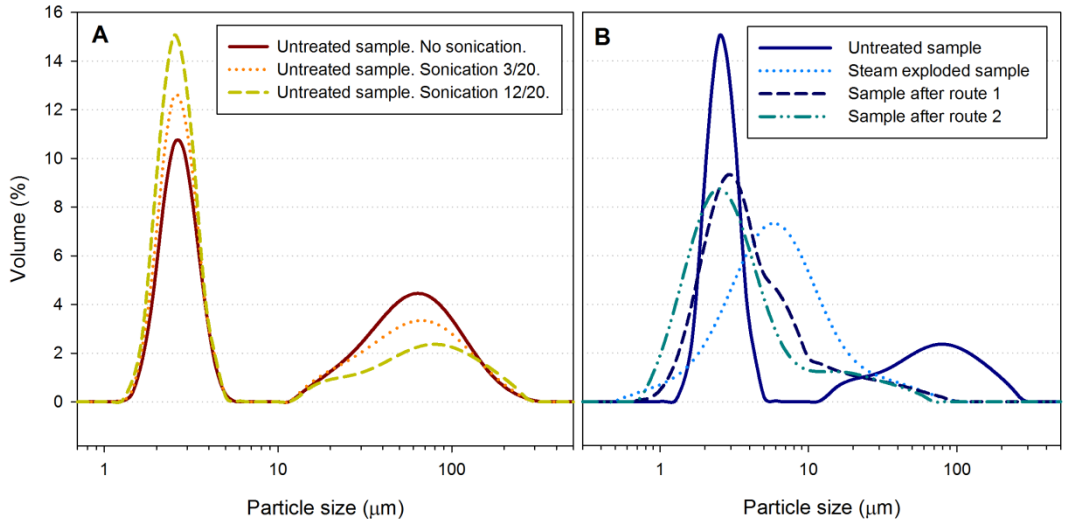


Figure 4.5: **Particle size distributions. A) Effect of sonication on the untreated sample. B) Results of different samples with sonication 12/20.**

The total lipid contents of the untreated microalga *Nannochloropsis gaditana* and the steam exploded sample, as determined by the Bligh and Dyer method, were found to be 22.2 % and 22.3 % (w/w, DAF of untreated microalga basis), respectively. Although it is known that the Bligh and Dyer method yields the highest lipid recoveries, the process is unsuitable for large scale industrial application due to environmental and health risks associated with the use of chloroform (73). For this reason, the use of n-hexane was considered as a better organic solvent candidate for lipid isolation from microalgae. n-Hexane was selected among other solvents because of its relatively low cost, low toxicity and easiness of recovery (74). The extraction experiment performed with the untreated microalgae showed very poor extraction capability of n-hexane, with 2.1 % (w/w, DAF basis) lipid yield. On the other hand, the amount of lipid extracted with n-hexane greatly enhanced as a result of applying the steam explosion technique. The steam exploded sample (at 150 °C, with 5 % sulfuric acid) yielded 17.6 % (w/w, DAF of untreated microalga basis) lipid recovery, which represents 79 % of the total lipid as obtained by the Bligh and Dyer method. This result is in agreement with our previous study (69), and shows the importance of carbohydrate hydrolysis due to the present of acid, besides cell disruption to achieve a higher lipid extraction yield from microalgal biomass samples, using n-hexane as solvent.

Regarding the carbohydrates analysis, the total sugar content of the untreated microalga, obtained by analytical acid hydrolysis was found to be 18.8 % (w/w, DAF basis), and as a consequence of the acid catalysed steam explosion treatment, the measured concentration of sugar in the solution was 12.9 % (w/w, DAF of untreated microalga basis).

4.4.2. Fractionation Route 1

According to the fractionation strategies proposed in the present study (see Section 2.1 and Fig. 1), Route 1 consists in subjecting the steam exploded sample to membrane filtration and then to extract with solvent the retentate and permeate streams. The filtration of steam exploded microalgae was performed with conventional cross-flow and dynamic filtration equipment. Within several membranes tested with MWCO between 90 Da and 0.2 µm, PE5 (MWCO = 5,000 Da) and PV400 (MWCO = 100,000 Da) exhibited the best performance in the filtration experiments in terms of permeability as well as irreversible fouling properties. Therefore, filtration experiments with these two membranes were further studied.

Table 4.1: **Results of mass balance and lipid and sugar analysis of filtration experiments with conventional cross-flow set-up.**

		PE5		PV400	
	Steam exploded sample	Retentate	Permeate	Retentate	Permeate
Total weight (g)	1500 g	1172 g	328 g	788 g	712 g
DAF percentage	5.1 %	6.0 %	2.1 %	7.4 %	2.7 %
Lipid (w/w, DAF of untreated	17.6 %	17.8 %	0.03 %	16.9 %	0.05 %

microalga basis)					
Sugar (g/L)	6.8 g/L	6.8 g/L	6.6 g/L	6.6 g/L	6.9 g/L

Table 4.1 and Table 4.2 present some results of the filtration experiments with conventional cross-flow and dynamic set-ups, respectively, including the total weight and DAF percentage and the lipids and sugars content of each of the different streams involved in the process (steam exploded sample, retentate and permeate). From the values of the DAF percentage in Tables 1 and 2, we can observe that different levels of concentration of the retentate streams were obtained (from 6 % to 10 % DAF), depending on the membrane employed and the extent of the filtration, which is determined by the amounts of permeate.

Table 4.2: **Results of mass balance and lipid and sugar analysis of filtration experiments with dynamic set-up.**

		PE5		PV400	
	Steam exploded sample	Retentate	Permeate	Retentate	Permeate
Total weight (g)	6000 g	2400 g	3600 g	2465 g	3535 g
DAF percentage	5.1 %	10.1 %	1.8 %	8.9 %	2.5 %

Lipid (w/w, DAF of untreated microalga basis)	17.6 %	17.3 %	0.08 %	17.5 %	0.08 %
Sugar (g/L)	6.8 g/L	5.9 g/L	6.0 g/L	6.6 g/L	6.9 g/L

The amounts of lipid extracted with n-hexane from the steam exploded and the retentate and permeate are also included in Table 4.1 and Table 4.2. It must be noted that these values are expressed as a percentage of the DAF weight of the untreated microalgal sample, to allow for better comparison. In all the cases, the permeate streams have an almost negligible content of lipids. This result was also confirmed by optical density measurements. $OD_{750\ nm}$ of permeates were similar to that of filtered (0.45 μm) seawater (Table 4.3) and hence we assume that lipid rejection was obtained in all the experiments. The absence of lipids in permeate implies that the studied membranes (PE5 and PV400) are suitable to reject lipids.

Table 4.3: **Optical density at 750 nm after filtration of steam exploded microalgae. Raw values are compared to filtered (0.45 µm) seawater (blank). Values are expressed as mean and the standard deviation is provided in brackets.**

		OD_{750nm}	
Membrane commercial names	Filtration technique	Blank	Permeate
PE5	Cross-flow	0.070 (0.001)	0.082 (0.001)
	Dynamic	0.081 (0.001)	0.091 (0.003)
PV400	Cross-flow	0.082 (0.001)	0.105 (0.002)
	Dynamic	0.082 (0.002)	0.098 (0.001)

On the other hand, the extraction of lipids from the retentate streams provided a similar amount as in the extraction of the steam exploded sample before filtration. Therefore, we can confirm that the concentration of sample by filtration (up to the levels in this study) does not have an effect on the extraction ability by hexane. The importance of this result lies in the fact that by concentrating the sample, there is a reduction in the amount of solvent needed for extraction and thus a reduction in operating costs.

Concerning the sugar analysis, approximately the same values of concentration were obtained for the steam exploded sample and retentate and permeate streams, in the different filtration experiments (see Table 4.1 and Table 4.2). This means that neither of the employed membranes (PE5 and PV400) are able to retain sugars. As a result,

in order to get most of the carbohydrates as a separate fraction, it would be desirable to perform the filtration to a high extent to get a high amount of permeate.

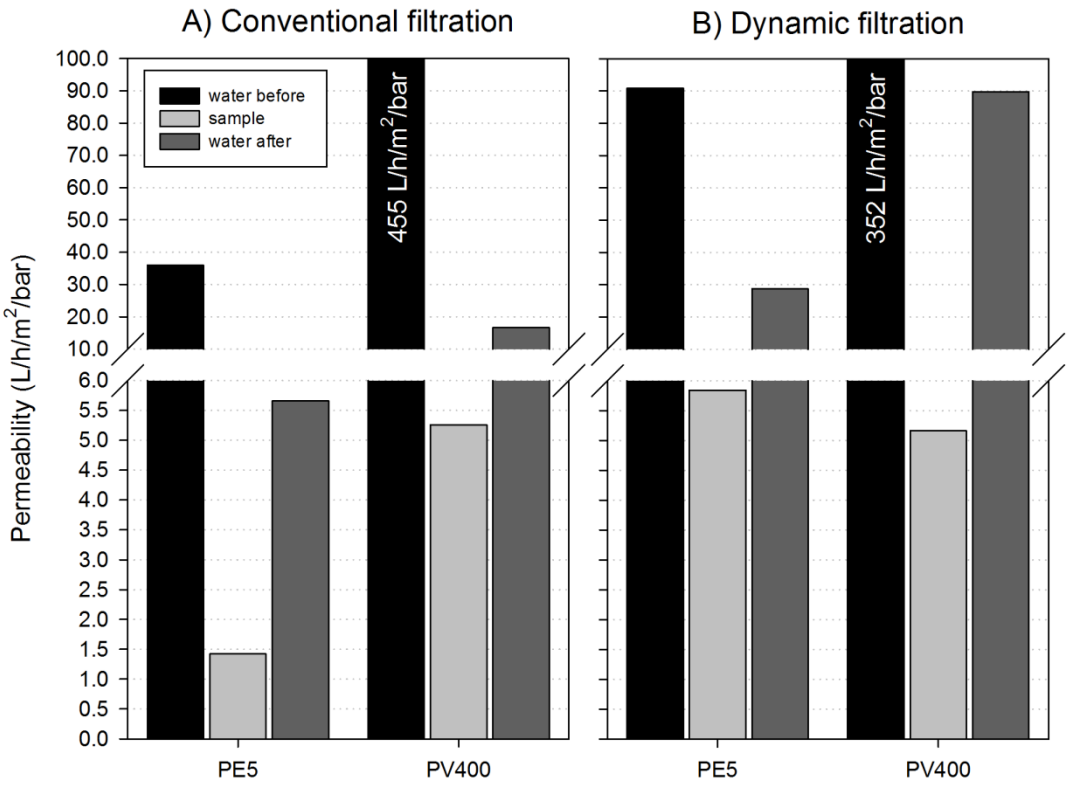


Figure 4.6: **Water and sample permeabilities with the membranes and set-ups used.**

Regarding the performance of the membranes during conventional cross-flow filtration, (Figure 4.6A) presents membrane permeabilities including water permeability with the new membrane and after the experiment. These results allow calculating irreversible fouling of materials. The permeabilities of steam exploded microalgae were measured and used in order to calculate the total fouling of membranes. PV400 showed greater water permeability than PE5, as could be expected after comparing the molecular weight cut-off (MWCO) of each membrane. For the virgin membranes PV400 had a permeability of 455 L/h/m^2/bar, when PE5 performed with the value of 36.0 L/h/m^2/bar. Regarding pretreated microalgae filtration, significant fouling was observed. PE5 exhibited microalgae permeability

of 1.42 L/h/m^2/bar, when PV400 performed with the value of 5.26 L/h/m^2/bar. However, in terms of total fouling, the results obtained for PE5 gave the value of 25.3, which is over three times lower than PV400 (TF = 86.5). It means that in the perspective of membrane lifetime, PE5 offered better performance due to less fouling. After performing the experiment and cleaning the system, the irreversible fouling was determined by measuring the water permeability for used membranes. Although PV400 had a permeability of 16.6 L/h/m^2/bar and PE5 exhibited the value of 5.66 L/h/m^2/bar, the membrane with higher irreversible fouling with the factor of 27.3 was PV400, as PE5 resulted in IF = 6.36. The reason of this difference might be easier pore blocking, as well as further cake formation over the surface of membrane with bigger MWCO.

On the other hand, Figure 4.6 B) presents membrane permeabilities obtained using vibratory shear enhanced processing setup. Results include water permeability with the virgin membrane and after the experiment. The permeabilities of steam-exploded biomass were measured and used in order to calculate the total fouling of materials. Concerning water permeability for dynamic filtration, higher value of 352 L/h/m^2/bar was obtained with PV400, when PE5 resulted in the permeability of 90.8 L/h/m^2/bar. Again, water permeability differences were those expected due to the MWCO of the membranes. In terms of pretreated microalgae filtration, PE5 resulted in microalgae permeability of 5.84 L/h/m^2/bar, when PV400 gave the value of 5.16 L/h/m^2/bar. When compared to the results of cross-flow filtration experiments, the TF of PE5 decreased almost two times, with the value of 15.55 In case of PV400, TF also decreased from the value of 86.51 in conventional technique to 68.21 in dynamic filtration. In order to calculate irreversible fouling, water permeability before and after the experiment was measured (including cleaning the system before

performing the water permeability measurements after the experiment). PE5 resulted in the permeability of 28.7 L/h/m^2/bar and PV400 performed with the value of 89.7 L/h/m^2/bar, therefore the membrane with lower irreversible fouling factor of 3.16 was PE5, while PV400 resulted in IF = 3.92. Huge improvement can be observed when compared to the conventional technique, irreversible fouling factor for PE5 is twice lower with dynamic filtration and for PV400 it decreases seven times. It means that dynamic filtration decreases irreversible fouling by reduction of cake formation over the membrane and pore blocking. Moreover, these results indicated an economic improvement considering that the extra energy required for the vibration setup represents only about 10% of the pumping cost.

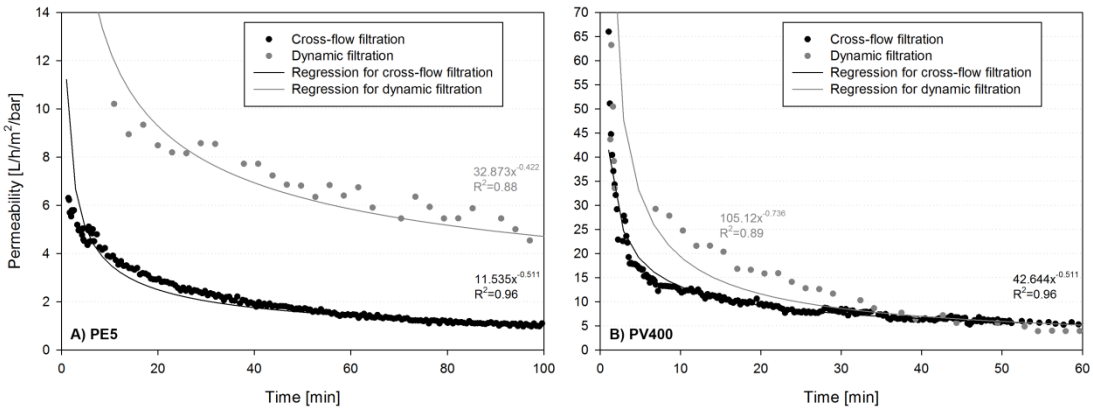


Figure 4.7: **Permeability profiles vs. time of filtration experiments performed with the membranes and set-ups used.**

In order to compare the filtration techniques employed, Figure 4.7 presents steam exploded microalgae permeability profiles with time for both conventional cross-flow and dynamic filtration. In all cases, fouling is observed and the permeability decrease with time follows a power correlation. Nevertheless in conventional cross-flow filtration the permeability decrease is higher at the beginning of the operation. Vibrational filtration offers a better performance in the case of the ultrafiltration membrane (PE5). For this membrane, the permeability value at the end of the experiment is three times higher than in the case of the conventional filtration. For

the case of the microfiltration membrane (PV400), although at the beginning the permeability is higher in vibrational filtration, at the end of the experiment the values are similar. Thus, the performance improvement (fouling reduction) that the vibrational system provides is significant in the case of ultrafiltration.

4.4.3. Fractionation Route 2

The fractionation strategy in Route 2 consisted in extracting the steam exploded sample with n-hexane to obtain the lipid fraction and then filtering the raffinate. The results obtained following Route 2 indicated that a similar amount of lipids was extracted from the sample as compared to the filtered samples in Route 1. However a much larger amount of hexane needed to be used in Route 2. This is an important drawback from the industrial point of view since the energy to recover the solvent (distillation) is much higher. Therefore, regarding the lipid extraction it is more interesting to follow the fractionation Route 1.

Membranes tested in this route included 1000 Da membrane. This membrane also rejected all the remaining lipids and allowed the sugars to keep the same concentration in the permeate as in the feed.

Particle size distribution results confirmed that permeates were particle-free. Further, the results (Fig. 4B) showed that the filtration process favors disrupting the aggregates formed during steam explosion, recovering the size of the original particles present in the sludge. There were no differences on size distribution in regard of the route followed.

In order to study the possibility of sugar concentration, in preliminary experiments three nanofiltration membranes were tested. The smallest pore size nanofiltration membranes (90 Da and 200 Da) did not perform properly due to fouling and small

membrane area. A 550 Da membrane in spite of having an acceptable flow rate permeate was not efficient enough to reject the sugars.

Nevertheless, we intend to experimentally investigate this path in the future, in order to evaluate the filtration process with special attention to the carbohydrates fraction separation.

4.5. Conclusions

Steam explosion produced a complete disruption of the microalgae cells allowing the extraction of the lipids with an organic solvent. At the same time a hydrolysis of carbohydrates was achieved producing an aqueous phase containing monomeric sugars. Membrane filtration allowed separating the aqueous phase (permeate) from the rest of the fractions retaining the lipids. Dynamic filtration provided a better permeability with just a little bit more of used energy compared to tangential cross-flow filtration. After filtration, lipid extraction can be performed in the retentate using a smaller amount of solvent keeping a high extraction yield.

Acknowledgements

This work was supported by the projects CTQ2014-56285-R "Cultivo, concentración, fraccionamiento y obtención de producto en refinería de microalgas" funded by the Spanish Ministry of Economy and Competitiveness and "Fuels from Biomass" funded by Excma. Diputació de Tarragona.

The research was also supported by the European Regional Development Funds (ERDF, FEDER Programa Competitividad de Catalunya 2007-2013).

Thanks to Oihana Núñez and Núria Descarrega for their collaboration in the experimental work.

5

CELL DISRUPTION AND FRACTIONATION OF SEVERAL MICROALGAE SPECIES[4]

This chapter describes the work regarding improvement of microalgae biorefining downstream operations. Experiments were focused on cell disruption and fractionation steps recovering lipids, sugars and proteins. Steam explosion and dynamic membrane filtration were used as unit operations. Species used were Nannochloropsis gaditana, Chlorella sorokiniana and Dunaliella tertiolecta with different cell wall characteristics.

[4] This chapter is based on the following publication:
E. Lorente, M. Hapońska, E. Clavero, C. Torras, J. Salvadó, *Steam Explosion and Vibrating Membrane Filtration to Improve the Processing Cost of Microalgae Cell Disruption and Fractionation,* Processes, Volume 6, Issue 4, 2018, Article number 28, ISSN 2227-9717, DOI: 10.3390/pr6040028

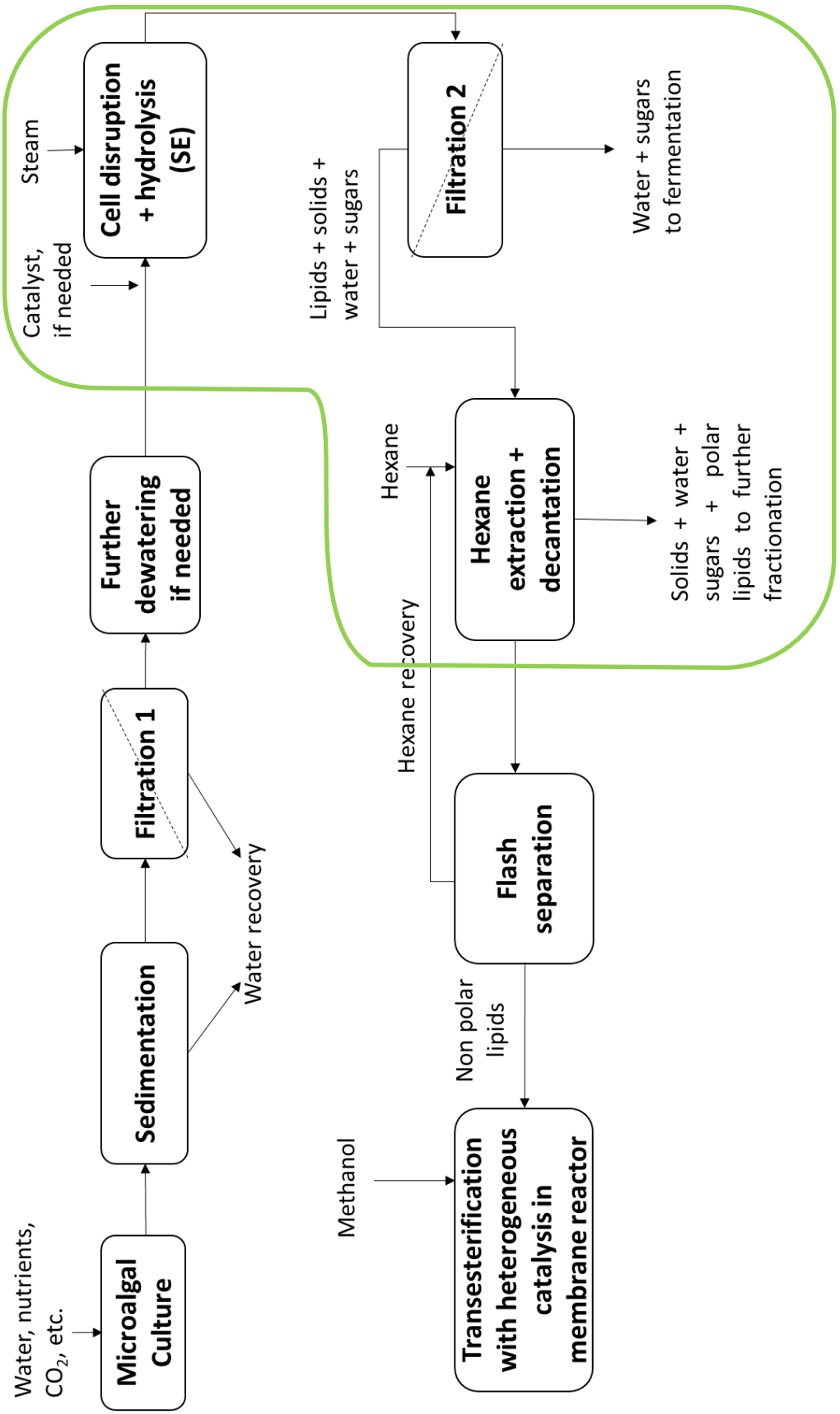


Figure 5.1: **Proposed scheme of microalgae biorefinery - steam explosion cell disruption and fractionation.**

5.1.Introduction

Around 10 years ago, the idea of using microalgae as a very efficient photosynthetic crop to provide energy was re-adopted (29), following the results obtained in earlier studies (75). Microalgae appeared as a good alternative to produce transportation fuels in the context of energy crisis and climate change.

Cost barriers in the several stages of mass production of energy vectors appeared. This resulted in having to re-address improvements in culture, harvesting, cell disruption, lipid extraction, and final production.

The production of biofuels from microalgae results in a variety of returns. These include a high lipid content, no competition for arable lands, and the use of a variety of water qualities, including wastewaters during the cultivation period (76). However, it has become clear that the option to produce only fuel from microalgae is not economically viable (77).

Researchers have learned from the preliminary results that, apart from reducing the costs of microalgae production, benefits have to be obtained from all fractions while also looking for other side paybacks in order to have an economically feasible production (78).

To achieve a positive economic balance, several matters should be taken into account such as CO2 capture (79,80), water quality improvement (81,82), procurement of commodities (83,84), and high-added value products (85,86).

The process unit operations needed in order to proceed to microalgae biorefining depend very much on the strain and products to be sought (i.e., commodity or high-added value products), but a typical sequence is: Culture in open ponds or

photobioreactors (87,88), dewatering (18,66,71,89–91), cell disruption (21,52,92–95), and fractionation (71,86,96).

In the present study, the focus is on the operation of cell disruption by using steam explosion, and secondly, in the process of fractionation.

Although steam explosion has been in use from the beginning of the 20th century, it has only been used in a few cases for microalgae biorefining, and therefore a comparison of the results can hardly be performed (92,97). As regards the results of previous work (21), steam explosion is used at relatively mild conditions to break the cell walls and produce the hydrolysis of carbohydrates.

Depending on the strain cell wall characteristics, cell disruption can be a cost-intensive operation and several procedures have been reported at the laboratory level, including the use of ultrasounds, microwave, or high pressure (94,98). Steam explosion is proposed in this work as an innovative technique for this application and is easy to scale-up with pilot plant results because of the nature of the equipment and because it is widely used industrially (52,92). Steam explosion has given the best results when compared with other methods for cell disruption such as ultrasonication, microwave, and autoclave (69). Beyond breaking the cell wall, if a low concentration of acid is used, steam explosion can hydrolysate the poly-saccharides in the cell and produce sugars in a first stage of fractionation (69). The main energy input for the steam explosion process is heat, thus reducing the cell disruption costs considering other techniques like sonication and that residual heat can be used. It should be stated that steam explosion is a commercial high-throughput available technology. A pilot plant with a capacity of 2 Tm/h has already been operated successfully with lignocellulosic materials from 1991 (99).

Membrane filtration and solvent extraction are methods to be used for fractionation (52,96). In a first unit operation, membrane filtration can be used to obtain two streams: a retentate containing lipids and proteins and a permeate containing water with the hydrolyzed monosaccharides (52). As in microalgae dewatering, fouling is a main drawback. To overcome this problem, dynamic filtration provides an adequate solution (100). Also, the use of ultrafiltration membranes (instead of microfiltration) increases permeability (71). In a second unit operation, sugars could be concentrated using nanofiltration membranes (101). To recover non-polar lipids from the retentate stream of the first operation, a hexane extraction is used. In our previous work (52), the microalga Nannochloropsis gaditana was selected to investigate the fractionation strategy for lipids and carbohydrates recovery. In this study, we intend to validate the selected fractionation path when different common microalgae species were used: Chlorella sorokiniana (102), Nannochloropsis gaditana (103), and Dunaliella tertiolecta (104). They are representative of different types of species of freshwater and marine strains. They have also been chosen because they represent different levels of strength in their cell walls. N. gaditana and C. sorokiniana are two species with recalcitrant cell walls, whereas D. tertiolecta lacks a cell wall. The cell wall of N. gaditana is primarily cellulose (75%) (105). This inner cellulose layer is protected by an algaenan layer which is assumed to be primarily responsible for the wall's recalcitrance to breakage (105). Besides, the C. sorokiniana cell wall contains little glucose (106) and therefore its cell wall may lack cellulose. On the other hand, the presence of algaenan in the C. sorokiniana cell wall may depend on the physiological state of the culture (107).

The study about the use of steam explosion will provide a basis of cost comparison with those technologies that use electrical power to operate.

5.2.Materials and methods

5.2.1. Microalgae samples

A semi-closed photobioreactor, with a 3050 L capacity and placed outdoors, was used for growing *Nannochloropsis gaditana* Lubián (strain CCMP1775, Provasoli—Guillard National Center for Marine Algae and Microbiota). A more detailed description of the photobioreactor is given in Nurra et al.(21). Cultures were performed between May and July, when the mean temperature ranged from 27 °C to 33 °C. The medium for the *N. gaditana* culture consisted of seawater enriched with 0.3 mL/L of Codafol 14.6.5 (Coda Sustainable Agro Solution S.A.). This plant fertilizer contains, in w/w, 14% nitrogen, 6% P2O5, 5% K2O, 0.1% Fe, 0.05% Zn, 0.05% Mn, 0.05% Cu, and 0.001% Mo.

Chlorella sorokiniana (strain CCAP 211/8k) and *Dunaliella tertiolecta* (strain CCAP19/6B) were grown indoors in column photobioreactors (300 L, 50 cm diam.) aerated with air and illuminated with Philips MASTER TLD 58 W/865 fluorescents giving an irradiance at the photobioreactor surface of 300 μmol photon/m2/s. C. sorokiniana was cultured at 22 ± 3 °C in tap water enriched with the following nutrients: NaNO3 (5.8 mM), K2HPO4·3H2O (0.092 mM), KH2PO4 (0.28 mM), Na2EDTA (0.045 mM), FeCl3·6H2O (17.9 μM), ZnSO4·7H2O (1.69 μM), MnCl2·4H2O (4.48 μM), Na2MoO4·2H2O (0.10 μM), CuSO4·5H2O (0.17 μM), and CoCl2·6H2O (0.06 μM). *D. tertiolecta* was cultured at 20 ± 3 °C in artificial seawater prepared with tap water and 37 g·L−1 of Aquaforest Reef Salt® enriched with NaNO3 (4.4 mM), Na2HPO4·2H2O (0.04 mM), and the same micronutrient concentrations as in *C. sorokiniana*. Phosphate was fed-batch to increase the concentration of the culture to3.2 μM to avoid precipitation, presumably with magnesium and calcium ions.

All the cultures were harvested some days after the stationary phase of growing was reached, except for the cultures of *D. tertiolecta* used in the steam explosion treatment without acid, which were harvested at the end of the log phase.

A continuous centrifuge (Clara 20 High Flow, Alfa-Laval, Lund, Sweden) was used to concentrate the microalgal biomass samples. The centrifuge was operated at 9060 rpm, using a counter pressure of 4 bar. A Seepex progressive cavity pump (BN series) was used to feed the sample with 1000 L/h of a nominal flow rate. After concentration, the samples *N. gaditana* and *C. sorokiniana* were frozen at −80 °C. For defrosting the samples, they were placed at 4 °C for two days, prior to the steam explosion procedure. *D. tertiolecta* was harvested and concentrated just before the biorefinery process to avoid extra actions that might break its naked cells.

5.2.2. Steam explosion

The equipment for the steam explosion of microalgae consisted of a 16 L reactor, operated in batch, and a collection vessel. The generation of steam was achieved with an electric boiler (Boreal, 380 V/82 kW) and thermally isolated high-pressure pipes were used to conduct the steam to the reactor. This was regulated by two valves placed in series, which were used to control the entrance of steam into the reactor. In the upper part of the reactor, there was a valve (2″ diameter) for feeding the sample. In the bottom of the reactor, a flash valve allowed a fast decompression to the collecting tank at atmospheric pressure. The tank consisted of a cylinder with a capacity of 100 L and a diameter of 50 cm. It had two valves, one for steam release and another for the collection of sample in liquid phase.

In each experiment, 4 kg of microalgae was introduced into the reactor, which had been preheated. Some samples were previously impregnated with sulphuric acid at a concentration of 5% (w/w, wet sample basis) by mixing for 2 h at room temperature.

The steam explosion pre-treatments were conducted at 150 °C (which corresponds to a saturated steam pressure of 4.7 bar) with a retention time of 5 min. The selection of the experimental conditions, which includes temperature, time, and acid concentration, was performed in a previous study [29]. After reaction and before the fractionation experiments, the exploded samples were collected and neutralized (to pH 5).

5.2.3. Filtration

A Vibratory Shear Enhanced Processing (VSEP, serie L, New Logic Research, Inc., Emeryville, CA, USA) system was used to perform dynamic membrane filtration experiments. A detailed description of this filtering system can be found elsewhere (18). Approximately 6.0 kg of microalgal sample was used for each experiment, with a transmembrane pressure of 5 bar and a vibrational frequency of 55.4 ± 0.1 Hz.

Experiments were performed with PE5, a commercial polymeric membrane (Nanostone, Eden Prairie, MN, USA), manufactured from polyether-sulfone and with a molecular weight cut-off (MWCO) of 5000 Da. The filtration area was 0.0446 m2.

Water flux measurements were performed in order to determine the permeability of virgin membranes. After that, the steam exploded microalgae biomass was filtered and measurements of permeability vs time were conducted during the experiment. The permeability with pre-treated algae was determined at the fixed time of 60 min. Finally, after cleaning, the system water permeability was measured again. The last step allowed for the determination of the irreversible fouling resistance of membranes. Also, two factors could be calculated, i.e., the irreversible fouling factor (IF), which is determined as the ratio of water permeabilities before and after the experiment, and total fouling factor (TF), consisting of the ratio between virgin

membrane permeability with water and microalgae sludge permeability. In all cases, permeability was calculated from measurements of permeate mass weight progress with time. Permeate output was driven to a vessel placed on a scale, which was connected to a computer. An own-made software was recording and calculating permeability in real time to assess experimentation. Permeability was determined as follows. For water, measurements were performed at three different transmembrane pressures between the recommended range given by the manufacturer to ensure that a linear correlation between both parameters was achieved. For microalgae sludge, flow rate measurements were being performed with an interval of 10 s..

5.2.4. Lipid extraction

The lipids from microalgal samples were extracted by contacting the same volume of sample and of n-hexane (20 mL). The extraction conditions were 60 °C and agitation at 800 rpm, for 2 h. After the contact time, separation was achieved by centrifugation at 4000 rpm for 10 min. The mixture partitioned into three fractions: organic phase, aqueous phase, and residual solid. To extract and quantify lipids, the top hexane phase was recovered and was then heated to complete dryness in the oven (at 70 °C).

5.2.5. Analytical techniques

Light microscope

A Zeiss Axio Scope A1 (Carl Zeiss Light Microscopy, Jena, Germany) microscope, equipped with Nomarski interference contrast optics, was used to check the effects of the steam explosion technique on cell morphology. A digital camera JENOPTIK ProgRes Speed Xtcore 3 was used to obtain the light micrographs. Objective magnifications from 10 to 100 were used.

Dry matter and ash content (TGA)

Thermogravimetric analyses (TGA), with a LECO instrument (TGA701), were performed in order to determine the dry ash free (DAF) weight of the samples which allows us to verify the mass balance during the steam explosion and membrane filtration processes. The samples were dried in a nitrogen atmosphere at 105 °C to constant mass, for the dry matter content determination. After that, the atmosphere was changed to oxygen and the temperature was increased up to 550 °C, in order to determine the ash content.

Total Lipid Extraction with Bligh and Dyer Method

The Bligh and Dyer method was used to extract the lipids from the fresh and steam exploded microalgal biomass. This method is the most commonly used at the analytical level for the quantitative extraction of lipids from microalgae (108).

Analytical acid hydrolysis

In order to determine the total extractable sugars, analytical acid hydrolysis experiments were conducted with the fresh microalgal samples, following a standard procedure (ASTM D1106-84). Although this method was originally used with lignocellulosic materials, microalgal biomass has also been previously analyzed [41]. The process consists of sulphuric acid hydrolysis in two stages. In the first stage, the freeze dried algal biomass sample (300 mg) is placed in contact with 72% (w/w, wet basis) sulphuric acid in a water bath at 30 °C, for 1 h. In the second stage, the sample is diluted to a concentration of 4% (w/w, wet basis) sulphuric acid and placed in an autoclave at 120 °C, for 45 min. After hydrolysis, filtration is performed using glass fiber filters in order to separate the acid insoluble residues from the hydrolysate. Finally, HPLC (high performance liquid chromatography) analyses were performed to quantitatively determine the sugar contents.

2.5.5. Monosaccharides analysis

HPLC analyses were conducted in order to identify and quantify the monosaccharides present in the microalgal samples in solution. A Biorad Aminex HPX-87H column (300 mm × 7.8 mm) at 50 °C was used, with a refraction index detector. Additionally, the mobile phase was a 5 mM solution of sulphuric acid with a flow rate of 0.5 mL/min. The identification of monomeric sugars was achieved by a comparison of retention times with those of the standards. The integration of peaks in the chromatograms allowed the quantification, using a calibration curve, which was previously prepared with the standards.

Protein analysis

Two different methods were used for protein analysis, namely solubilization and hot NaOH. To quantify the proteins released by the steam explosion treatment, the solubilization method was used. In this method, proteins were suspended by mixing 0.2 mL of sample in 1 mL 0.1 N NaOH. After 1 h of incubation at room temperature, samples were centrifuged at 4000 rpm for 10 min. Protein in the supernatant was precipitated with trichloroacetic acid (TCA) to avoid interfering substances. Following Barbarino and Lourenço [42], proteins were precipitated with 25% TCA at the ratio of 2.5:1 (TCA:homogenate) and centrifuged at 4000 rpm. Pellets were consecutively re-suspended in 10% and 5% TCA and finally solubilized in 0.1 N NaOH for the Bicinchoninic acid protein assay (BCA kit, Sigma-Aldrich, St. Louis, MO, USA). Color development was measured as absorbance at 562 nm using a microplate reader (INFINITE M200 PRO, Tecan, Männedorf, Switzerland). Absorbance values were read against a standard curve generated with a protein standard (bovine serum albumin), and percentage protein was calculated on a dry weight basis.

Since cell disruption was not expected using the solubilization method, a stronger method (hot NaOH) that allowed cell wall disruption was also applied to the concentrated culture and the steam exploded sample to evaluate the effects of steam explosion. In this procedure, 0.5 mL samples were extracted with 0.5 mL 2 N NaOH with 0.5% β-mercaptoethanol (v/v) at 90 °C for 10 min and centrifuged at 4000 rpm. Proteins were precipitated with TCA and solubilized in 0.1 N NaOH for the Bicinchoninic acid assay, as explained previously. Both extraction methods were performed in triplicate.

Particle size distribution

A Malvern Mastersizer 2000 piece of equipment with the Hydro 2000 MU module for liquid samples was used for particle size distribution measurements. A blue laser light was used. The medium consisted of 500 mL of demineralized water and sludge sample drops were added without further treatment until obtaining an appropriate obscuration level (as stated by the equipment instructions).

Two different levels of sonication: 6 kHz and 24 kHz, were used in the measurements, in order to check if aggregation had occurred with particles present in the sludge.

Optical density

Absorbance measurements at 750 nm were performed to estimate the turbidity of the permeate, which can confirm total particle or oil rejection after membrane filtration. Absorbance was measured using a microplate reader (INFINITE M200 PRO, Tecan), and 96 well plates were used for the absorbance determinations. The optical density (OD750 nm) values were obtained by dividing the raw values over the path-length, and using as a reference the OD750 nm of filtered (0.45 µm) seawater.

5.3.Results and discussion

5.3.1. Steam Explosion Treatment of Studied Strains

A steam explosion experiment was performed for each microalgae sample, at 150 °C, for 5 min and using 5% sulphuric acid to impregnate the samples. An additional experiment was performed with D. tertiolecta, to analyze the effect of steam explosion without acid impregnation, since this microalga has no cell wall. By comparing the dry ash free weight values of the samples before and after the steam explosion treatment, good balance closures (>97%) were obtained for all the experiments.

5.3.2. Cell Morphology

The examination of cell morphology by light microscopy showed that *C. sorokiniana*, *N. gaditana*, and *D. tertiolecta* had experienced high levels of cell disruption after the steam explosion pretreatment (Figure 5.2). Original samples consisted of isolated cells, except for *C. sorokiniana*, which contained both single cells and cell aggregates, hence the bimodal distribution in Figure 5.3 B. Sonication dispersed cells and most aggregates were disintegrated. Accordingly, after sonication, the peak centered in ca. 3 µm, matching the *C. sorokiniana* cell size, was much higher, and the peak centered at ca. 20 µm which corresponds to aggregates almost disappeared.

Although *C. sorokiniana* appeared slightly damaged after thawing, with the cytoplasm slightly shrunken and retracted from the smooth cell wall, it was the less injured of the three species after steam explosion. *C. sorokiniana* cells showed three different patterns of disruption. Cells could be totally disrupted, algal material appearing as granulated aggregates. Cells could also maintain their unity but have

granular cytoplasm and wrinkled margins. In this condition, cells had a low contrast appearance, which reveals that shapes may be flatter, probably due to a thinner and softer cell wall. More often, *C. sorokiniana* cells maintained their unity and high contrast appearance with smooth margins, but the cellular content was homogeneous except for a central depression, and no intracellular organelles (like chloroplast or pyrenoid) could be detected. After thawing, *N. gaditana* cells had the same morphology as live cells. However, after steam explosion treatment, algal material was mostly unevenly distributed in aggregates. They correspond to particles of different sizes. Some of them presented a yellow-brown color and could correspond to chloroplast remains. In a few cases, cells were detected, but then they appeared with granular cytoplasm and wrinkled margins as the intermediate disruption pattern of *C. sorokiniana*. It should be noted that the cell disruption effect of steam explosion was not apparently enhanced by freezing because *N. gaditana*, the cell walled species whose morphology appeared more altered after thawing, was less affected by steam explosion. Naked cells of *D. tertiolecta* were strongly sensitive, even to the centrifugation process. After centrifugation, cells lost their internal structure or were totally disrupted. The steam explosion treatment further disintegrated the algal material and formed granulated aggregates. The same kind of cell debris was observed in the treatments with and without acid.

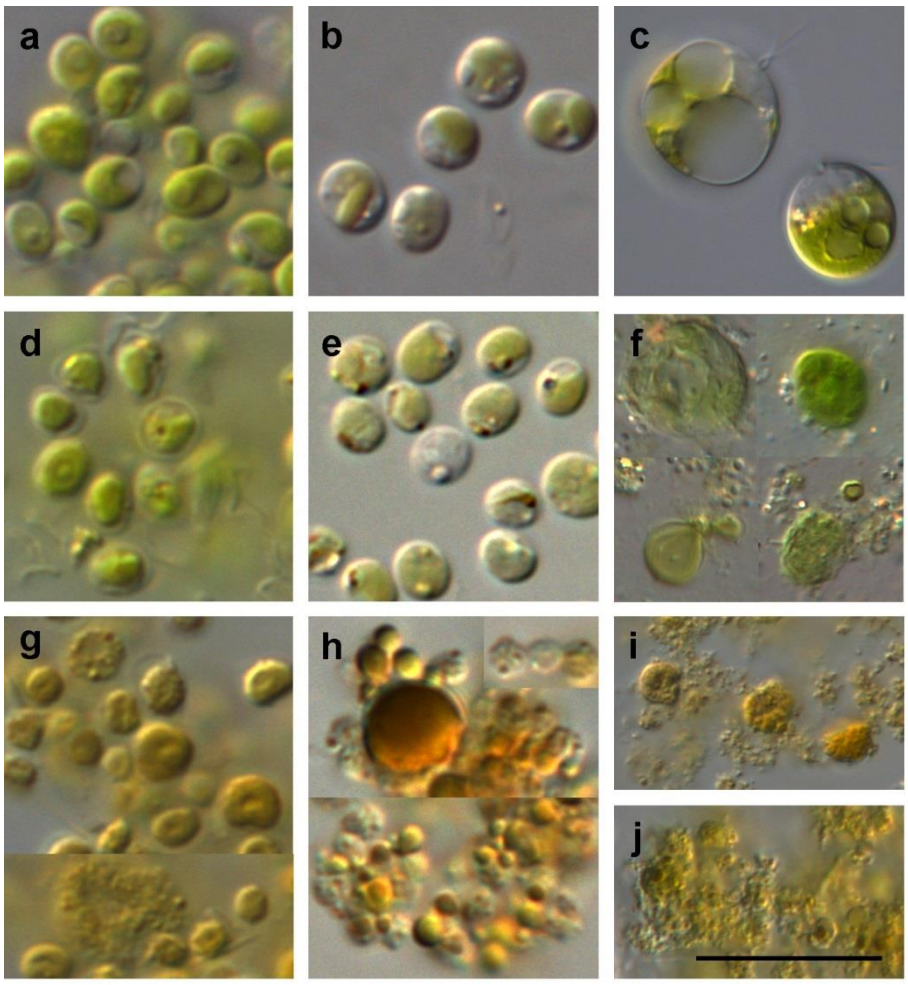


Figure 5.2: **Light micrographs of Chlorella sorokiniana (a,d,g), Nannochloropsis gaditana (b,e,h), and Dunaliella tertiolecta (c,f,i,j) before and after steam explosion. (a,b,c) Live cells; (d,e) Thawed material; (f) D. tertiolecta after centrifugation; (g,h,i) Algal material after steam explosion with acid; (j) D. tertiolecta after steam explosion without acid. Scale bar corresponds to 10 µm in (a–f) and to 20 µm in (g–j).**

5.3.3. Particle Size Distribution

Morphological characterization by means of microscopy was confirmed by the results obtained from particle size distribution (Figure 5.3).

Steam explosion produces aggregates when used with N. gaditana and *C. sorokiniana*. These aggregates disappear after filtration, probably due to the pump effect and the stress this caused. This effect is observed in almost all cases where these species were used. But this aggregation effect does not occur with *D.*

tertiolecta, where the particle size distributions are always similar. Nevertheless, a smooth shift of the unique existent peak occurs, indicating some mass aggregation as the microscopy images show. The mean size ranges from 3 µm to 30 µm, whereas the size of the nominal microalgae cell is around 15 µm. The sample regarding the filtration retentate is the one with a smaller mean particle size due to the disaggregating role of the pump. The samples related to steam explosion treatment performed with acid have mean particle sizes which are slightly smaller than those performed without acid. Concerning *D. tertiolecta*, it is interesting to note the ability of sonication to break the microalgae cells. This only happens with this species and is probably due to the fact that *D. tertiolecta* does not have a cell wall. With other species, sonication only breaks aggregates. This is only observed with the sample after being harvested, but not with samples after steam explosion and membrane filtration. The reason for this is that at those stages, cells are almost totally unstructured, in agreement with microscopy images.

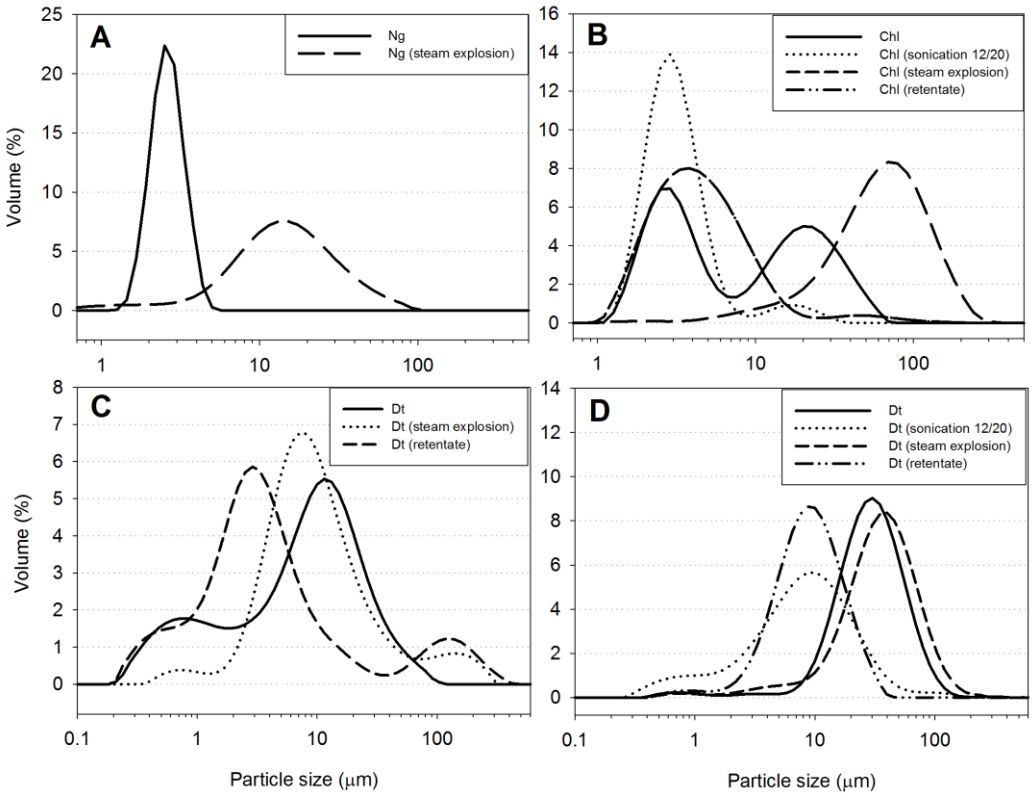


Figure 5.3: **Particle size distribution results. (A) Nannochloropsis gaditana (B) Chlorella sorokiniana (C) Dunaliella tertiolecta (steam explosion with acid) (D) D. tertiolecta (steam explosion without acid). In all cases except those indicated, sonication was 0/12. All plots were obtained from an average of three measurements.**

5.3.4. Lipid, Sugar, and Protein Contents

The Table 1 shows the results of the steam explosion experiments. The amount of lipid extracted (by Bligh and Dyer and n-hexane), sugar, and protein contents are indicated. For the purpose of comparison, the values of lipid, total sugar content, and proteins from the fresh untreated samples are also included.

Table 5.1: **Results of lipid, sugar, and protein analysis of steam explosion experiments (150 °C, 5 min and 5% *w*/*w*. H_2SO_4 except sample *D. tertiolecta* (II) with no acid). Values are expressed as the mean and the standard deviation is indicated in brackets.**

		Lipids		**Sugar**	**Protein**	
		Bligh & Dyer	**Hexane**		**Hot NaOH**	**Solubilization**
Nannochloropsis gaditana	Untreated	22.2% (0.4)	2.1% (0.3)	18.8% (0.8)	17.3% (0.8)	1.4% (0.1)
	Steam exploded	22.3% (0.1)	17.6%(0.2)	12.9% (0.6)	8.4% (0.6)	9.1% (0.4)
Chlorella sorokiniana	Untreated	13.0% (0.2)	0.6% (0.0)	23.5% (1.3)	19.2% (0.3)	2.2% (0.0)
	Steam exploded	11.8% (0.1)	4.8% (0.2)	18.6% (0.9)	9.2% (0.1)	10.7% (0.1)
Dunaliella tertiolecta (I)	Untreated	26.6% (0.8)	2.8% (0.7)	26.1% (2.2)	14.5% (0.5)	12.0% (0.3)
	Steam exploded	29.7% (3.2)	10.6% (0.1)	19.2% (0.8)	2.6% (0.0)	5.1% (0.3)
Dunaliella tertiolecta (II)	Untreated	11.4% (1.2)	1.6% (0.1)	25.8% (2.4)	10.5% (0.0)	5.9% (0.1)
	Steam exploded No acid	11.9% (0.1)	2.1% (0.0)	8.6% (0.6)	4.8% (0.1)	4.4% (0.4)

By comparing the total lipid contents, as determined by the Bligh and Dyer method, of the untreated and steam exploded samples, we can observe that similar values are obtained in all the cases. This is because the Bligh and Dyer method yields the highest lipid recoveries, because it is a stronger method. But the use of n-hexane was considered as organic solvent for lipid isolation from microalgae to avoid the use of chloroform, which presents environmental and health risks, especially when it is used at an industrial scale. The experiments performed with the untreated microalgae samples showed the low extraction capability of n-hexane, with a maximum of 2.8% (*w*/*w*, DAF basis) lipid yield in the case of *D. tertiolecta*. But the amount of lipid extracted with n-hexane improved with the application of the steam explosion technique. Among the three microalgae species studied, *N. gaditana* yielded the maximum amount of lipid recovery of the steam exploded sample (at 150 °C, with 5% sulfuric acid), with 17.6% (*w*/*w*, DAF of untreated microalga basis). It signifies 79% of the total lipid as obtained by the Bligh and Dyer method. For *C. sorokiniana*, the amount of lipid extracted after steam explosion (at 150 °C, with 5% sulfuric acid) was only 4.8% (*w*/*w*, DAF of untreated microalga basis), representing 41% of the total amount of lipids of this microalga. In the case of *D. tertiolecta*, the extraction of lipids with n-hexane greatly enhanced due to the use of acid in the steam explosion process. A lipid yield of 2.1% (*w*/*w*, DAF of untreated microalga basis) was obtained when steam explosion was applied without acid impregnation, whereas this value increased to 10.6% (*w*/*w*, DAF of untreated microalga basis), as a consequence of using 5% sulphuric acid in the steam explosion experiment. This result is in agreement with our previous study (69), and shows the importance of carbohydrate hydrolysis to achieve a higher lipid extraction yield from microalgal sludge, using n-hexane as the solvent.

Concerning carbohydrates, the total sugar content of the untreated microalga, obtained by analytical acid hydrolysis, was determined for each microalgae species and the specific values are presented in Table 1. These values can be compared with the measured concentration of sugar in the solution of the steam exploded samples, which are also included in Table 1. For the steam explosion experiments performed with acid impregnation, a high percentage, between 70% and 80%, of the total sugar content of the microalga was found in solution after steam explosion. Contrary to this, the experiment performed with *D. tertiolecta* without the use of acid resulted in a low sugar concentration, representing 33% of the total sugar content of the untreated sample.

The protein concentration of the untreated microalgal samples ranged between 10% and 19% of DAF in the three species (Table 5.1). These values are in the range reported for species of the same genera in the stationary phase of culture.

The protein contents of *D. tertiolecta* detected after solubilization with dilute NaOH or after extraction at a high temperature were similar (Table 5.1). Thus, proteins were already available for solubilization in the harvested cultures of this naked microalgae species, meaning that it was not necessary to apply a disruption treatment. On the other hand, the protein contents detected after solubilization with dilute NaOH of both *N. gaditana* and *C. sorokiniana* were much higher after steam explosion. This rise in the detected protein revealed the cell disruption effect of steam explosion. However, the number of proteins detected after extraction at high temperature was lower in the steam exploded material than in the untreated sample for the three species. This protein loss may be explained by the occurrence of protein hydrolysis during steam explosion. The color reaction that is measured in the bicinchoninic acid assay is due to the reduction of Cu^{2+} to Cu^{+} by the oxidation of

aromatic residues and peptide bonds in the protein in the reaction solution. Therefore, a lighter coloration may evidence a reduction in the number of peptide bonds due to protein hydrolysis.

5.3.5. Fractionation of Steam Exploded Samples by Means of Membrane Filtration

According to the results of a previous study (52), the fractionation strategy followed in the present work consists of filtrating the exploded sample with a membrane set-up and then extracting the retentate and permeate streams with solvent. The filtration was performed with dynamic filtration, which allowed for a much better permeability with just a little more energy compared to conventional cross-flow filtration. This was because fouling is highly reduced. Not only are less pores blocked, but, primarily, the cake molding over the surface of the membrane that occurs in conventional filtration is hardly produced in dynamic filtration. Therefore, vibrating filtration highly reduces microalgae attachment on the membrane surface. A PE5 membrane (MWCO = 5000 Da) was used, since it exhibited the best performance in the filtration experiments regarding permeability and irreversible fouling.

5.3.6. Rejection

Table 5.2 presents the results of the filtration experiments including the total weight and DAF percentage and the lipids, sugars, and protein content of each of the different streams. From the values of the DAF percentages, it can be observed that different concentrations of the retentate streams were attained (from 3% to 10% DAF). This mainly depended on the concentration of the starting material.

Table 5.2: **Results of total mass balance, and lipid, sugar, and protein analysis of filtration experiments.**

	Nannochloropsis gaditana			***Chlorella sorokiniana***		
	Steam Exploded Sample	**Retentate**	**Permeate**	**Steam Exploded Sample**	**Retentate**	**Permeate**
Total weight (g)	6000	2400	3600	6000	2240	3760
DAF percentage	5.1 (0.1)	10.1 (0.2)	1.8 (0.05)	2.7 (0.02)	5.8 (0.08)	0.9 (0.01)
Lipid (g/L)	9.2 (0.3)	22.7 (0.5)	0.07 (0.01)	1.3 (0.05)	3.9 (0.09)	0.05 (0.01)
Sugar (g/L)	6.8 (0.3)	5.9 (0.2)	6.0 (0.2)	5.1 (0.1)	5.2 (0.2)	4.9 (0.1)
Protein (g/L)	4.7 (0.2)	5.65 (0.15)	n.d.	2.92 (0.04)	4.8 (0.14)	n.d.
	Dunaliella tertiolecta			***Dunaliella tertiolecta* (No Acid)**		
	Steam Exploded Sample	**Retentate**	**Permeate**	**Steam Exploded Sample**	**Retentate**	**Permeate**
Total weight (g)	6000	2290	3710	6000	2400	3600
DAF percentage	1.7 (0.01)	3.2 (0.04)	0.9 (0.01)	1.6 (0.01)	3.0 (0.02)	0.7 (0.01)
Lipid (g/L)	1.8 (0.02)	3.5 (0.08)	0.07 (0.01)	0.34 (0.03)	1.3 (0.01)	0.07 (0.01)
Sugar (g/L)	3.3 (0.2)	2.9 (0.1)	3.2 (0.2)	1.4 (0.1)	1.3 (0.1)	1.2 (0.1)
Protein (g/L)	0.89 (0.05)	1.46 (0.06)	n.d.	0.71 (0.06)	1.13 (0.07)	n.d.

The amount of lipid extracted with n-hexane and the proteins obtained with the solubilization method from the steam exploded and the permeate and retentate are included in Table 5.2. These values are expressed as a concentration of each stream, to allow for a better comparison. The permeate streams have a negligible content of lipids and no proteins. This result was also confirmed by optical density measurements. OD750 nm of permeates were like that of filtered (0.45 μm) seawater (Table 5.3). Therefore, it was assumed that lipid rejection was obtained in all the experiments. The absence of lipids and proteins in the permeate implies that the membrane PE5 is suitable for rejecting lipids and proteins from different microalgae

species. On the other hand, the concentration of lipids and proteins in the retentate streams is much higher than that of the steam exploded sample before filtration.

Concerning the sugar analysis, approximately the same values of concentration were obtained for the steam exploded sample and retentate and permeate streams, for the different microalgae species. This means that the employed membrane (PE5) is unable to retain sugars.

Table 5.3: **Optical density at 750 nm after filtration of steam exploded microalgae. Raw values are compared to filtered (0.45 µm) seawater (blank). Values are expressed as the mean and the standard deviation is provided in brackets.**

	OD_{750nm}	
	Blank	**Permeate**
Nannochloropsis gaditana	0.081 (0.001)	0.091 (0.003)
Chlorella sorokiniana	0.081 (0.001)	0.101 (0.002)
Dunaliella tertiolecta	0.083 (0.001)	0.085 (0.001)
Dunaliella tertiolecta **(no acid)**	0.083 (0.001)	0.083 (0.000)

5.3.7. Permeability

Regarding the performance of the membrane in using dynamic filtration, Figure 5.4 shows membrane permeabilities including water permeability with the new (unused) membrane and after the experiment, for the different microalgae species studied. The permeabilities of steam-exploded biomass were measured. With them, the total fouling of materials was calculated. Concerning the permeability for the water of new PE5 membranes, the values between 30.4 L/h/m2/bar (for *D. tertiolecta* exploded without acid) and 90.8 L/h/m2/bar (for *N. gaditana*) were obtained. In an ideal system where a liquid that does not provide fouling is used and virgin

membranes perfectly manufactured are used, the same permeabilities would be obtained. But in laboratory or pilot-scale scenarios, both conditions hardly occur. As checked earlier with the help of a scanning electron microscope, membrane thicknesses differ within the same sample. Following Darcy's law, this makes the permeability change accordingly. If enough surface of membrane is used, a mean permeability value with a low deviation is normally obtained. But this is not the case with a pilot unit as the one used in this work.

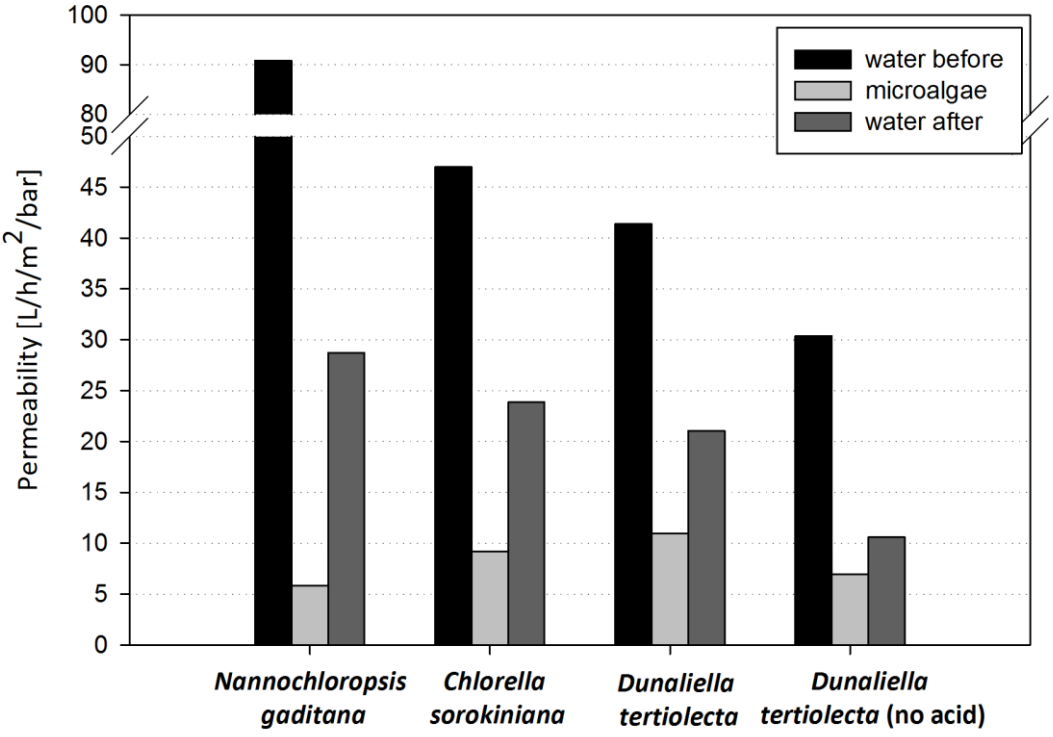


Figure 5.4: **Water and sample permeabilities for the different microalgae samples.**

In terms of pretreated microalgae filtration, the *N. gaditana* sample resulted in a microalgae permeability of 5.84 L/h/m2/bar, the lowest value among the samples. With *D. tertiolecta* exploded without acid, a microalgae permeability of 6.93 L/h/m2/bar was obtained, and with *C. sorokiniana*, a permeability of 9.18 L/h/m2/bar was reached. The best membrane performance was obtained when

filtrating the sample of *D. tertiolecta* exploded with acid, with the permeability value of 10.97 L/h/m2/bar.

The total fouling factor (TF) of PE5 was the highest for *N. gaditana*, with the value of 15.55. In the case of *C. sorokiniana*, TF was lower with the value of 5.12 and with D. tertiolecta exploded without acid, where TF = 4.39. The best performance in terms of TF was obtained with *D. tertiolecta* exploded with acid, where the value of 3.77 was given.

5.3.8. Irreversible fouling

To calculate irreversible fouling, membrane permeability with water before and after the experiment was measured (the system was cleaned before performing the water permeability measurements after the experiment). PE5 with *N. gaditana* resulted in the permeability of 28.7 L/h/m2/bar, *C. sorokiniana* performed with the value of 23.85 L/h/m2/bar, *D. tertiolecta* exploded with acid gave the value of 21.07 L/h/m2/bar, and finally, *D. tertiolecta* exploded without acid performed with the value of 10.61 L/h/m2/bar. Therefore, the experiment with the lowest irreversible fouling factor of 1.96 was *D. tertiolecta* exploded with acid, while N. gaditana, *C. sorokiniana*, and *D. tertiolecta* exploded without acid resulted in IF = 3.16, IF = 2.86, and IF = 1.97, respectively.

Figure 5.5 presents the exploded microalgae permeability profiles vs time for dynamic filtration with *N. gaditana* and *C. sorokiniana*. In the filtration of *C. sorokiniana*, a steady state was reached after 30 min of the experiment with the permeability value of 9.5 L/h/m2/bar. On the contrary, in the filtration of *N. gaditana*, the plateau was not reached, even though the experiment lasted longer than *C. sorokiniana*. After 130 min of filtrating, the value of permeability with *N. gaditana* was 4.2 L/h/m2/bar and still decreasing.

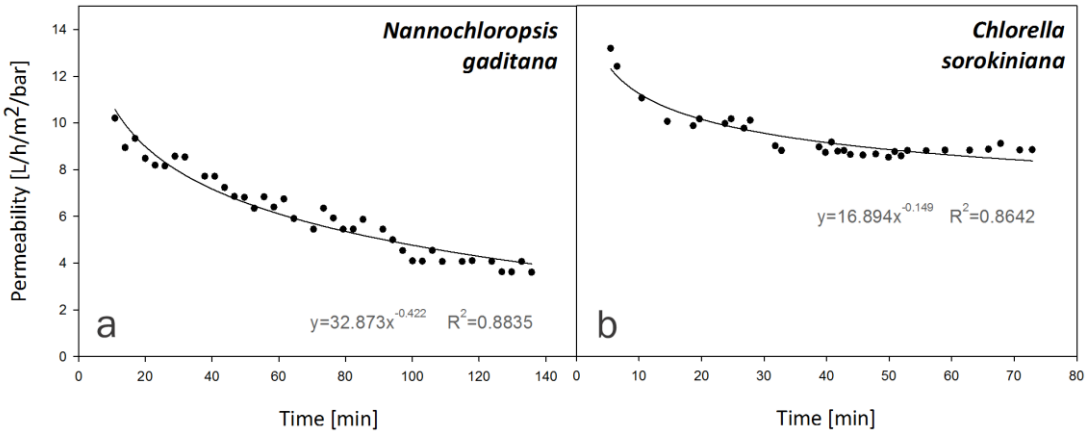


Figure 5.5: **Permeability profiles vs. time of filtration experiments performed with (a) Nannochloropsis gaditana and (b) Chlorella sorokiniana.**

5.4. Conclusions

Steam explosion has the potential to become a broad-spectrum microalgae cell disruption, as well as pre-fractionation, treatment. It provided proper availability of organic compounds and carbohydrate hydrolysis into sugars with all the various kinds of used microalgae and it is particularly effective when the strains have recalcitrant cell walls.

The use of steam explosion, besides breaking the cell wall, partially hydrolyzes proteins.

With all the tested strains, dynamic membrane filtration offers an excellent performance regarding permeability by rejecting lipids.

The sequence of steam explosion, dynamic membrane filtration, and solvent extraction as downstream unit operations in a microalgae biorefinery clearly allows for the reduction of process costs. All the mentioned technologies for all the stages are already commercially available.

Acknowledgements

This study was funded by the Spanish Ministry of Economy and Competitiveness (grant number CTQ2014-56285-R). The research was also supported by the European Regional Development Funds (ERDF, FEDER Programa Competitividad de Catalunya 2007–2013) and by the project “Fuels from Biomass” funded by Excma. Diputació de Tarragona. M. Hapońska is grateful to the Universitat Rovira i Virgili (URV) for her PhD scholarship.

6

TRANSESTERIFICATION

This chapter describes the performance of different membrane reactors combined with heterogeneous catalysis. The main objectives were to identify a proper catalyst, to choose the proper immobilization technique, to establish the membrane with the adequate pore size and to control the reaction and separation process. Amberlyst®15 with acid sites and different types of Strontium Oxide with basic sites were tested as heterogeneous catalysts. Two catalytic membrane reactors were produced and tested confirming the production of several types of methyl esters.

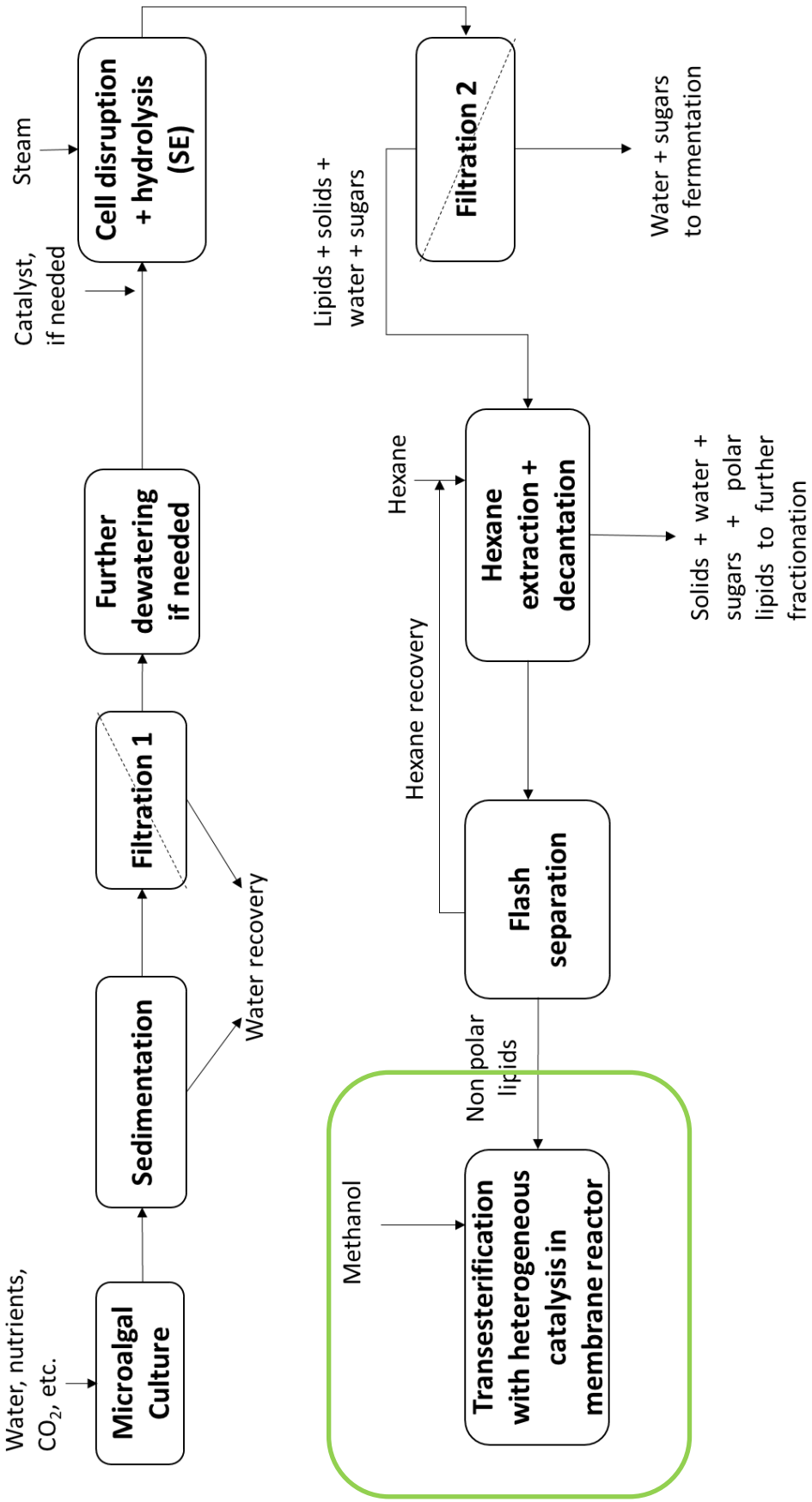


Figure 6.1: **Proposed scheme of microalgae biorefinery – transesterification.**

6.1.Introduction

There are many raw material sources to produce the biodiesel from vegetable crops (first generation feedstock being discarded due to food competition) (109) to microalgae (third generation feedstock) (21,110). Transesterification with methanol is the most common process used for biodiesel production. This process is generally carried out by using homogeneous catalysts (usually alkali-catalyst) in a stirred batch reactor (111). Due to the low cost of raw materials, sodium or potassium hydroxides are usually used as the homogeneous catalyst. They are the most economic because the process is carried out under low temperature and pressure and high conversion is attained with no intermediate steps (25). However, this procedure implies several by-products like soap and water, generated due to the need of a washing step for catalyst removal (112), which entails the necessity of more energy and higher investment. For this reason, substitution of homogeneous catalysts by solid "anchored" heterogeneous ones could be an alternative, allowing an easier separation of the catalyst (for example, by filtration) for further reuse, and no water need. In addition, heterogeneous catalysts can simultaneously catalyse the transesterification and esterification reactions, which can advantageously avoid the pre-esterification step (25). Several catalysts have been already tested for this purpose as CaO, MgO or SrO (113–116).

In this study a strong basic SrO catalyst (insoluble in methanol, vegetable oils and fatty acid methyl ester) (117,118) was selected, based on their feasibility in the transesterification reaction (119).

In the biodiesel production it is necessary to remove residual triglycerides, free fatty acids, and glycerol. One method is to drive the reaction as close as possible to complete conversion, however transesterification is an equilibrium reaction and there

are limits to this approach. Other approaches employ multiple water washing steps, which can give rise to a treatment problem in the wastewater stream (120). FAME, methanol and glycerol in the final reaction mixture (after batch transesterification) can be separated by settling. A membrane reactor can be a unique piece of reactor/separation design for the transesterification process (121,122), to facilitate the separation of products in a continuous process. There are two types of membrane reactors which combine the activity of a catalyst and the separation of products: catalytic membrane reactor (CMR) and inert membrane reactor with catalyst on a feed side (IMRCF) (120–123).The difference between those two reactors lies in the location of the reaction zone. In the CMR the catalyst is attached to the membrane surface either forms part of a membrane matrix. In the IMRCF the catalyst is neighbouring with the inert membrane on the feed side of the module (124). This system can improve the catalytic performance without needing catalyst recovery and products separation. In the particular case of biodiesel production, the large oil droplets are not able to cross the membrane contrarily of FAME and methanol. This permits to remove the products from the reactor, thus overcoming equilibrium limitations. Membrane contactors have also been tested as devices capable to modify equilibrium 20. Moreover, both methanol and catalyst can be reused in further reactions. Membrane reactors for the biodiesel attainment have been investigated showing the potentiality of the technique (120,121).

This work attempts to design and evaluate the potentiality of membrane reactors. To achieve it, 1) the selected catalyst was tested in the batch reaction; 2) the immobilization of the solid catalyst was performed in a polymeric membrane; 3) catalytic tests were conducted over the synthesized CMRs and 4) combination of the catalyst-filled bag together with commercial membranes in the novel IMRCF was

studied . Therefore, this work is a novel one presenting a catalytic membrane reactor with SrO to carry-out a transesterification process.

6.2.Experimental

6.2.1. Materials

For the transesterification reactions commercial regional sunflower oil from Borges Company was used, because of its similar characteristics to a microalgae oil and its widespread distribution. Methanol (99.9 % grade, Scharlau). Commercial biodiesel (FAME) was kindly provided by Stocks del Valles, S.A.

Heterogeneous catalysts were selected from a literature review. Strontium oxide was selected as basic catalyst and two types of products were purchased: one with technical grade from Alfa Aesar and a more pure one from Sigma-Aldrich (99.9 % grade).

For GC analysis, n-Heptane (>99%, VWR), methyl heptadecanoate (standard for GC, Sigma-Aldrich) and F.A.M.E. MIX, C8-C24 (Sigma-Aldrich) were used.

For the experiments with the novel CMR module two commercial microfiltration membranes were tested: PTFE/Freudenburg with the MWCO (molecular weight cut-off) of 0.05 µm (Donaldson) and PTFE/PP with the MWCO of 0.2 µm (Donaldson) both provided by New Logic Research. CMRs were manufactured using polysulfone purchased from Sigma-Aldrich (Mw = 35,000). Solvent employed for polymeric membrane synthesis was: Dimethylformamide 99.9 % Multisolvent® (DMF) and Dimethylacetamide 99.5 % (DMA) were purchased from Scharlab. Demineralised water was used in the coagulation bath as non-solvent for the CMR membrane preparation.

6.2.2. Methods

Transesterification with conventional CMR

In the previous study three configurations were investigated for the transesterification reaction using conventional CMR: (1) traditional reaction using the heterogeneous catalysts dispersed in the bulk solution, followed by separate standard phases partition; (2) reaction with the heterogeneous catalysts dispersed in the bulk solution coupled with in situ continuous filtration performed with a commercial membrane (0.2 μm); and finally, (3) reaction with the immobilized catalyst on a synthesized polymeric membrane. (125)

Table 6.1: **Variables values from literature in distinct applications**.

Catalyst	**Strontium Oxide** (126)
Catalyst loading	3%
Temperature	65
Methanol-oil molar ratio	12:1
Time of conversion	30 minutes

Basing on the results obtained, in this work an influence of the SrO particle size for a transesterification reaction was studied for a configuration 1. For this purpose the catalyst was milled and sieved in order to obtain the powder containing of a particles with a desired size. The sieve with a mesh size of 500 μm and 100 μm was used. A comparison between two magnetic agitation speeds (800 rpm and 1000 rpm) was also studied for this configuration.

Experimental conditions for the transesterification reaction were adopted from previous publications in the literature (Table 6.1): 65 ºC, 3 wt. % of catalyst with

respect to the sunflower oil-methanol mixture, methanol-to-oil ratio of 12:1. The reactions were maintained for 60 minutes.

Briefly, sunflower oil and methanol were weighted in a round bottom flask and pre-heated in a glycerol bath with magnetic stirring. Once the desired temperature was reached, the catalyst was added to the reaction mixture. When the reaction was stopped at the corresponding reaction time, the round bottom flask was cooled and allowed to stand for phase separation.

Membrane reactor configurations

Catalyst immobilization inside the novel membrane reactor was studied. Two variants were tested: a) reaction with the immobilized SrO catalyst on a synthesized polymeric membrane (CMR) and b) reaction with the non-woven bag filled with SrO catalyst combined with commercial membrane filtration (IMRCF). For the variant a) membranes were synthesized by immersion precipitation (a type of phase inversion). It is a standard well-known technique described in literature (127). A polymeric solution consisting of 10 wt. % PSf is dissolved in DMF under magnetic stirring for 24 hours at room temperature. The solution was then casted onto a glass plate using a casting knife. The knife was pushed over the glass thanks to an automatic film applicator (BYK-Gardner Automatic Film Applicator L) at constant rate of 11 mm/s and the glass plate was immersed into a coagulation bath containing water as non-solvent to obtain the membrane. Regarding the variant b) two commercially available membranes with different pore size were tested. Water permeability for virgin commercial membranes was measured.

Experimental conditions for the transesterification reaction were adapted to the limitations of the equipment: 65 ± 3 ºC, 2 wt. % of catalyst with respect to the sunflower oil-methanol mixture, methanol-to-oil ratio of 12:1. The reactions were

maintained for 2 hours with the trans-membrane pressure of 1.5 bar and the permeate flow between 40.0 ml/min and 48.5 ml/min.

Sunflower oil and methanol were weighted and poured into two 2 L Erlenmeyer flasks and pre-heated separately with magnetic stirring to the temperature of 60 ± 5 oC. In order to obtain the homogeneous temperature inside the whole set-up including the membrane module, hot sunflower oil was being pumped through the system until the desired temperature inside the membrane module was reached. Next, pre-heated methanol was added to the feed flask. The first sample of permeate was taken for the analysis after 10 minutes of the reaction. The samples were taken each 15 minutes during two hours.

In order to remove the residual methanol from the product, the samples were lyophilized in the temperature of -80.0 oC for two hours, with the pressure of 1.00 mbar.

Analytics

Triglycerides and fatty acid methyl esters were characterized off-line by a gas chromatograph (Agilent Technologies 7890A) using a FID detector and a HP-INNOWax column 19091N-113 (30 m x 0.32 mm x 0.25 μm). Ester (C14-C24) content was determined according to the European standard test EN 14103 method. Triglycerides content was calculated from the results obtained by the GC and using the following expression: wt% = (triglycerides area in the initial sample – triglycerides area in the actual sample) / triglycerides area in the initial sample.

6.2.3. Equipment

The set-up varied depending on the configuration used.

In the configuration 1, when the influence of the SrO particle size on the reaction was investigated, the reaction products (methanol, FAME, oil and glycerol) were continuously pumped (400 ml/h rate) from the top layer and returned to the recirculation vessel. This system enhanced the contact between both reactants and the catalyst.

Experiments with the CMR and IMRCF were carried out using the cross-flow filtration setup. (Figure 2.5 a). Two configurations were tested: a) reaction with the immobilized SrO catalyst on a synthesized polymeric membrane supported by commercial membrane (in order to ensure total glycerol rejection) and b) reaction with the non-woven bag filled with SrO catalyst combined with commercial membrane filtration. For the configuration a) self-prepared PSf membrane with the SrO catalyst inside the matrix was placed inside the novel CMR together with the spacer. In the configuration b) the non-woven bag filled with 15.0 g of SrO catalyst was placed inside the membrane cell together with the commercial membrane. In both configurations, the feed tank was placed over the hot plate magnetic stirrer with a thermocouple. The reaction components (methanol, FAME, oil and glycerol) were recirculated through the system using a membrane pump. The reaction mixture was pumped from the feed tank towards a catalytic membrane cell system equipped with heating plates and thermocouples. The temperature inside the module during the experiments was regulated using thermocontroller. A transmembrane pressure was regulated with a compact back pressure regulator. Transmembrane pressure was fixed at 1.5 bars. The volume of oil and methanol used as the feed was 800 mL and 443 mL, respectively.

6.3.Results

6.3.1. Catalyst particle size influence on the transesterification reaction

Table 6.2: **Methyl esters composition in sunflower oil biodiesel and the results obtained in this work using SrO as catalyst**

		Typical composition of sunflower oil (128)	**Measured composition of the used oil**	**Experimental values - SrO 99.9%**
		% weight		
Methyl Palmitate	16:0	6	6-7	5-6
Methyl Stearate	18:0	3-5	4-5	3-4
Cis-9-oleic Methyl Ester	18:1	17-22	29	25-29
Methyl Linoleate	18:2	67-74	59-60	61-65

Regarding SrO with 99.9 % grade, almost complete yield of methyl esters was achieved. In this case, the particle size (500 μm and 100 μm) and agitation speed (800 and 1000 rpm) were also considered as variables. In the first minutes of the experiment the reaction progressed much faster when working with lower particle size of the catalyst. The lower the particle size the bigger catalytic active surface area is accessible for the reactives, therefore the FAME yield increased faster in the case of SrO particle size < 100 μm. Regarding the different stirring rate the FAME yield increased faster when working with higher agitation speed (1000 rpm) reaching the value of 93 ± 4 wt. % after 10 minutes of the reaction. At the same time, the reaction

performed with lower agitation speed (800 rpm) gave the FAME yield of 3 ± 2 wt. %, which increased to 85 ± 10 wt. % after 20 minutes of the reaction. Figure 6.2 shows the particle size distribution for the strontium oxide depending on the agitation speed applied in the experiment. The stirrer in the contact with the catalyst causes disintegration of the particles providing better access to catalytic active surface. The higher the agitation the faster the disintegration of the particles and the reaction starts sooner. Regarding methyl esters composition (Table 6.2), results showed that the methyl esters distribution was very similar to the one obtained from applying the EN ISO 5509 norm, with a slight increment of the methyl linoleate in this case.

As glycerol was generated during the transesterification reaction, three phases (two liquids and one solid) were spontaneously separated. The upper phase contained the esters formed, while most of the excess methanol was dragged to the glycerol phase in the middle phase and the solid catalyst to the bottom phase. As expected, when using the SrO immobilized in the CMR (third configuration) only two phases were clearly discerned.

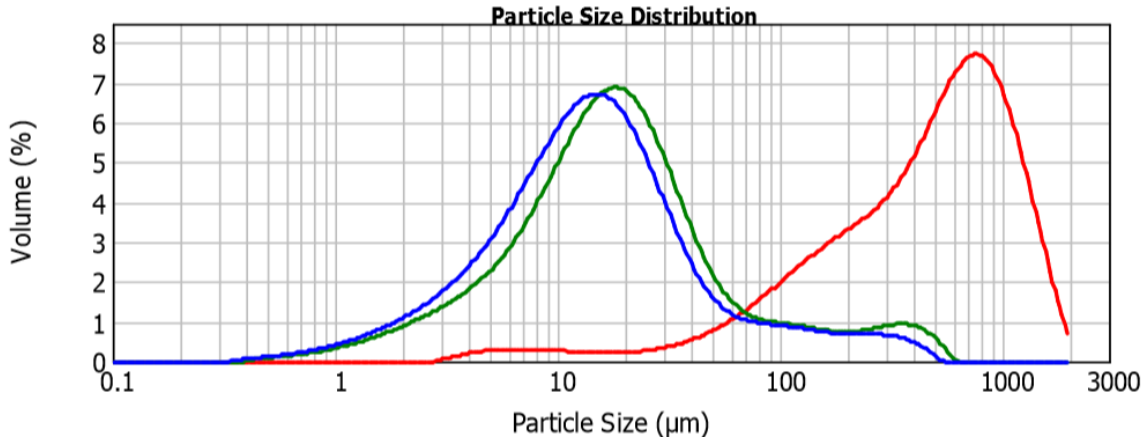


Figure 6.2: **Particle size distribution of SrO catalyst**

6.3.2. Catalytic membrane reactor

Catalyst immobilization selection using conventional CMR

Two procedures were carried-out in order to obtain the CMR with the catalyst immobilized on the membrane surface (catalyst dispersed over the polymeric solution after casting) or inside the polymeric matrix (catalyst added into the polymeric solution).

The only configuration showing conversion was that with the catalyst inside the membrane matrix. The limitations caused by the temperature loss inside the system together with small membrane area resulted in low conversions. Methyl esters obtained in these experiments were methyl palmitate, cis-9-oleic methyl ester, and methyl linoleate. Methyl Stearate was not detected, probably due to the overall low conversion and the low fraction in which it normally performs. These results also indicated that the CMR configuration might allow tuning the composition of methyl esters obtained in the process by applying different contact times. This result can be interesting for other applications seeking for higher added value products.

Transesterification with the novel IMRCF

In the first configuration studied the transesterification was performed using self-prepared PSf membrane with the SrO catalyst immobilized inside the membrane matrix supported by 0.05 Teflon/Freudenberg. The conversion obtained was very low (< 1.0 %) due to low catalyst to methanol/oil ratio. Higher catalyst load inside the membrane matrix was impossible to achieve and the membrane cell size limited the membrane size to be incorporated. In the second configuration a non-woven bag filled with the catalyst was used in order to increase the catalyst load. Additionally,

two commercially available membranes were tested in this configuration. First, water permeability of the virgin membranes was measured. The water permeability for PTFE 0.2 was of 152.7 L h-1 m-2 bar-1 and for 0.05 Teflon/Freudenberg 30.9 L h-1 m-2 bar-1. Since the FAME yield obtained with both membranes was similar, the one chosen for the further experiments was PTFE 0.2 showing higher permeability.

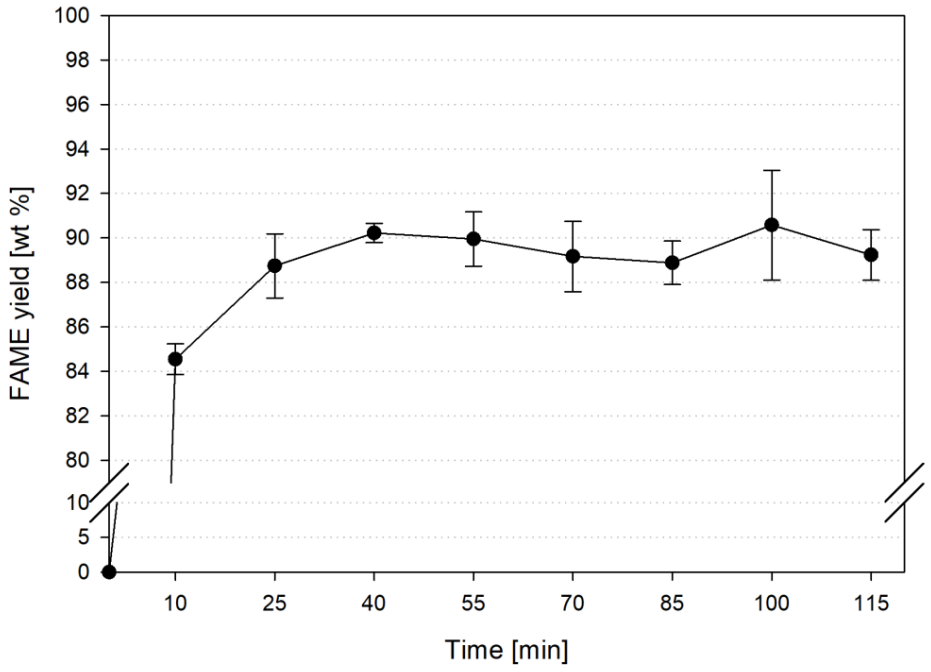


Figure 6.3: **FAME yield during the transesterification reaction with the SrO catalyst bag and PTFE 0.2 membrane in the novel CMR.**

Figure 6.3 shows the FAME yield obtained during the transesterification reaction with the SrO catalyst bag and PTFE 0.2 membrane. The FAME content was increasing until reaching the value of 90.2 wt % after 40 minutes of the reaction. Comparing to the results with self-prepared PSf membrane with the SrO catalyst immobilized in the membrane matrix huge improvement was achieved using the bag with bigger amount of the catalyst. Also the possibility of maintaining the homogeneous temperature inside the whole system allowed obtaining the proper conditions for the reaction to be performed.

6.4. Conclusions

The potentiality of using membrane reactors for performing transesterification reactor was investigated. The process was divided in key stages and each one was checked.

It was confirmed that a membrane with a mean pore size of 0.2 μm rejects oil and glycerol and allows the permeance of methyl esters. Methanol cannot be rejected but further recuperation from methyl esters is easy to recycle to the reaction system.

Although some catalytic activity was observed when working with self-prepared polymeric membranes with the catalyst immobilized in membrane matrix, significant improvement was achieved when combining catalyst-filled bag and commercial membrane. Novel membrane reactor with the cell heating system ensures the homogeneous temperature inside the whole set-up providing proper conditions for the conversion. Within the commercial materials tested with the IMRCF better results were obtained with the membrane of bigger MWCO. Since the FAME yield obtained was similar in both cases, the membrane with higher permeability was chosen as a better one for this purpose.

A CMR/IMRCF using SrO as catalyst is a promising method to transesterify triglycerides into methyl esters enabling process intensification. It avoids the use of a homogeneous catalyst that should be further recovered and eludes a washing procedure that may cause soap formation.

Acknowledgements

This work was supported by the projects CTQ2014-56285-R “Cultivo, concentración, fraccionamiento y obtención de producto en refinería de microalgas”

funded by the Spanish Ministry of Economy and Competitiveness and ‘‘Fuels from Biomass’’ (research program funded by Excma. Diputació Tarragona).

The research was also supported by the European Regional Development Funds (ERDF, FEDER Programa Competitividad de Catalunya 2007-2013).

Authors are grateful to Stocks del Vallès, S.A. for kindly donating commercial biodiesel.

M. Hapońska is grateful to Universitat Rovira i Virgili (URV) for her PhD scholarship.

C. Nurra is grateful to the Catalonia Institute for Energy Research (IREC) for her PhD scholarship and to Universitat Rovira i Virgili (URV) for her mobility scholarship that allowed an internship at Delft University of Technology, where part of the research was carried out at.

Authors are also grateful the technicians and personal of the Catalysis Engineering Department of Delft University of Technology.

SUMMARY OF RESULTS AND GENERAL CONCLUSIONS

The study presented in this thesis concerns the application of membrane technology for microalgae biorefining. This complex process requires several improvements due to the relatively high operational costs of each step. The idea of using membranes for this purpose may lead to general cost reduction and simplification of the procedures. The technical improvement and optimization of harvesting, cell disruption, fractionation and transesterification steps was performed.

In the harvesting stage:

- The production and application of novel polymeric membrane materials together with vibratory technology led to the performance improvement of microalgae *Chlorella sorokiniana* and *Dunaliella tertiolecta* dewatering.
- It was showed that vibrational membrane filtration improves performance compared to cross-flow filtration resulting in a doubled permeability. Also, when using dynamic filtration, the performance continued to be satisfactory with sludge concentration increment.
- Successful production of ABS membranes for the vibratory filtration, knowing that the polymer price is three orders of magnitude lower than the price of commercially available high-grade polymers such as polysulfone and polyacrylonitrile, already gave a huge advantage over existing, commonly used membranes.

- It was also proofed that polymeric composition and the temperature of the coagulation bath are important parameters for preparation of ABS membranes with desired mechanical properties.
- Further study showed that substantial energy and cost reduction can be achieved when combining pH induced sedimentation with dynamic filtration for microalgae harvesting.
- The high concentration factors reached in the pilot scale experiments (CF of 205 and 245 for the studied strains) proofed that this method could lead to concentrations high enough to proceed to cell disruption with no need for further operations.

Regarding the cell disruption and fractionation stage:

- Satisfactory results were obtained when using the sequence of steam explosion, dynamic membrane filtration, and solvent extraction.
- For all the microalgae strains treated (*Nannochloropsis gaditana, Chlorella sorokiniana and Dunaliella tertiolecta),* the access to organic compounds and carbohydrate hydrolysis into sugars was obtained by acid-catalyzed steam explosion.
- The separation of the lipids from the aqueous phase was reached by membrane filtration. Again, dynamic filtration provided better results than conventional technique.

Concerning the transesterification step:

- The comparison of novel catalytic and inert membrane reactors for biodiesel production with strontium oxide as a heterogeneous catalyst was performed.

- Some catalytic activity was detected for self-prepared polymeric membranes with the catalyst immobilized in membrane matrix, but much better performance was observed for the combination of catalyst-filled bag and commercial membrane in the novel IMRCF with the cell heating system.
- The transesterification process intensification can be obtained by the application of a CMR/IMRCF using SrO as a heterogeneous catalyst.

Microalgae biorefining in the terms of industrial scale needs modernization leading to final cost reduction of the process. Since this work focuses on the technical improvement of each step of the microalgae treatment for biofuel production, the scope of the future work would be to evaluate economically the impact of the application of the techniques proposed. Further study of harvesting implying sedimentation combined with dynamic filtration of larger volumes of microalgae suspension should be performed to check the maximum concentration possible to be obtained in a pre-industrial test. The possibility of direct processing the concentrate obtained by the proposed steam explosion cell disruption and fractionation techniques should be considered. The investigation of higher SrO catalyst load for the transesterification using IMRCF should be performed.

BIBLIOGRAPHY

1. Piasecka A, Krzemińska I, Tys J. Physical methods of microalgal biomass pretreatment. Int Agrophysics. 2014;28(3):341–8.

2. Tan XB, Lam MK, Uemura Y, Lim JW, Wong CY, Lee KT. Cultivation of microalgae for biodiesel production: A review on upstream and downstream processing. Chinese J Chem Eng. 2018;26(1):17–30.

3. Im H, Lee H, Park MS, Yang J-W, Lee JW. Concurrent extraction and reaction for the production of biodiesel from wet microalgae. Bioresour Technol. 2014;152:534–7.

4. Cheng J, Yu T, Li T, Zhou J, Cen K. Using wet microalgae for direct biodiesel production via microwave irradiation. Bioresour Technol. 2013;131:531–5.

5. Lam GP ’t, Vermuë MH, Eppink MHM, Wijffels RH, van den Berg C. Multi-Product Microalgae Biorefineries: From Concept Towards Reality. Trends Biotechnol. 2018;36(2):216–27.

6. Straathof AJJ. 2.57 - The Proportion of Downstream Costs in Fermentative Production Processes. In: Moo-Young M, editor. Comprehensive Biotechnology (Second Edition). Second Edi. Burlington: Academic Press; 2011. p. 811–4.

7. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renew Sustain Energy Rev. 2010;14(1):217–32.

8. Barros AI, Gonçalves AL, Simões M, Pires JCM. Harvesting techniques applied to microalgae: A review. Renew Sustain Energy Rev. 2015;41:1489–

500.

9. Brennan L, Owende P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev. 2010;14(2):557–77.

10. Chatsungnoen T, Chisti Y. Harvesting microalgae by flocculation-sedimentation. Algal Res. 2016;

11. Şirin S, Clavero E, Salvadó J. Potential pre-concentration methods for Nannochloropsis gaditana and a comparative study of pre-concentrated sample properties. Bioresour Technol. 2013;132:293–304.

12. Gerardo ML, Hende S Van Den, Vervaeren H, Coward T, Skill SC. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. Algal Res. 2015;11:248–62.

13. Grima EM, Belarbi E-H, Fernández FGA, Medina AR, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv. 2003;20(7):491–515.

14. Gerardo ML, Oatley-Radcliffe DL, Lovitt RW. Integration of membrane technology in microalgae biorefineries. J Memb Sci. 2014;464(0):86–99.

15. Chen JP, Mou H, Wang LK, Matsuura T. Membrane Filtration. In: Wang LK, Hung Y-T, Shammas NK, editors. Advanced Physicochemical Treatment Processes. Totowa, NJ: Humana Press; 2006. p. 203–59.

16. Bilad MR, Arafat HA, Vankelecom IFJ. Membrane technology in microalgae cultivation and harvesting: A review. Biotechnol Adv. 2014;32(7):1283–300.

17. Ríos SD, Salvadó J, Farriol X, Torras C. Antifouling microfiltration strategies

to harvest microalgae for biofuel. 2012;119:406–18.

18. Nurra C, Clavero E, Salvadó J, Torras C. Vibrating membrane filtration as improved technology for microalgae dewatering. Bioresour Technol. 2014;157(0):247–53.

19. Lee SY, Cho JM, Chang YK, Oh Y-K. Cell disruption and lipid extraction for microalgal biorefineries: A review. Bioresour Technol. 2017;244:1317–28.

20. Khanra S, Mondal M, Halder G, Tiwari ON, Gayen K, Bhowmick TK. Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: A review. Food Bioprod Process. 2018;

21. Nurra C, Torras C, Clavero E, Ríos S, Rey M, Lorente E, et al. Biorefinery concept in a microalgae pilot plant. Culturing, dynamic filtration and steam explosion fractionation. Bioresour Technol. 2014;163(0):136–42.

22. Halim R, Webley PA, Martin GJO. The CIDES process: Fractionation of concentrated microalgal paste for co-production of biofuel, nutraceuticals, and high-grade protein feed. Algal Res. 2016;19:299–306.

23. Kwan TA, Tu Q, Zimmerman JB. Simultaneous Extraction, Fractionation, and Enrichment of Microalgal Triacylglyerides by Exploiting the Tunability of Neat Supercritical Carbon Dioxide. ACS Sustain Chem Eng. 2016 Nov 7;4(11):6222–30.

24. Ramachandran K, Suganya T, Nagendra Gandhi N, Renganathan S. Recent developments for biodiesel production by ultrasonic assist transesterification using different heterogeneous catalyst: A review. Renew Sustain Energy Rev. 2013;22(0):410–8.

25. Leung DYC, Wu X, Leung MKH. A review on biodiesel production using catalyzed transesterification. Appl Energy. 2010;87(4):1083–95.

26. Gao L, Xu W, Xiao G. Modeling of biodiesel production in a membrane reactor using solid alkali catalyst. Chem Eng Process Process Intensif. 2017;122:122–7.

27. Aransiola EF, Ojumu T V, Oyekola OO, Madzimbamuto TF, Ikhu-Omoregbe DIO. A review of current technology for biodiesel production: State of the art. Biomass Bioenerg. 2014;61(0):276–97.

28. Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: A review. Renew Sustain Energy Rev. 2011;15(1):584–93.

29. Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007;25(3):294–306.

30. Lam MK, Lee KT. Microalgae biofuels: A critical review of issues, problems and the way forward. Biotechnol Adv. 2012;30(3):673–90.

31. Kang G, Cao Y. Application and modification of poly(vinylidene fluoride) (PVDF) membranes – A review. J Memb Sci. 2014 Aug;463:145–65.

32. Rios SD, Clavero E, Salvadó J, Farriol X, Torras C. Dynamic Microfiltration in Microalgae Harvesting for Biodiesel Production. Ind Eng Chem Res. 2011 Feb 16;50(4):2455–60.

33. Nurra C, Franco E, Maspoch M, Salvadó J, Torras C. Cheaper membrane materials for microalgae dewatering. J Mater Sci. 2014;49(20):7031–9.

34. Ríos SD, Salvadó J, Farriol X, Torras C. Antifouling microfiltration strategies to harvest microalgae for biofuel. Bioresour Technol. 2012;119(0):406–18.

35. Jaffrin MY. Dynamic shear-enhanced membrane filtration: A review of rotating disks, rotating membranes and vibrating systems. J Memb Sci. 2008;324(1):7–25.

36. Shi W, Benjamin MM. Effect of shear rate on fouling in a Vibratory Shear Enhanced Processing (VSEP) RO system. J Memb Sci. 2011;366(1):148–57.

37. Slater CS, Savelski MJ, Kostetskyy P, Johnson M. Shear-enhanced microfiltration of microalgae in a vibrating membrane module. Clean Technol Environ Policy. 2015;

38. Olivera S, Muralidhara HB, Venkatesh K, Gopalakrishna K, Vivek CS. Plating on acrylonitrile–butadiene–styrene (ABS) plastic: a review. J Mater Sci. 2016;51(8):3657–74.

39. Díez-Pascual AM, Gascón D. Carbon nanotube buckypaper reinforced acrylonitrile-butadiene-styrene composites for electronic applications. ACS Appl Mater Interfaces. 2013;5(22):12107–19.

40. Ohno H, Kawamura Y. Analysis of acrylonitrile, 1,3-butadiene, and related compounds in acrylonitrile-butadiene-styrene copolymers for kitchen utensils and children's toys by headspace gas chromatography/mass spectrometry. J AOAC Int. 2010;93(6):1965–71.

41. Cole DP, Riddick JC, Iftekhar Jaim HM, Strawhecker KE, Zander NE. Interfacial mechanical behavior of 3D printed ABS. J Appl Polym Sci. 2016;133(30):43671.

42. Sanaeepur H, Ebadi Amooghin A, Moghadassi A, Kargari A, Moradi S, Ghanbari D. A novel acrylonitrile-butadiene-styrene/poly(ethylene glycol)

membrane: preparation, characterization, and gas permeation study. Polym Adv Technol. 2012 Aug 18;23(8):1207–18.

43. Boricha AG, Murthy ZVP. Acrylonitrile butadiene styrene/chitosan blend membranes: Preparation, characterization and performance for the separation of heavy metals. J Memb Sci. 2009;339(1):239–49.

44. Bandehali S, Kargari A, Moghadassi A, Saneepur H, Ghanbari D. Acrylonitrile-butadiene-styrene/poly(vinyl acetate)/nanosilica mixed matrix membrane for He/CH 4 separation. Asia-Pacific J Chem Eng. 2014 Sep 20;9(5):638–44.

45. Pruvost J, Van Vooren G, Cogne G, Legrand J. Investigation of biomass and lipids production with Neochloris oleoabundans in photobioreactor. Bioresour Technol. 2009;100(23):5988–95.

46. Torras C, Pitol-Filho L, Garcia-Valls R. Two methods for morphological characterization of internal microcapsule structures. J Memb Sci. 2007 Nov;305(1–2):1–4.

47. Torras C, Garcia-Valls R. Quantification of membrane morphology by interpretation of scanning electron microscopy images. J Memb Sci. 2004 Apr;233(1–2):119–27.

48. New Logic Research. Membrane Filtration of Waste Oil Case Study. A cost-effective and environmentally sound processing solution [Internet]. 2001. p. 1–5. Available from: http://www.vsep.com/pdf/WasteOil.pdf

49. Förch R, Schönherr H, Tobias A, Jenkins A. Appendix C: Contact Angle Goniometry. In: Surface Design: Applications in Bioscience and

Nanotechnology. 2009. p. 471–3.

50. Zhang X, Hu Q, Sommerfeld M, Puruhito E, Chen Y. Harvesting algal biomass for biofuels using ultrafiltration membranes. Bioresour Technol. 2010 Jul;101(14):5297–304.

51. Tansel B, Dizge N, Tansel IN. Analysis of high resolution flux data to characterize fouling profiles of membranes with different MWCO under different filtration modes. Sep Purif Technol. 2017;173:200–8.

52. Lorente E, Hapońska M, Clavero E, Torras C, Salvadó J. Microalgae fractionation using steam explosion, dynamic and tangential cross-flow membrane filtration. Bioresour Technol. 2017 Aug 1;237:3–10.

53. Moreno-Garcia L, Adjallé K, Barnabé S, Raghavan GS V. Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. Vol. 76, Renewable and Sustainable Energy Reviews. 2017. p. 493–506.

54. Jankowska E, Sahu AK, Oleskowicz-Popiel P. Biogas from microalgae: Review on microalgae's cultivation, harvesting and pretreatment for anaerobic digestion. Renewable and Sustainable Energy Reviews. 2017.

55. Sukenik A, Shelef G. Algal autoflocculation?verification and proposed mechanism. Biotechnol Bioeng. 1984 Feb 1;26(2):142–7.

56. Şirin S, Trobajo R, Ibanez C, Salvadó J. Harvesting the microalgae Phaeodactylum tricornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. J Appl Phycol. 2012;24(5):1067–80.

57. Chen C-Y, Yeh K-L, Aisyah R, Lee D-J, Chang J-S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. Bioresour Technol. 2011;102(1):71–81.

58. Japar AS, Takriff MS, Yasin NHM. Harvesting microalgal biomass and lipid extraction for potential biofuel production: A review. J Environ Chem Eng. 2017 Feb 1;5(1):555–63.

59. Dassey AJ, Theegala CS. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresour Technol. 2013;128(0):241–5.

60. Mo W, Soh L, Werber JR, Elimelech M, Zimmerman JB. Application of membrane dewatering for algal biofuel. Algal Res. 2015;11:1–12.

61. Marbelia L, Mulier M, Vandamme D, Muylaert K, Szymczyk A, Vankelecom IFJ. Polyacrylonitrile membranes for microalgae filtration: Influence of porosity, surface charge and microalgae species on membrane fouling. Algal Res. 2016;19:128–37.

62. Singh B, Guldhe A, Rawat I, Bux F. Towards a sustainable approach for development of biodiesel from plant and microalgae. Renew Sustain Energy Rev. 2014 Jan;29:216–45.

63. Pavez J, Cabrera F, Azócar L, Torres A, Jeison D. Ultrafiltration of non-axenic microalgae cultures: Energetic requirements and filtration performance. Algal Res. 2015;10:121–7.

64. Larronde-Larretche M, Jin X. Microalgal biomass dewatering using forward osmosis membrane: Influence of microalgae species and carbohydrates

composition. Algal Res. 2017;23:12–9.

65. Bilad MR, Marbelia L, Naik P, Laine C, Vankelecom IFJ. Direct comparison of aerated and vibrated filtration systems for harvesting of Chlorella vulgaris. Algal Res. 2014;6:32–8.

66. Hapońska M, Clavero E, Salvadó J, Torras C. Application of ABS membranes in dynamic filtration for Chlorella sorokiniana dewatering. Biomass and Bioenergy. 2018;111:224–31.

67. Fret J, Roef L, Diels L, Tavernier S, Vyverman W, Michiels M. Implementation of flocculation and sand filtration in medium recirculation in a closed microalgae production system. Algal Res. 2016;13:116–25.

68. Günerken E, D'Hondt E, Eppink MHM, Garcia-Gonzalez L, Elst K, Wijffels RH. Cell disruption for microalgae biorefineries. Biotechnol Adv. 2015;33(2):243–60.

69. Lorente E, Farriol X, Salvadó J. Steam explosion as a fractionation step in biofuel production from microalgae. Fuel Process Technol. 2015 Mar;131:93–8.

70. Gilbert-Lopez B, Mendiola JA, Fontecha J, van den Broek LAM, Sijtsma L, Cifuentes A, et al. Downstream processing of Isochrysis galbana: a step towards microalgal biorefinery. Green Chem. 2015;17(9):4599–609.

71. Rossignol N, Vandanjon L, Jaouen P, Quéméneur F. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. Aquac Eng. 1999;20(3):191–208.

72. Templeton DW, Quinn M, Wychen S Van, Hyman D, Laurens LML. Separation and quantification of microalgal carbohydrates. J Chromatogr A. 2012;1270:225–34.

73. Molina Grima E, Ibañez Gonzalez MJ, Gimenez GA. Solvent extraction for microalgae lipids. Algae for Biofuels and Energy. 2013;187–206.

74. Ryckebosch E, Bruneel C, Termote-Verhalle R, Muylaert K, Foubert I. Influence of extraction solvent system on extractability of lipid components from different microalgae species. Algal Res. 2014;3:36–43.

75. Sheehan J, Dunahay T, Benemann J, Roessler P. Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae; Close-Out Report. United States; 1998.

76. Zhu L, Ketola T. Microalgae production as a biofuel feedstock: risks and challenges. Int J Sustain Dev World Ecol. 2012;19(3):268–74.

77. Zhu L. Biorefinery as a promising approach to promote microalgae industry: An innovative framework. Renew Sustain Energy Rev. 2015;41:1376–84.

78. Wijffels RH, Barbosa MJ, Eppink MHM. Microalgae for the production of bulk chemicals and biofuels. Biofuels, Bioprod Biorefining. 4(3):287–95.

79. Aslam A, Thomas-Hall SR, Mughal TA, Schenk PM. Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas. Bioresour Technol. 2017;233:271–83.

80. Zhao B, Su Y. Process effect of microalgal-carbon dioxide fixation and biomass production: A review. Renew Sustain Energy Rev. 2014;31:121–32.

81. Gupta PL, Lee S-M, Choi H-J. Integration of microalgal cultivation system for

wastewater remediation and sustainable biomass production. World J Microbiol Biotechnol. 2016 Jun;32(8):139.

82. Kumar KS, Dahms H-U, Won E-J, Lee J-S, Shin K-H. Microalgae – A promising tool for heavy metal remediation. Ecotoxicol Environ Saf. 2015;113:329–52.

83. Lupatini AL, Colla LM, Canan C, Colla E. Potential application of microalga Spirulina platensis as a protein source. J Sci Food Agric. 97(3):724–32.

84. Rizza LS, Smachetti MES, Nascimento M Do, Salerno GL, Curatti L. Bioprospecting for native microalgae as an alternative source of sugars for the production of bioethanol. Algal Res. 2017;22:140–7.

85. Reyes FA, Mendiola JA, Ibañez E, del Valle JM. Astaxanthin extraction from Haematococcus pluvialis using CO2-expanded ethanol. J Supercrit Fluids. 2014;92:75–83.

86. Uquiche E, Antilaf I, Millao S. Enhancement of pigment extraction from B. braunii pretreated using CO2 rapid depressurization. Brazilian J Microbiol. 2016;47(2):497–505.

87. Cuello JL, Hoshino T, Kuwahara S, Brown CL. Chapter 19 - Scale-Up—Bioreactor Design and Culture Optimization. In: Eckert CA, Trinh CT, editors. Biotechnology for Biofuel Production and Optimization. Amsterdam: Elsevier; 2016. p. 497–511.

88. Thomassen G, Egiguren Vila U, Van Dael M, Lemmens B, Van Passel S. A techno-economic assessment of an algal-based biorefinery. Clean Technol Environ Policy. 2016 Aug;18(6):1849–62.

89. Ersahin ME, Ozgun H, Dereli RK, Ozturk I, Roest K, van Lier JB. A review on dynamic membrane filtration: Materials, applications and future perspectives. Bioresour Technol. 2012;122:196–206.

90. Kim J, Yoo G, Lee H, Lim J, Kim K, Kim CW, et al. Methods of downstream processing for the production of biodiesel from microalgae. Biotechnol Adv. 2013;31(6):862–76.

91. Zhao F, Chu H, Zhang Y, Jiang S, Yu Z, Zhou X, et al. Increasing the vibration frequency to mitigate reversible and irreversible membrane fouling using an axial vibration membrane in microalgae harvesting. J Memb Sci. 2017;529:215–23.

92. Cheng J, Huang R, Li T, Zhou J, Cen K. Physicochemical characterization of wet microalgal cells disrupted with instant catapult steam explosion for lipid extraction. Bioresour Technol. 2015;191:66–72.

93. Grimi N, Dubois A, Marchal L, Jubeau S, Lebovka NI, Vorobiev E. Selective extraction from microalgae Nannochloropsis sp. using different methods of cell disruption. Bioresour Technol. 2014;153:254–9.

94. Mendes-Pinto MM, Raposo MFJ, Bowen J, Young AJ, Morais R. Evaluation of different cell disruption processes on encysted cells of Haematococcus pluvialis: effects on astaxanthin recovery and implications for bio-availability. J Appl Phycol. 2001 Feb;13(1):19–24.

95. Yap BHJ, Dumsday GJ, Scales PJ, Martin GJO. Energy evaluation of algal cell disruption by high pressure homogenisation. Bioresour Technol. 2015;184:280–5.

96. Safi C. Microalgae biorefinery: proposition of a fractionation process. École Doctorale Sciences de la Matière (Toulouse); 154236152; 2013.

97. Lorente E, Hapońska M, Clavero E, Torras C, Salvadó J. Steam Explosion and Vibrating Membrane Filtration to Improve the Processing Cost of Microalgae Cell Disruption and Fractionation. Processes. 2018;6(4).

98. Lee AK, Lewis DM, Ashman PJ. Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. Biomass and Bioenergy. 2012;46:89–101.

99. Heitz M, Capek-Ménard E, Koeberle PG, Gagné J, Chornet E, Overend RP, et al. Fractionation of Populus tremuloides at the pilot plant scale: Optimization of steam pretreatment conditions using the STAKE II technology. Bioresour Technol. 1991;35(1):23–32.

100. Verwijst T, Baggerman J, Liebermann F, van Rijn CJM. High-frequency flow reversal for continuous microfiltration of milk with microsieves. J Memb Sci. 2015;494:121–9.

101. Malmali M, Stickel JJ, Wickramasinghe SR. Sugar concentration and detoxification of clarified biomass hydrolysate by nanofiltration. Sep Purif Technol. 2014;132:655–65.

102. Kumar K, Das D. Growth characteristics of Chlorella sorokiniana in airlift and bubble column photobioreactors. Bioresour Technol. 2012;116:307–13.

103. Chua ET, Schenk PM. A biorefinery for Nannochloropsis: Induction, harvesting, and extraction of EPA-rich oil and high-value protein. Bioresour Technol. 2017;244:1416–24.

104. Francavilla M, Kamaterou P, Intini S, Monteleone M, Zabaniotou A. Cascading microalgae biorefinery: Fast pyrolysis of Dunaliella tertiolecta lipid extracted-residue. Algal Res. 2015;11:184–93.

105. Scholz MJ, Weiss TL, Jinkerson RE, Jing J, Roth R, Goodenough U, et al. Ultrastructure and Composition of the Nannochloropsis gaditana Cell Wall. Eukaryot Cell . 2014 Nov 1;13(11):1450–64.

106. Takeda H. Taxonomical assignment of chlorococal algae from their cell wall composition. Phytochemistry. 1993;34(4):1053–5.

107. Kodner RB, Summons RE, Knoll AH. Phylogenetic investigation of the aliphatic, non-hydrolyzable biopolymer algaenan, with a focus on green algae. Org Geochem. 2009;40(8):854–62.

108. Bligh EG, Dyer WJ. A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION. Can J Biochem Physiol. 1959;37(1):911–7.

109. Torres CM, Ríos SD, Torras C, Salvadó J, Mateo-Sanz JM, Jiménez L. Sustainability analysis of biodiesel production from Cynara Cardunculus crop. Fuel. 2013;111:535–42.

110. Ríos SD, Castañeda J, Torras C, Farriol X, Salvadó J. Lipid extraction methods from microalgal biomass harvested by two different paths: Screening studies toward biodiesel production. Bioresour Technol. 2013;133(0):378–88.

111. Akutu K, Kabashima H, Seki T, Hattori H. Nitroaldol reaction over solid base catalysts. Appl Catal A Gen. 2003;247(1):65–74.

112. Baroutian S, Aroua MK, Raman AAA, Sulaiman NMN. A packed bed membrane reactor for production of biodiesel using activated carbon

supported catalyst. Bioresour Technol. 2011;102(2):1095–102.

113. Mierczynski P, Ciesielski R, Kedziora A, Maniukiewicz W, Shtyka O, Kubicki J, et al. Biodiesel Production on MgO, CaO, SrO and BaO Oxides Supported on (SrO)(Al2O3) Mixed Oxide. Catal Letters. 2015 May;145(5):1196–205.

114. Gandía LM, Reyero I, Bimbela F, Moral A, Radosevic J, Sanz O, et al. Metallic monolithic catalysts based on calcium and cerium for the production of biodiesel. Fuel. 2016;182:668.

115. Vahid BR, Haghighi M. Urea-nitrate combustion synthesis of MgO/MgAl2O4 nanocatalyst used in biodiesel production from sunflower oil: Influence of fuel ratio on catalytic properties and performance. Energy Convers Manag. 2016;126:362–72.

116. Camacho Jesus N, Reyna N, E GMG, Ivan G-O, Ramiro B, Rubi R. Comparative Study of Quick Lime and CaO as Catalysts of Safflower Oil Transesterification. Vol. 14, International Journal of Chemical Reactor Engineering. 2016. p. 909.

117. Chen C-L, Huang C-C, Tran D-T, Chang J-S. Biodiesel synthesis via heterogeneous catalysis using modified strontium oxides as the catalysts. Bioresour Technol. 2012;113(0):8–13.

118. Wang Y-Y, Chou H-Y, Chen B-H, Lee D-J. Optimization of sodium loading on zeolite support for catalyzed transesterification of triolein with methanol. Bioresour Technol. 2013 Oct;145:248—253.

119. López DE, Goodwin JG, Bruce DA, Lotero E. Transesterification of triacetin

with methanol on solid acid and base catalysts. Appl Catal A Gen. 2005;295(2):97–105.

120. Dubé MA, Tremblay AY, Liu J. Biodiesel production using a membrane reactor. Bioresour Technol. 2007;98(3):639–47.

121. Cao P, Tremblay AY, Dubé MA. Kinetics of Canola Oil Transesterification in a Membrane Reactor. Ind Eng Chem Res. 2009;48(5):2533–41.

122. Cao P, Tremblay AY, Dubé MA, Morse K. Effect of Membrane Pore Size on the Performance of a Membrane Reactor for Biodiesel Production. Ind Eng Chem Res. 2007;46(1):52–8.

123. Carvalho CML, Cunnah P, Aires-barros MR, Cabral JMS. Performance Of A Membrane Bioreactor For Enzymatic Transesterification: Characterization And Comparison With A Batch Stirred Tank Reactor. Biocatal Biotransformation. 2000;18(1):31–57.

124. Sun YM, Khang SJ. A catalytic membrane reactor: its performance in comparison with other types of reactors. Ind Eng Chem Res. 1990 Feb 1;29(2):232–8.

125. Nurra C. Separation processes in microalgae biorefining. Universitat Rovira i Virgili; 2014.

126. Liu X, He H, Wang Y, Zhu S. Transesterification of soybean oil to biodiesel using SrO as a solid base catalyst. Catal Commun. 2007 Jul;8(7):1107–11.

127. van de Witte P, Dijkstra PJ, van den Berg JWA, Feijen J. Phase separation processes in polymer solutions in relation to membrane formation. J Memb Sci. 1996;117(1):1–31.

128. J. C. Pasqualino, PhD, Universitat Rovira i Virgili, 2007.

THESIS OUTPUTS

Publications

1) **M. Hapońska**, E. Clavero, J. Salvadó, X. Farriol, C. Torras, *Pilot scale dewatering of Chlorella sorokiniana and Dunaliella tertiolecta by sedimentation followed by dynamic filtration*, Algal Research, Volume 33, 2018, Pages 118-124, ISSN 2211-9264, https://doi.org/10.1016/j.algal.2018.05.007

2) **M. Hapońska**, E. Clavero, J. Salvadó, C. Torras, *Application of ABS membranes in dynamic filtration for Chlorella sorokiniana dewatering*, Biomass and Bioenergy, Volume 111, 2018, Pages 224-231, ISSN 0961-9534, http://doi.org/10.1016/j.biombioe.2017.03.013.

3) E. Lorente, **M. Hapońska**, E. Clavero, C. Torras, J. Salvadó, *Steam Explosion and Vibrating Membrane Filtration to Improve the Processing Cost of Microalgae Cell Disruption and Fractionation*, Processes, Volume 6, Issue 4, 2018, Article number 28, ISSN 2227-9717, DOI: 10.3390/pr6040028

4) E. Lorente, **M. Hapońska**, E. Clavero, C. Torras, J. Salvadó, *Microalgae fractionation using steam explosion, dynamic and tangential cross-flow membrane filtration*, Bioresource Technology, Volume 237, 2017, Pages 3-10, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2017.03.129.

Pending:

5) **M. Hapońska**, C. Nurra, S. Abelló, M. Makkee, J. Salvadó, C. Torras, *Comparison of novel catalytic and inert membrane reactors for biodiesel production with strontium oxide as a heterogeneous catalyst*, final manuscript preparation

Oral presentations

1) **M. Hapońska**, E. Clavero, J. Salvadó, C. Torras, *Dunaliella Tertiolecta microalgae harvesting using ABS membranes in vibratory filtration,* 25th European Biomass Conference and Exhibition EUBCE 2017, Stockholm, Sweden

2) **M. Hapońska**, E. Clavero, J. Salvadó, C. Torras, *Polymeric membranes in biodiesel production from microalgae,* III Symposium of Young Scientists, Poznań, Poland

3) **M. Hapońska**, E. Clavero, J. Salvadó, C. Torras, *Application of ABS membranes in dynamic filtration for microalgae dewatering*, 24th European Biomass Conference and Exhibition EUBCE 2016, Amsterdam, Netherlands

4) E. Lorente, C. Torras, E. Clavero, **M. Hapońska**, O. Núñez, J. Salvadó, *Improvement of microalgae fractionation using steam explosion and tangential cross-flow filtration,* 1st International Conference on Bioresource Technology for Bioenergy, Bioproducts & Environmental Sustainability Biorestec 2016, Sitges, Spain

Poster presentations

1) **M. Hapońska**, J. Salvadó, C. Torras, *Biorefining of microalgae: from oil extraction to biofuel production,* 12th Doctoral day, URV Tarragona, Spain, 20th May 2015

2) **M. Hapońska**, E. Lorente, J. Salvadó, C. Torras, *Application of steam explosion and membrane filtration for cell disruption and fractionation of several microalgae species,* 14th Doctoral day, URV Tarragona, Spain, 24th May 2017

ABOUT THE AUTHOR

Monika Hapońska was born on 12 June 1990 in Poland. She was raised in a small village close to Gniezno, where she finally moved together with her family when she was seven. After finishing high school, she moved to Poznań to study Chemistry at the Adam Mickiewicz University.

After the first year of her MSc studies she had an opportunity to do an internship in the CTQC in Tarragona, Spain. During the second year she performed her master project at the Universitat Rovira I Virgili in Tarragona. She obtained her MSc degree on 7 August 2014 and afterwards on September 2014 she started her PhD thesis at the URV in the collaboration with the Bioenergy and Biofuels division of Catalonia Institute for Energy Research. Her project entitled “Biorefining of microalgae: from harvesting to biofuel production” was supervised by Dr. Carles Torras Font and Prof. Joan Salvadó Rovira. She participated in the several European and National projects.

The research she performed during her PhD thesis resulted in four scientific articles already published in the prestigious international journals and another one ready to be published. Her work has been presented at the numerous international conferences, held either as an oral or poster presentations.

UNIVERSITAT ROVIRA I VIRGILI
BIOREFINING OF MICROALGAE: FROM HARVESTING TO BIOFUEL PRODUCTION
Monika Haponska

UNIVERSITAT
ROVIRA i VIRGILI