CZECH TECHNICAL UNIVERSITY IN PRAGUE

FACULTY OF MECHANICAL ENGINEERING

DEPARTMENT OF PROCESS ENGINEERING

TECHNOLOGY TO SEPARATE MICROALGAE FROM WATER BATCH

BACHELOR'S THESIS

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2020

ZADÁNÍ BAKALÁŘSKÉ PRÁCE

I. OSOBNÍ A STUDIJNÍ ÚDAJE

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Technologie separace mikrořas z vodného prostředí

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Technology to separate microalgae from water batch

Pokyny pro vypracování:

Zpracujte literární, patentovou a průmyslovou rešerši zaměřenou na možnosti separace mikrořas z vodného prostředí. Otestujte různé přístupy s ohledem na možnosti využití laboratorního vybavení U12118 a proveďte prvotní testy účinnosti separace.

Seznam doporučené literatury:

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Jméno a pracoviště vedoucí(ho) bakalářské práce:

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III. PŘEVZETÍ ZADÁNÍ

Student bere na vědomí, že je povinen vypracovat bakalářskou práci samostatně, bez cizí pomoci, s výjimkou poskytnutých konzultací. Seznam použité literatury, jiných pramenů a jmen konzultantů je třeba uvést v bakalářské práci.

29.06.2020
Datum převzetí zadání

Krupica
Podpis studenta

STATEMENT OF ORIGINALITY

I hereby declare that the present bachelor's thesis was composed by myself and that the work contained herein is my own. All the assistance received in preparing this thesis and sources have been acknowledged.

Prague ……. ……

Adam Krupica

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I would like to thank my supervisor doc. Ing. Lukáš Krátký who has introduced me to this topic and have helped me throughout the whole project. There have never been a bad time for me to have a question and for that, I am truly grateful.

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Annotation sheet

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Annotation - Czech: Účelem této práce je zhodnocení současných metod separace mikrořas. Součástí práce je literární a patentová rešerše, během které byly nalezeny tři hlavní skupiny metod separace. Těmi jsou gravitační, odstředivé a filtrační metody. Zároveň bylo provedeno několik experimentů za účelem zhodnocení využitelnosti těchto metod ve školní laboratoři.

Annotation - English: The aim of this thesis is to review current methods of microalgal harvesting and their evaluation. Literature and patent review have been carried out and three main mechanisms of separation were found. These are gravitational, centrifugal, and filtration methods. Subsequently, several experiments were performed to evaluate the possible utilization of these methods in the school laboratory.

Keywords: Microalgae, Harvesting, Chlorella, Filtration, Flocculation

Utilization: General overview of microalgal harvesting technologies.

Contents

Abbreviations

CCS	Carbon capture and storage	PAC	Polyaluminum chloride
CCU	Carbon capture and utilization	PAN	Polyacrylonitrile
CFF	Cross flow filtration	PBR	Photobioreactor
CTAB	Cetrimonium bromide	PES	Polyethersulfone
DAF	Dissolved air flotation	PTFE	Polytetrafluoroethylene
DIF	Dispersed air flotation	PVDF	Polyvinylidene fluoride
EPS	Extracellular polymeric substances	RPM	Rotations per minute
IAF	Induced air flotation	TOE	Ton of oil equivalent
IONPs	Iron oxide nanoparticles	TSS	Total suspended solids
MCE	Mixed cellulose esters	UF	Ultrafilter

Physical quantities

v	Settling velocity	(m s^{-1})	v_L	Velocity of segment ascent velocity	(m s^{-1})
g	Local gravitational field	(m s^{-2})			
d	Diameter of a particle	(µm)	E_x	Efficiency of a membrane	(-)
ρ_p	Density of a particle	(kg m^{-3})	N_u	Number of particles upstream	(-)
ρ	Density of a fluid	(kg m^{-3})			
c_v	Concentration of particles in a fluid	(m^{-3})	N_d	Number of particles downstream	(-)
η	Dynamic viscosity	(Pa s)	β	Beta ratio	(-)
h_0	Height of fluid at the beginning	(m)	φ	Mass concentration	(kg m^{-3})
			ω_i	Mass fraction	(-)
h	Height at a given time of sedimentation	(m)	m_{si}	Weight of the dried biomass	(g)
			V_i	Volume sample	(ml)
			m_i	Suspension weight	(g)

1 Introduction

The rising amount of carbon dioxide (CO_2) in the atmosphere and subsequent global temperature rise has in recent decade sparked a debate on how to tackle this phenomenon. Several solutions have emerged from this debate. The main two are CCS and CCU.

The focus of CCS (carbon capture and storage) technologies is to gather CO_2 from the atmosphere and store it somewhere for a long period. Such a solution is not a permanent one as there is only a limited amount of storage space and CO_2 levels are expected to continue rising as is well apparent from Fig. 1. and Fig. 2. Additionally, associated upfront costs, necessary transportation of CO_2, and overall resistance of locals further impede these technologies [3]. In contrary to CCS, the goal of CCU (carbon capture and utilization) technologies is to manage the CO_2 level by converting it into products that could be utilized by society. Examples of which could be biofuels or other chemical compounds, or food source/supplement. Presumably less effective in the beginning but in the long run CCU technologies will be necessary to get CO_2 levels under control.

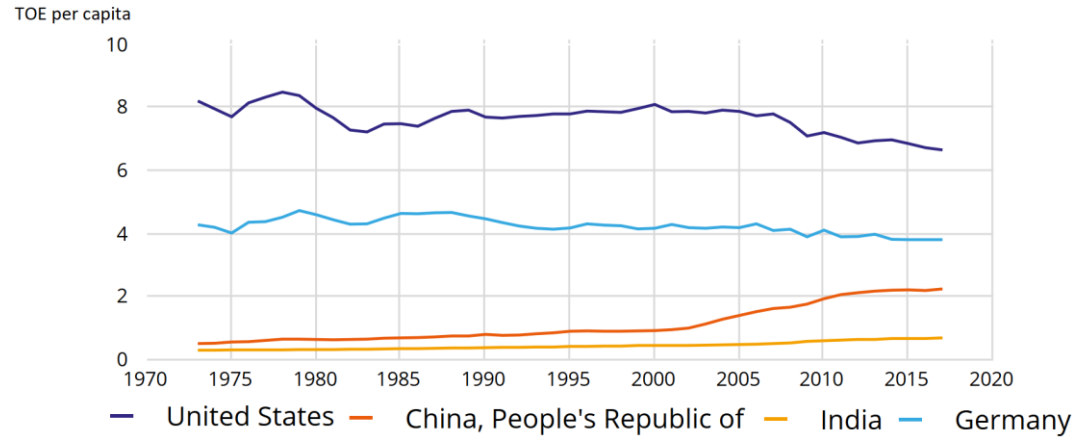

Fig. 1. *Total primary energy supply [1].*

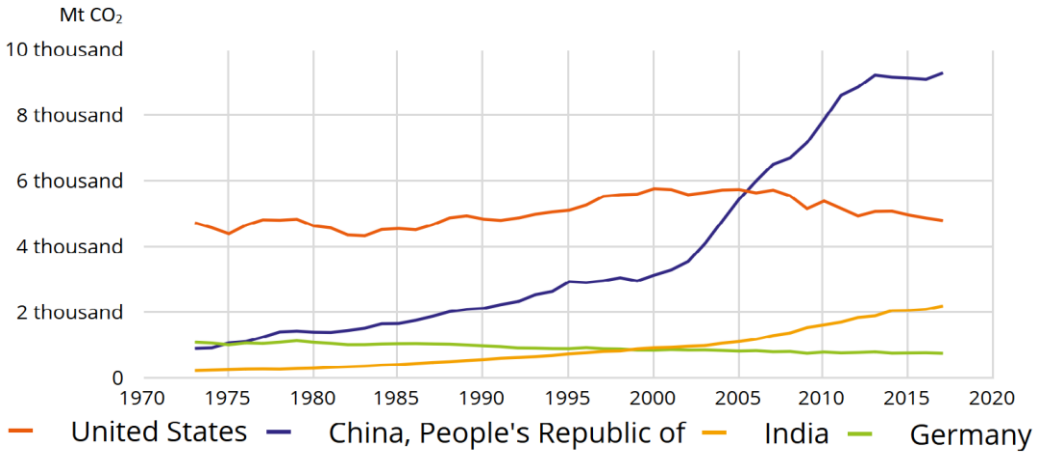

Fig. 2. *CO_2 emissions [2].*

The biggest drawback of the CCU is the energy potential of CO_2. Since it is a final product of a chain of reactions it requires an energy input to transform the gas into something utilizable [3]. Some synthetic solutions are presented in Fig. 3. Production of an intermediate product that could be further processed into something more desirable is also a possibility. An example of such a process is microalgal cultivation during which CO_2 is 'transformed' into microalgal biomass from which other more valuable products could be produced. The benefits of this solution will be discussed in the subsequent chapter.

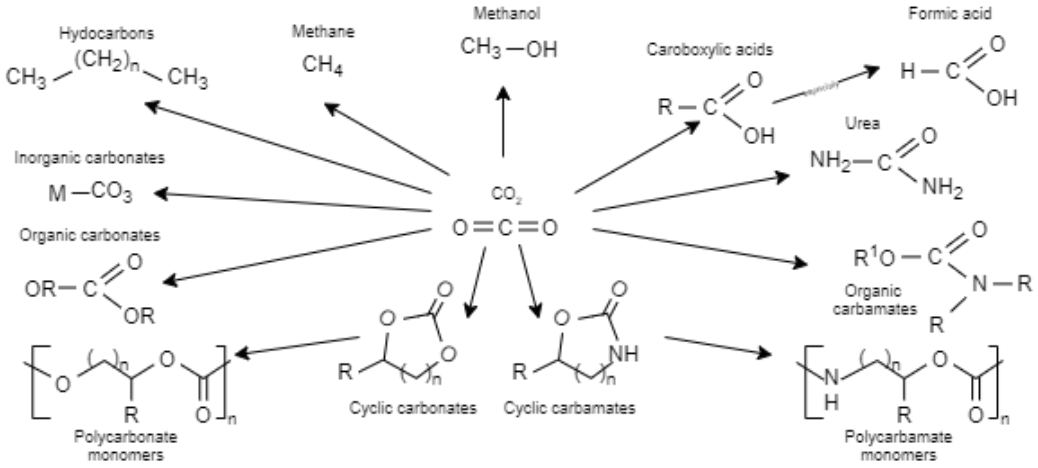

Fig. 3 *CCU-Synthethic products [3]*

1.1 Microalgae

Microalgae, a subclass of algae, is a group of eukaryotic plants that have an average size of around 5-100 μm. The cells are typically spherical but other shapes such as needles or cylinders are also common. Microalgae have a density similar to water and negative surface charge [4, 5]. In contrary to regular crops microalgae grow faster and do not require arable land therefore they can produce more biomass even under conditions where other plants would not survive [3]. Annually over 5000 tons of dry microalgae are being produced. Most of them are used as feedstock [6].

All microalgae species are slightly different in their composition and are therefore suitable for a production of different products some of them may be seen in Fig. 4. Of those mentioned high-value products such as food, proteins, and fatty acids are more desirable. The production process is similar for all microalgal species and consists of three main steps. The process starts with the cultivation during which a culture medium is produced. Raceway ponds, flat panel, tube, or biofilm photobioreactors are commonly used for this process. It is reported that the type of cultivation system can affect the properties of the culture medium namely the size of the microalgal cells and the number per liter of the medium [7]. After the cultivation stage, the microalgae is then harvested

from the culture medium. The reason is that with a smaller processed volume, the overall cost of the subsequent processes are reduced which may lead to lower overall costs. The type of subsequent process may vary by the nature of the desired product but drying, disintegration, or substance extraction seem to be the most common [3]. As there are many genera of microalgae with vastly different properties this thesis is aimed at the genus *Chlorella* as it is the microalgae cultivated at the faculty.

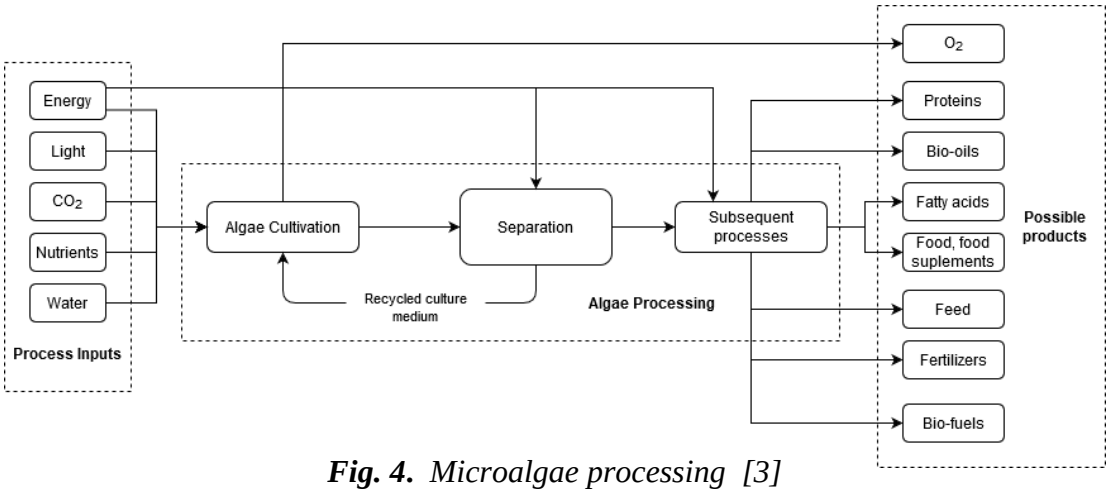

Fig. 4. *Microalgae processing [3]*

An average *Chlorella* cell has a diameter of 5.3 μm but it can vary from 3-10 μm. Its density is around 1070 kg m^{-3} [8] which is similar to the density of water. The concentration of cells in a culture medium highly depends on the source of the microalgae. Cultivated *Chlorella* has a rather low average concentration of 0.4 g L^{-1} [9, 10], with a maximum around 10 g L^{-1} [11]. This property heavily depends on the type of cultivation method as each of them produces microalgal suspension with different concentration [12].

1.2 Goals of the thesis

Microalgal separation is one of the key components of the process and is therefore necessary for its successful and efficient implementation. The goal of this thesis is to gain a better insight into the subject and to prepare the ground for further research. The first part of this thesis is therefore an overview of the common methods found in the literature with their assessment at its end. In the second part, several ‘proof of concept’ experiments shall be conducted to verify some of the information gained from the literature review and to evaluate which methods are feasible with the available equipment.

2 Methods of separation

Microalgal harvesting is one of the key components in microalgal processing and along with disintegration it is one of its main bottlenecks. The reason is its energy-demanding nature. It is estimated that 20-30 % of total production costs are invested in microalgal separation and thickening [13]. Therefore an advancement in this field may greatly improve the overall rentability of the whole process, which is currently negative [14].

Nowadays, there are several possible strategies on how to harvest microalgal cells from their culture medium. All of them have several advantages and disadvantages and each of them has its field of applicability.

Gravitational methods are generally used in water treatment facilities due to their lower costs, high efficiency, and overall reliability. Due to the necessity of flocculant, which is often toxic, microalgae separated through this method tend to be of low value.

Centrifugation, on the other hand, is an expensive, energy-demanding process that needs large upfront investment but is well suited and has been used in the production of high-value microalgal food and food supplements. The reason is that it does not decrease the value of the product [15, 16].

Filtration should not be as expensive as centrifugation [9] and theoretically without the necessity to use a flocculant, it may be the most universal method of microalgae separation. Its main handicap is the problem with fouling which needs to be addressed first [10].

2.1 Gravitational methods

2.1.1 Sedimentation

Sedimentation is the easiest, least energy-demanding method of microalgal separation. Due to the gravitational pull, the particles of microalgae slowly drift towards the bottom of the vessel.

The velocity with which an object sinks in a fluid is described by Stokes law (1) where g (m s^{-2}) is the local gravity constant, d (m) and ρ_p (kg m^{-3}) are the diameter of the sedimenting particle and its density, ρ (kg m^{-3}) and η (Pa s) are the density and dynamic viscosity of the fluid. Hence an estimated sedimentation velocity of a large *Chlorella* cell can be calculated. The resulting velocity is around 14 mm h^{-1}. This means that the time necessary to fully sediment a 1 m high tank would be close to 3 days.

$$v = \frac{g \cdot d^2 \cdot (\rho_p - \rho)}{18\eta} \quad (1)$$

The main contributor to the sedimentation velocity is the square of the diameter of a particle. That is why microalgal cells, which have a microscopic diameter, tend to sediment so slowly. The fact that the density of microalgae is similar to that of water does not help either.

The real sedimentation velocity is even lower. The diameter used for this estimate was 10 μm and the calculation does not consider that the cells can slowly move on their own. Furthermore, this equation describes only a part of the sedimentation process and is only valid for the low concentration of particles that neither interact with one another nor with the surface of the vessel. Real microalgal cells do not fulfill either of those criteria.

Moreover, during this process, the tank cannot be agitated. This leads to the starvation of the cells as they are not properly supplied with nutrients. Deterioration may occur as a consequence and thus it may limit the efficiency of subsequent processes [12].

By modifying the diameter of the particles, the sedimentation velocity can be increased. This can be achieved by binding the cells together, by flocculating them. During the flocculation electrostatic forces that keep the cells separated are overcome and flocks can be produced [4].

Due to the complex geometries and different properties between the flocks the velocity with which the flocks shall sink cannot be easily described. Unfortunately, this velocity is one of the key parameters when a settling or centrifugation device is being designed [17].

An example of such a calculation is given in Eq. 2. By this equation, a minimal outer diameter R_2 (m) of a sedimentation tank is calculated. In this equation, R_1 (m) is the inner diameter of the vessel and $\dot{V}_{su}$(m^3 s^{-1}) demanded flow rate and v (m s^{-1}) the sedimentation velocity of microalgal cells. The schematic of such a device is presented in Fig. 5.

$$R_2 = \sqrt{R_1^2 + \frac{\dot{V}_{su}}{\pi v}} \qquad (2)$$

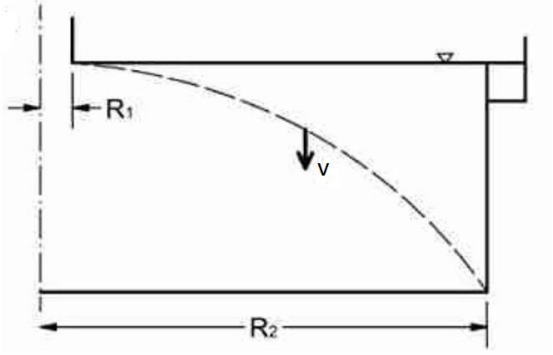

Fig. 5. *Schematic of a settler [17]*

A method through which it can be determined utilizes a sedimentation curve, which is shown in Fig. 6. Depending on the properties of the suspension the curve has a different shape but several pieces of information can be obtained from it. The concentration at the boundary c_v (kmol m^{-3}) is described by Eq. 3. where h_0 (m) and c_{v0} (kmol m^{-3}) are the height and concentration at the beginning. h_t (m) is the specific height of the boundary for which the concentration is calculated. Furthermore, sedimentation velocity v (m s^{-1}) and velocity with which a layer with given concentration rises v_L (m s^{-1}) can also be described by Eq. 4. and 5. Therefore, an experiment with different flocculants should be conducted to determine their efficiency, sedimentation velocity, toxicity, and final concentration.

$$c_v = c_{v0}\frac{h_0}{h_t} \tag{3}$$

$$v = \tan\alpha \tag{4}$$

$$v_L = \tan\beta \tag{5}$$

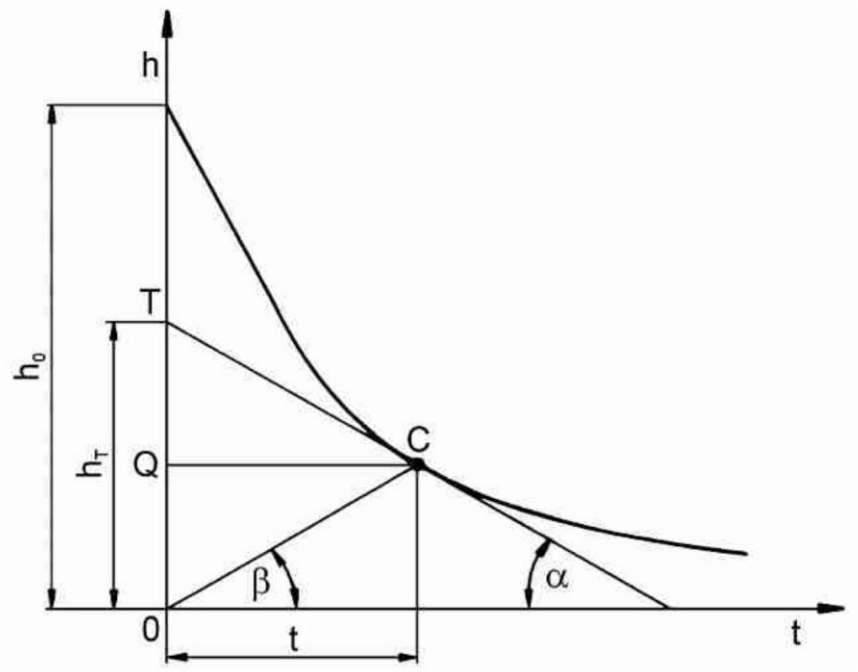

Fig. 6. *Concentration curve during sedimentation [18]*

Such a test was carried out by Vandamme et al. [19] and its results are displayed in Fig. 7. Fastest flocculation occurred when aluminum salt was used with an average velocity of 150 mm h^{-1} with 5 times higher concentration than that at the beginning. When cationic starch was used the resulting concentration was 7.5 times higher than at the beginning but the velocity of sedimentation was only 40.8 mm h^{-1}. Both of the results are 1.5 to 150 times faster than sedimentation without flocculant that can vary from 1 to 26 mm h^{-1} [12, 20].

In conclusion, sedimentation may be a cheap, reliable way to separate microalgal cells from water. What holds this method back is the necessary time (tenths of minutes for larger vessels with the addition of flocculant [21]) and low recovery rate without it (60% to 65% [22]), low output concentration (up to 1.5 % [20, 5], 6% with flocculant [23] TSS), and flocculant toxicity. Given that the necessary dose of flocculant for swift sedimentation is 10 to 50 times higher than the dose for flotation, it is a far less popular method as more efficient flocculants can be expensive. Due to these facts sedimentation tanks are not used widely in the industry. Their energy requirement is around 0.9 kWh t^{-1} [7].

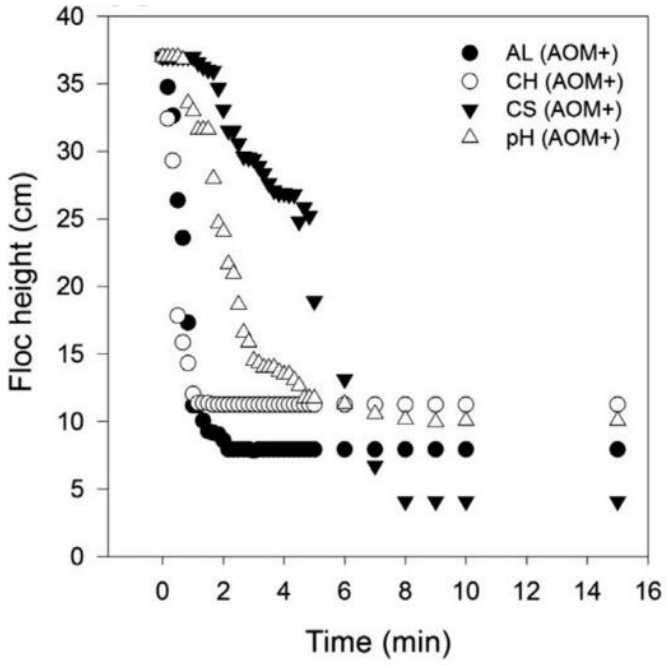

Fig. 7. *Sedimentation curves for flocculation induced by Aluminum (AL), Chitosan (CH), Cationic starch (CS) and pH [19]*

2.1.2 Flotation

In contrary to sedimentation during flotation microalgal cells are brought towards the surface of the vessel. This type of separation can be achieved by binding gas bubbles to the cell's surface. For this to occur the cells must be destabilized by a flocculant which results in subsequent binding of the cell/flock to the bubbles [24]. To the benefit of this effect, microalgal cells tend to float as they have a relatively low density [25]. Compared to sedimentation, flotation is faster and the flocks are easier to gather but it is dependent on the addition of gas bubbles into the liquid. Their size and distribution largely influence the efficiency of the system. Poor design may lead to additional maintenance costs [26]. This method is for those reasons frequently used in water treatment and other large scale applications [13]. The expected output solid content is similar for all types of flotation and is around 7% TSS [23].

Several types of flotation appliances, derived by the mechanism of bubble creation, can be distinguished: dissolved air flotation, dispersed air flotation, and electrolytic flotation are the most common ones.

a. Dissolved air flotation

DAF in short, works on the principle of air solubility described by Henry's law (Eq. 6.), wherein a separate saturator at higher pressure air is dissolved into the water. This solution is then pumped into the tank with microalgal suspension where due to the pressure drop air bubbles are formed [13]. A schematic of such a device can be seen in Fig. 8. These bubbles have a diameter between 10-100 μm with an average of around 40 μm [8, 5]. The recommended saturator pressures are between 400-600 kPa [27].

$$H^{cp} = \frac{c_a}{p} \tag{6}$$

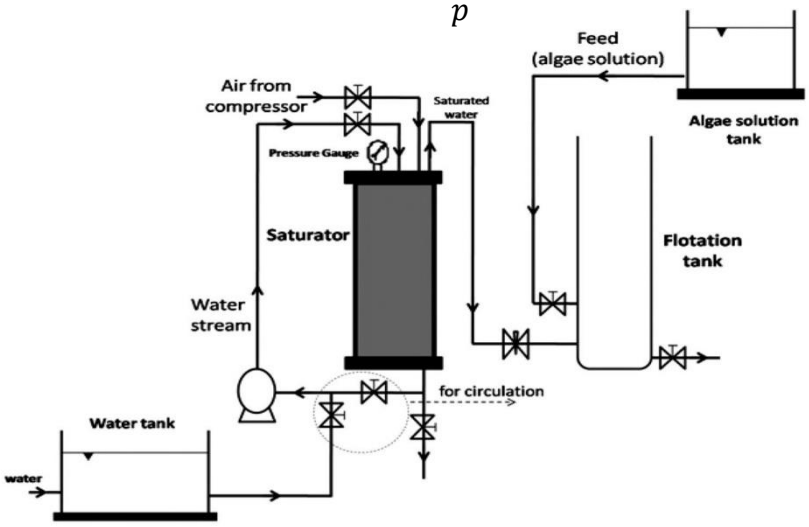

Fig. 8. *Schematic of DAF unit [13]*

b. Induced air flotation / Dispersed air flotation

Both methods utilize a rotating agitator. In IAF air is pumped into the agitator from which it is released through small pores on its surface wherein DIF the air is pumped beneath it and is then dispersed by the blades. This difference is well apparent from Fig. 9. and Fig. 10. The diameter of the bubbles ranges from 700-1500 μm depending on the properties of the agitator [13]. The benefits of these methods are that it is simpler and cheaper to make. But DAF is often considered to be more efficient [13].

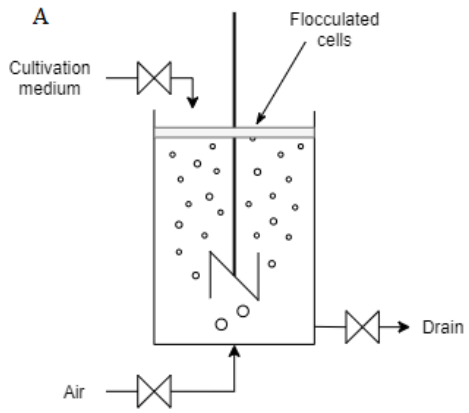

Fig. 9. *DIF unit scheme (A)*

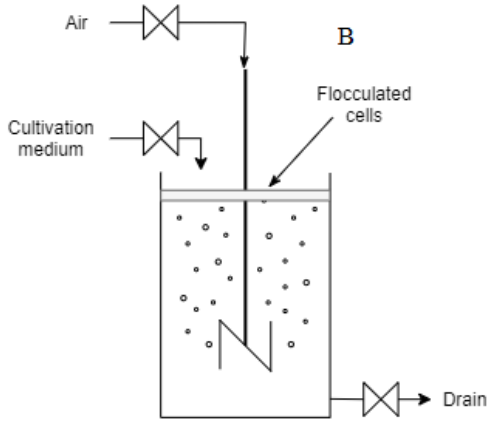

Fig. 10. *IAF unit scheme (B)*

c. Electrolytic flotation

Based on Faraday's law of electrolysis, electrolytic flotation is suitable for conductive microalgal suspensions such as seawater. The gas bubbles of hydrogen and oxygen are formed on the cathodes and anodes. Subsequently, the bubbles adhere to the microalgal cells or flocks and carry them towards the surface [5]. Combination with electrolytic coagulation seems like a preferred solution as flocks are generally easier to separate and the disposition of such a device would already have all the necessary equipment installed. Furthermore, such a configuration is already used in some water treatment facilities to remove heavy metals, dyes, and other chemicals [28]. The required energy, necessary temperature control, and scalability remain a problem.

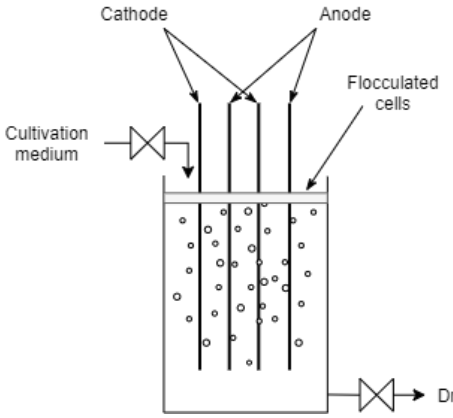

Fig. 11*. Electrolytic flotation unit scheme*

2.1.3 Flocculants

Terms coagulants and flocculants are often considered as synonyms and hence only term flocculant will be used in this thesis. But in general, flocculants are polymeric chemicals which after their addition to the suspension bind to the cell's surface and create bigger, less dense flocks whereas coagulants are electrolytes (mostly salts) whose purpose is to destabilize the surface charge of the cells creating smaller more dens coagula [10].

There are three main mechanisms of flocculation patching, bridging, and sweeping. Patching means that a flocculant is patched to the surface of the cell. As a consequence, the surface charge is neutralized which allows other cells to bind to it.

In bridging the flocculant is capable to adhere to several cells at once hence binding them together.

Sweeping flocculation is when the cells are trapped inside of a mineral precipitate [20].

Only two forces are generally considered in mechanisms of flocculation. Long-range electrostatic forces, which are the reason why we need flocculants in the first place, as the cells tend to be all of a similar charge and therefore repulse one another, and short-range Van der Waals force which enables binding between polymers and cells [29].

When applied to the microalgal separation several criteria emerge. First and foremost, this process should be efficient and its cost reasonable. Secondly, the flocculant should not be toxic as it would decrease the market value of the product and add a necessary step of water purification. In an ideal scenario, the culture medium should be reused again in the cultivation stage [15]. These requirements severely limit the number of suitable flocculants.

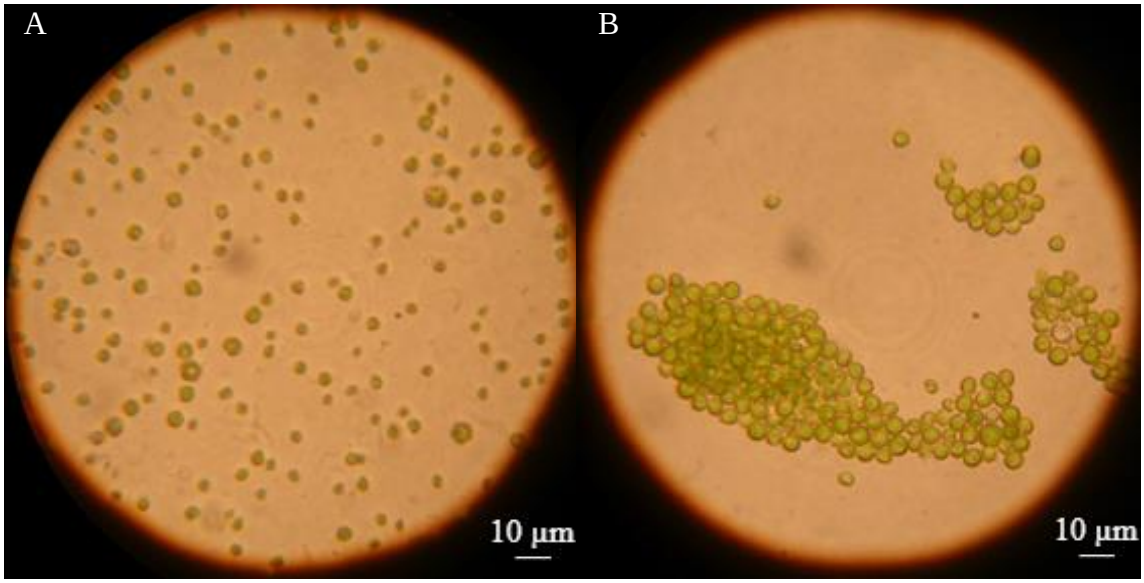

Fig. 12. *Unflocculated cells (A)*

Fig. 13. *Flocculated cells (B)*

Flocculants can be distinguished into several categories, some of which are closely bound together, and their definition is often enlarged or interchanged. The efficiency of a flocculant is determined by its ability to neutralize the surface charge. Parameter evaluating this ability is either ζ potential or ionic strength [20], both of them also depend on the pH of the suspension and the type of algae.

In Fig. 12. and Fig. 13. the difference caused by the addition of flocculant into a culture medium can be seen. At the end of the process, the flocks can be so densely packed that light is not permitted to pass through the flock.

There are two similarly working categories of flocculants which are often interchanged, bio-flocculants and auto-flocculants. The difference between them will be explained in subsequent paragraphs.

a. Bio-flocculants

Bioflocculation is induced by a separate microorganism such as bacteria or fungi which coexist with the microalgae. This type of flocculation is a naturally occurring phenomenon and is therefore often presented as non-toxic [30] and environmentally friendly solution to the problems of flocculants. The best-case scenario proposed by Vandamme et al. [24] is the cocultivation of microalgae and flocculant in one tank as part of a water treatment plant. Even though promising there are some hindrances that should be mentioned. First of them is the fact that it is a biological process that is very hard to predict and therefore hard to control [20]. Secondly, every microalgal species may react differently to the same microorganism, therefore more amount of research will be necessary before the implementation of this method. Lastly, a large-scale plantation with semi flocculated microalgae slowly circulating through the pipes may be hard to achieve as even unflocculated cells tend to stick to a surface rather well.

In Fig. 14 the results of experiments carried out by Lee et al. [30] are presented. Lee et al. have tested several bacteria produced bio-flocculants with a base concentration of 9 g L^{-1}. Bacterial strain *AM49* had the best results and was therefore subjected to further testing. In these tests dependency on pH value was presented with the best efficiency of 86 % for pH 11. With pH-neutral culture medium efficiency of 81 % was reached. Thus an alkaline co-flocculant would be needed for the highest efficiency. $CaCl_2$ was suggested, but this might lessen the value of produced microalgae.

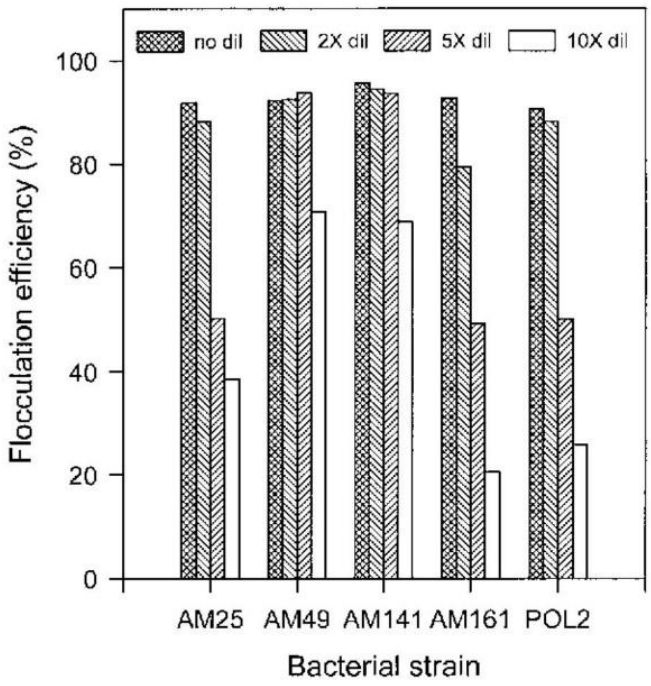

Fig. 14. *Some bioflocculant strains with a base concentration of 7 g L^{-1} [30]*

b. Auto-flocculants

The main difference between autoflocculants and bioflocculats is that during autoflocculation the microalgal cells tend to bind together because of self-secreted biopolymers (EPS) [24] which are excreted as a form of protection from the environment. This is mostly achieved by increasing the pH values of the suspension. It is possible to do so either by intentionally increasing the pH value above 9 by adding alkaline substances such as calcium or phosphate [12] or by lowering the amount of CO_2 in the suspension resulting in subsequent precipitation of calcium and magnesium [5]. In consequence, the surface charge of microalgal cells is neutralized by the excreted EPS and hence flocculation may be achieved [12].

There are several problems with this method. The mechanics of autoflocculation are not fully understood yet and further research is needed. Also, high pH values may damage the cells and devalue the desired products. The precipitates may also be of low toxicity which may hinder subsequent processes [10].

In comparison compiled by Mathimani and Malick [20], autoflocculation caused by several different chemicals was tested. It is apparent from Tab. 1. that to flocculate 95 % of all cells in culture medium pH value of 11 must be achieved. Unfortunately, the price could not be determined as the amount of each chemical was not stated.

Tab. 1. *Comparison of auto-flocculants* [20]

Chemical	***pH***	***Efficiency (%)***
Sodium carbonate	Increased to 11	0
Potassium hydroxide	Increased to 11	≥ 95
Calcium hydroxide	Increased to 11	≥ 95
Magnesium hydroxide	Increased to 11	≥ 95

c. Organic flocculants

As the name suggests organic flocculants are organic compounds of polymeric nature, some of which are natural such as chitosan, potato starch, or guar gum while others are synthetic – polyacrylamides and polyelectrolytes being the most common ones. Other means of division are by the nature of their surface charge as anionic, cationic, or non-ionic [13, 15].

Following on the topic of the surface charge, cationic flocculants turned out to be superior by quite a margin over the other two categories. This is due to the nature of the surface charge of the cell, which is negative, and the way the flocculants work i.e. neutralizing the negative charge. Therefore, substances which are in nature cationic will work better than the other two groups [15].

Comparing natural and synthetic flocculants, they both have their pros and cons. Although natural flocculants are overall the most expensive flocculants on the market they benefit from their usually low toxicity, low growth inhibition when the culture medium is reused, and biodegradability [10].

On the contrary synthetic flocculants are cheaper but often toxic, especially acrylamides which are used in the industry today [15]. This toxicity is not only dangerous for other aquatic species but may also hamper subsequent extractions of lipids and other valuable substances. Additionally, it would make the produced microalgae unsuitable as a feed or as a food supplement [31].

In Tab. 2 a comparison of organic flocculants is presented. Price per gram of flocculant and liter of culture medium, necessary dose per liter of culture medium, and efficiency were compared. Efficiency is defined by the number of flocculated cells per number of cells in the culture medium. The prices of several flocculants were not published but there is a striking difference in efficiency between the two polyacrylamide flocculants. Comparing the two natural flocculants moringa oleifera flour seems like the worse of the options as it is both ineffective and expensive.

Tab. 2. *Comparison of organic flocculants [20]*

Flocculant	***Price (€ g^{-1});(€ L^{-1})***	***Type***	***Dose (mg L^{-1})***	***Efficiency (%)***
Chitosan	1.5; 0.045	Natural	30	92
Zetag 8185	-	Polyacrylamide	5	100
Magnafloc LT225	-	Polyacrylamide	35	72
POLY SEPAR© CFL25	-	Tanin, quaternary ammonium salt	30	95
Moringa oleifera	0.2; 0.2	Natural	1000	88

d. Inorganic flocculants

Flocculants of this type are mostly salts of metals Al, Fe, Zn, or of alkaline earth metals such as Mg or Ca [29]. From those mentioned, salts of Al and Fe are considered as the strongest and most efficient as their electronegativity is bigger than that of the remaining compounds [12]. To benefit from this property salts with high oxidation numbers for example $FeCl_3$ or $Al_2(SO_4)_2$ are preferred [32] while they fully use the whole potential of the metal. Those salts can be also pre-polymerized to improve their efficiency [27].

Even though inorganic flocculants are cheaper than their organic counterparts, the costs of using inorganic flocculants can be higher as their efficiency is rather poor. Moreover, similar problems to synthetic flocculants are also present. Aluminum in particular is considered toxic to humans [15]. Furthermore, most salts tend to inhibit methanogenesis and other processes that might be used down the stream [12]. This also means that such water is not recyclable in the growth process.

In a study carried out by Papazi et al. [29], commonly used inorganic flocculants were compared. The main points of comparison were the necessary time, the amount of flocculant per culture medium, and the efficiency of the flocculant. Efficiency is defined by the number of flocculated cells per number of cells in the culture medium. The price estimates were set for each flocculant based on the retail prices of the chemical manufacturer. The first price is per gram of flocculant and the second price is per liter of culture medium. For the reason of comparison, the price per liter was divided by the efficiency of the flocculant. Based on the comparison, salts with chloride anion performed far better than the rest. Due to the low efficiency of $FeCl_3$, $AlCl_3$ seems like the better of the two with an average price of 0.006 € per liter of culture medium.

Tab. 3. *The efficiency of inorganic flocculants [29]*

Flocculant	**$Al_2(SO_4)_3$**	**$AlCl_3$**	**$Fe_2(SO_4)_3$**	**$FeCl_3$**	**$Zn(SO_4)_3$**	**$ZnCl_2$**
Time (s)	300	300	300	300	500	500
Dose (g L^{-1})	0.5	0.5	0.75	0.5	1	0.5
Efficiency (%)	90	90	70	80	80	70
Price (€ g^{-1})	0.05	0.01	0.08	0.05	0.06	0.11
Price (€ L^{-1})	0.028	0.006	0.086	0.031	0.075	0.079

e. Electrolytic flocculants

Electrolytic flocculation or coagulation is very similar to electrolytic flotation as it uses suspension's conductivity as one of its key mechanisms [28]. The difference between flocculation and coagulation is that during coagulation the anode is sacrificed and metal ions are released into the suspension. These ions then act as an inorganic flocculant and thus they neutralize the surface charge of cells. Consequently, flocculation can occur [28]. On the contrary, during flocculation due to the negative nature of the microalgal cells, they are attracted towards the positively charged cathode where their surface charge is neutralized and can form flocks on its surface [20]. The fact that they bind to the surface of the electrode is not the desired one as with time it will lower the efficiency of the process. A successful large-scale experiment was conducted during which at least 80 % of microalgal cells were removed. This method also does not need any flocculant added and requires a lesser amount of energy. If stable electrodes would be used this type of separation could be used to separate non-toxic microalgae. However, the cost of such equipment may be high and the electrodes are prone to fouling [5].

In Tab. 4. the efficiency of several electrolytic methods is presented. Unfortunately, the economic side of the experiments could not be compared as neither time nor power were available.

Tab. 4. *Electrolytic flocculants [20]*

Type	***Material and specification***	***Efficiency (%)***
Electrolytic flocculation	Aluminum 3 V cm^{-1}	90
Electrolytic coagulation	Aluminum 3 mA cm^{-2}	≤81
	Iron 3 mA cm^{-2}	≤91
Magnetic separation	Iron oxide microparticles	95
	DEAE magnetic beds pH -4	90

DEAE - Diethylethanolamine

2.2 Centrifugation

Like sedimentation but faster, centrifugation substitutes gravitational acceleration with a centrifugal one. As a method, it can produce sludges with the highest concentration of microalgae from all the listed methods with a recovery efficiency of over 90 % [16]. Its main disadvantage is the fact, that it also requires the largest amount of energy to do so (apart from drying). For this reason, it is unsuitable for culture medium with a low concentration of microalgae which is commonly produced by a cultivation stage and is also expensive to add as a second stage method. Hence it is considered unsuitable for bulk production [26].

On the other hand, centrifugation is the only method that is currently used at a larger scale to produce microalgal concentrate certified for humans [4]. The appliance itself is easy to clean and maintain [20], the method is swift and reliable which are all desired properties.

According to Al Hattab [21], optimal particle size for disc centrifuge should be between 3 and 30 μm and the input solid content should vary from 2% to 25%. For decanter centrifuge the diameter should be greater than 15 μm and the solid content at least 15%.

Unfortunately, only a handful of studies were found that are aimed at Chlorella separation. All of those were of laboratory scale, conducted on swinging bucket centrifuge which is very unsuitable for large scale production.

Bělohlav and Jirout [17] have theoretically compared decanter (A) and disk centrifuges (B) which are both shown in Fig. 15. Disk centrifuge was selected as a better solution as it was smaller in size, more efficient and its performance was easier to control through rotation speed and number of disks.

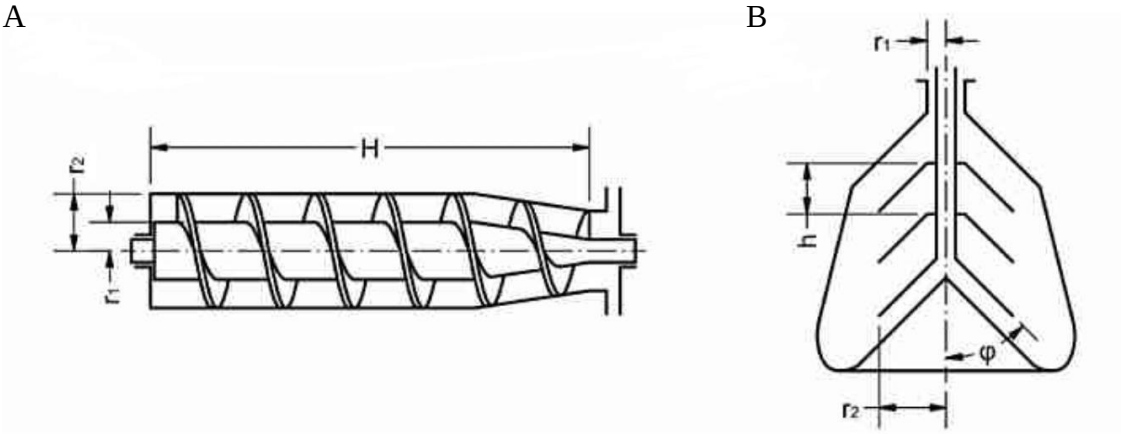

Fig. 15. *Decanter centrifuge (A) and disk centrifuge (B) [17]*

Other types of centrifuges could be used as could be a centrifuge equipped with a filter membrane. Unfortunately, no data were found regarding any of these types of centrifuges. Therefore an experiment should be performed to evaluate their potential.

2.3 Filtration

Filtration is a method which separates solids from liquids on the base of size exclusion where smaller particles are let through the membrane and large ones are retained on its surface [33]. Due to its energy demand, which is lower than that of centrifugation [9], reasonable process speeds, and the option of continual separation, filtration is one of the most promising and widely researched methods in microalgae separation. The fact that it is easy to scale up and has a low impact on output properties is also beneficial. Moreover, the use of flocculants is only optional therefore microalgae separated through this method could be used in the feed or food processing industry [20]. However, membrane pores must be of size smaller than the smallest microalgal cell or a flock.

Due to the small size of the cells, the membrane suffers from fouling which greatly reduces its flux capabilities. Hence finding a method that mitigates this phenomenon is one of the most important challenges in the current membrane development [34]. Another problem is the recovery itself. A commonly applied method, backwashing, is not suitable for this process as it would dilute the suspension again [26].

A couple of different configurations are often presented. Namely dead-end filtration and cross-flow/tangential flow filtration (CFF) [33]. The main difference is well portraited in Fig. 16. From these two CFF is considered more suitable as it is better at dealing with fouling which is the main problem when it comes to microalgal filtration. Dead-end filtration is more suitable for larger microalgae species as it is more prone to fouling [20].

It is possible to use several different driving forces to achieve filtration. Using gravitation is the cheapest option as it does not require any energy input but is often insufficient due

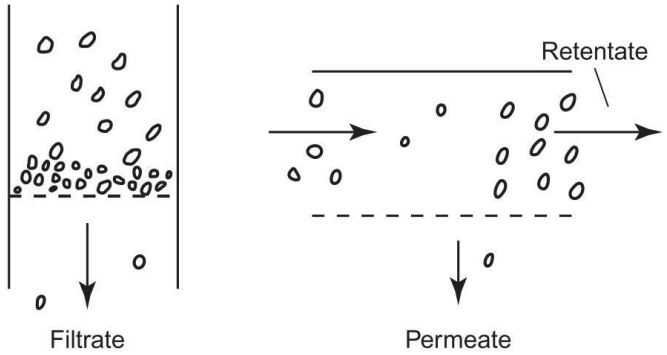

Fig. 16. *Dead-end filtration (left) and CFF (right) [35]*

to losses on the membrane. A pressure difference, made either by vacuum on the outside or by higher pressure on the inside of the membrane, can also be used. The latter being the more common one. The only drawback is the necessary use of a pump which could destroy the cells/flocks. Lastly, we could combine centrifuge with a membrane system, which is not ideal due to the power requirements of the centrifuge but it is certainly a possibility [5].

2.3.1 Cross-flow filtration (CFF)

Also called tangential flow filtration is characterized by feed flow which is tangent to a membrane surface. Particles that are smaller than the pore size of the membrane pass through due to the transmembrane pressure while the large particles pass along the membrane surface and are let out with a retentate. The retentate can be further recirculated or passed to the second stage of the separation. This method is therefore suitable for continual separation as the cells should not clog the surface of the membrane as is common during dead-end filtration [36]. The whole process is controlled by the pressure gradient and flow velocity. From the literature review conducted by Al Hattab [21], the energy consumption can vary between 0.38 up to 10 kWh per m^3 with TSS from 2% up to 25%. The output solid content highly depends on the type of the filter and can vary from 2% to 27%. Small pore size tends to perform worse in this regard [23].

Even though this method should be immune to fouling, several research papers have reported that it occurs. According to Rossi and al. [34], higher tangential fluid velocity increases membrane flux until the tensile stress disrupts the surface of microalgal cells. A subsequent release of chemicals creates a gel-like substance which dramatically decreases its flux potential.

Due to the fouling problems, there is also a considerable irreversible degradation of membrane over time which means that the filtration cartridge is going to need replacement. This could be slightly mitigated by cleaning or coating the membrane surface but as of now, there is not a permanent solution to this problem [5].

The benefits of this method are the facts that it does not affect the microalgal cells, their properties, or chemical composition and that it can produce microalgae suitable for human use. The need for backwashing is also reduced as most of the cells should ideally stay in the retentate. By this method, it is possible to recover cells of various sizes while permeate is also entirely reusable [37] which further decreases the operational costs. It is also efficient and reasonably expensive [5].

2.3.2 Filter types

Filter selection is one of the main criteria which influences the properties of the whole system. There are many types of filter systems with different properties e.g. pore size, thickness, density, permeability, material, construction, and others. The two main specifications are described more in detail. Moreover, advantages and drawbacks are specified.

a. Pore size and material

Membranes may be differentiated by the size of their pores into several categories, namely reverse osmosis (0.001 μm), ultra-filtration (0.02-0.2 μm), microfiltration (0.1-10 μm) and macrofiltration (≥10 μm). For microalgae separation, microfiltration seems adequate given their average size [20]. When flocculated, macrofiltration could be used. In Fig. 17. several membrane surfaces and their cross-sections are shown.

The size of pores influences pressure loss, flux, and fouling. Generally, with bigger pores the pressure loss is decreased, while flux and fouling grow [38]. When it comes to surface porosity Marbelia et al. [39] have shown that it does not significantly affect membrane permeance. More importantly, Marbelia et al. have also shown that more porous membranes are more prone to irreversible fouling. Pores with gradient size change were also found to be unsuitable as the cells tend to get stuck in the neck of the pore [40].

Most of the membranes are made from organic polymers such as polyvinylidene fluoride, polysulfone, polyethersulfone, polyacrylonitrile, and polyethylene. They are mostly very durable and are commonly on the cheaper side of the spectrum.

Inorganic materials e.g. ceramic, alumina silicon carbide, and others are expensive, but very stable and are often used in processes with aggressive chemicals. Hence they are not very suitable for microalgae separation [33].

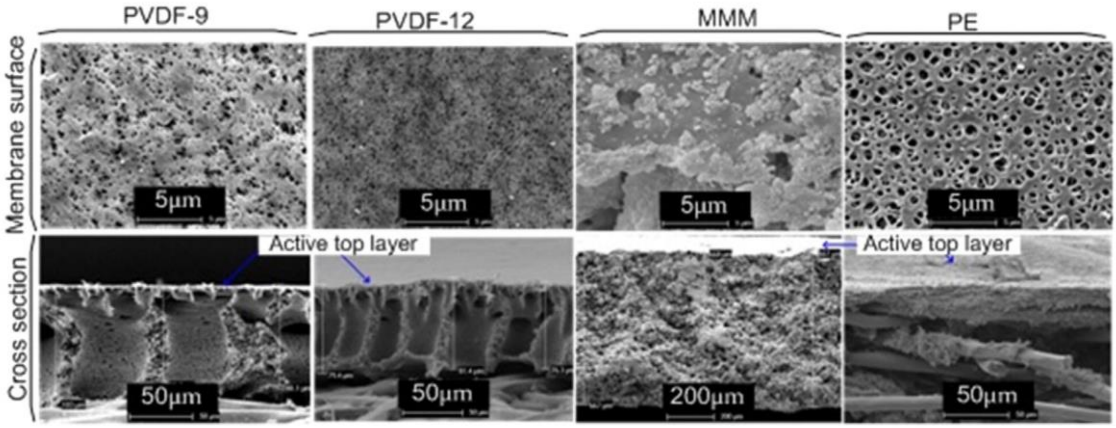

Fig. 17. *Membrane surface and cross-section under a microscope* [41]

There is also a possibility to coat the membrane with a thin layer of a different substance which is mostly another polymer. This modification can improve the property of the membrane, enhance its antifouling capabilities, and make cleaning and regeneration easier [38]. It has been shown that hydrophilic materials are more resistant towards fouling as most residues are hydrophobic [42].

b. Design

The main parameter of a filter is the area through which the filtrate can pass through. Consequently, manufacturers try to invent such construction that would increase this value. There are several styles of construction from which a sleeve, a cartridge, and a desk variant are commonly used. All of these have been used with varying degrees of efficiency for each of the methods.

Sleeves and desks are easier to manufacture and are usually cheaper, easier to clean and maintain. On the other hand, they have a higher space requirement for an equal amount of filtration area and tend to clog up faster as they are often a dead-end type of filter [35]. In short, cartridge filters are filters stuffed inside a tube. There are several possible configurations. A hollow fiber filling is prone to fouling due to cells getting stuck inside of the tiny fibers [37] but on the other hand, has the highest area per volume. The tubular design is similar to the hollow fiber but the holes for retentate are straight and much larger. And last but not least there is a spiral wound membrane which is practically a plane filter rolled inside of a tube [33]. All cartridge filters tend to work in CFF configuration.

Fig. 18. *Spiral wound membrane* [43]

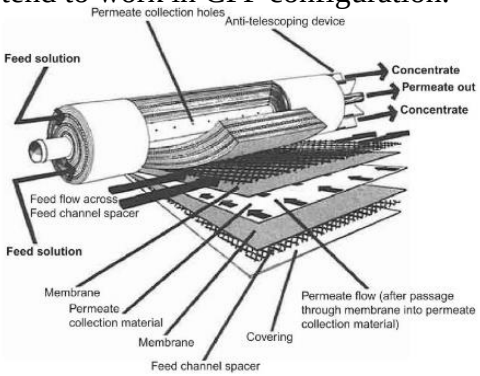

Fig. 19. *Membrane cross-section* [35]

Fig. 20. *Hollow fiber membrane* [44]

Fig. 21. *Tubular membrane* [45]

Of the designs mentioned above, the tubular design seems like the least prone to fouling due to its construction as there is a lesser amount of space for the microalgae to easily adhere to. The straight tubular holes through which feed goes (Fig. 21.) may be easier to clean compared to feed chamber spacer (Fig. 19.) or hollow fiber in general (Fig. 20.). There are several more styles of construction, some of which are already used in water purification plants. Machines such as rotary drum filters or belt filters have been used since 1988 [16] but are generally considered too big and expensive [20].

2.3.3 Fouling

It is the bottleneck of the whole process. There are several different types of fouling which all decrease the efficiency of the filtration. The main type of fouling is the deposition of material, cells, or EPS [46] along the membrane surface resulting in a reduction of flux and in an increase in the amount of demanded energy (Fig. 22.). With higher microalgal content the fouling gets worse [47]. This may be a limiting factor when higher output concentration is desired because of the diminishing efficiency [33]. This type of fouling is reversible and can be cleaned from the surface [48]. The second irreversible type of fouling happens when the cells get stuck inside of pores. It is difficult, even with chemical compounds, to fully restore such a deposition and due to this, over time membranes tend to slowly deteriorate [34].

It is suggested that the main mechanism of fouling is the creation of EPS by the microalgal cells. This substance, mostly composed of proteins, lipids, polysaccharides and cell parts [46] creates a gel-like structure on the surface of the membrane which is due to high pressure pressed into the membrane and clogs it [34].

Fig. 22. *Membrane surface clogged by flocculated algal cells*

Several antifouling methods, which are partly capable of mitigating this problem, have been presented. The membrane may be protected by a surface coating which can prevent the adhesion of particles to its surface, or by removing the cake by other means such as air bubbles or mechanical system. The use of flocculant has been tried with varying efficiency where in one study it is a beneficial modification while in another no significant difference has been observed [40, 39].

There is also the possibility of backwash, and cleaning cycles but that would result in a pause during production. Such a procedure is inevitable but it should not be the main antifouling tool because of how uneconomical such a process would be [38].

2.3.4 Parameters

Filtration is mainly controlled by two parameters, i.e. flow velocity and transmembrane pressure. In theory, with higher velocity and pressure, the flux (volume of liquid which passes through the membrane per its area [36]) should also increase. However, there is a point when while those parameters are increased the flux starts to diminish [38]. This point is called a critical flux and it signals that irreversible fouling is occurring on the membrane surface [34]. That is due to high shear forces induced upon microalgal cells which release more EPS content into the water [34] resulting in more and worse fouling. Therefore it is prudent to work in slightly milder conditions to diminish the effect of this phenomenon [9].

The efficiency of a membrane E_x is defined for a given particle size of x by Eq. (7). Where N_u is the number of particles upstream and N_d downstream of the membrane [35].

$$E_x = \frac{N_u - N_d}{N_u} \tag{7}$$

Another commonly displayed parameter is the beta ratio. This ratio compares the number of particles upstream to the number of particles downstream [35].

$$\beta = \frac{N_u}{N_d} \tag{8}$$

2.4 Method comparison

To simplify the final comparison a table of results from the literature was compiled (Tab. 5.). Four main parameters are compared, namely speed of the process where a volume of the culture medium separated per second is stated, process costs where energy requirements per liter and price of chemicals per liter of culture medium are taken into account, an output concentration which is often described by TSS (total suspended solids), and process efficiency which describes the percent of cells gathered from the culture medium compared to the total amount of cells in the medium.

However, not all sources contained all the necessary information or included only a part of it. For gravitational methods, power demand for the appliance was rarely mentioned therefore only the price of flocculants was taken into the account. The price of chemicals is based on the retail price of a chemical supplier and thus could be lower when bought in bulk. Where power consumption has been mentioned a price per liter of microalgae was calculated. An average cost for European Union (0.1 € per kWh) was used. If the price was lower than 10^{-4} € L^{-1}, 0 is written instead. Lastly, all the experiments were of laboratory-scale ranging from hundreds of mL to 2-3 L hence their efficiency and power consumption may change when applied on a larger scale.

From the gravitational methods, DIF with chitosan as flocculant seems like the best option as it has high efficiency of over 90 % and a low necessary dose of flocculant. The power demand of the electrolytic method was surprisingly low. This might be because the scale of the experiment was rather small and might be higher for large device.
Unexpectedly, the power demand of centrifugation was also low even though an unsuitable device was used. Compared with the costs of flocculants the price of 0.04 € L^{-1} seems insignificant when the fact that centrifugation separates microalgae of higher quality is also considered.
All membrane units performed with high efficiency over 90 % and when flocculant was used the final flux was higher. Other results are hard to deduce as the data vary significantly since the experiments focused on different aspects of filtration and were performed under different conditions. A common aspect of all the experiments is the significant drop in flux due to the fouling but again it is difficult to evaluate this data as the timescale is often missing.

Tab. 5. *Literature results comparison*

Method	***Throughput (mL s^{-1})***	***Costs (€ L^{-1})***	***Conc. (g L^{-1})***	***Eff.***	***Conditions***	***Ref.***
Gravitational						
Sedimentation	0.25 mm s^{-1}	0.375 0.075 0.125	1.2 0.5 1.2	91.9 92.3 92.4	Chitosan 250 mg L^{-1} Chitosan 50 mg L^{-1} $Al_2(SO_4)_3$ 2.5 g L^{-1}	[49]
DIF	-	0.0075 0.02	-	≥90	Chitosan 5 mg L^{-1} Saponin 20 mg L^{-1}	[25]
DAF	-	0.0025 -	-	75 85	$Al_2(SO_4)_3$ 50 mg L^{-1} PAC50 50 mg L^{-1}	[50]
DAF	0.3 2	0.005 0.015	-	80 90	CTAB 20 mg L^{-1} Chitosan 10 mg L^{-1}	[50]
Electrolytic flocculation	2.1 1.4 1	0 0 0	- - -	98 98 98	22.2 A m^{-2} 44.4 A m^{-2} 66.6 A m^{-2}	[28]
Magnetic microparticles	-	0.11 0.22 0.67 1.34	-	65 80 85 90	IONPs 25 mg L^{-1} 50 mg L^{-1} 150 mg L^{-1} 300 mg L^{-1}	[51]
Centrifugation						
Swinging bucket	0.1	-		98	10 000 RPM 30 ml scale	[52]
Swinging bucket	0.625	0.0404	750	-	8000 RPM	[53]
Swinging bucket	0.12	-	-	35 68 75 96	1000 RPM 3000 RPM 5000 RPM 7000 RPM	[51]

Continuation of the Tab. 5. on the next page.

Filtration	***Flux*** ***($L\ m^{-2}\ min^{-1}$)***	***Costs*** ***(€ L^{-1})***	***Conc.*** ***(g L^{-1})***	***Eff.***	***Conditions***	***Ref.***
0.22 µm pore Plane MCE	0.17 1.13 mL s^{-1}	-	-	99.9 99.5	Fresh Chitosan 10 mg l^{-1}	[32]
8 µm Plane Filter paper	20 3.57 mL s^{-1}	-	-	6.5 93.5	Fresh Chitosan 10 mg l^{-1}	[32]
0.008 µm *0.013 µm* *0.036 µm*	0.47 0.5	0.0001	3.5 - -	99 99 100	Submerged aerated PVDF ultrafilter	[9]
10000 Dalton	8.3 mL s^{-1}	0.0002	1.5	-	Tangential, PES UF	[53]
0.91 µm PFTE Plane + Chitosan	5→0.3[1] 5→0.3[1] 5→0.4[1] 5→0.5[1]	-	-	-	0 mg L^{-1} 50 mg L^{-1} 100 mg L^{-1}, best reversibility 200 mg L^{-1}	[54]
0.91 µm PFTE Plane + Chitosan, presettled	3→0.1[1] 10→1[1] 15→1.5[1] 20→4[1]	-	-	-	0 mg L^{-1} 50 mg L^{-1} 100 mg L^{-1} 200 mg L^{-1}	[54]
0.036 µm PVDF-9, *0.013 µm PVDF-12*	0.7→0.5[1] 0.6	0	1.43	90 99	Vibrated membranes 45 Hz 6.4 W tank volume1.6 L	[47]
0.91 µm PTFE + PACl	11→0.5[1] 11→0.5[1] 11→1[1] 14→3[1] 14→3[1] 15→4.2[1]	-	18%[2] 34%[2] 25%[2] 24%[2] 21%[2] 22%[2]	-	0 ppm 10 ppm 50 ppm 200 ppm 300 ppm 500 ppm	[55]
PAN8 90 % *PAN9 81 %* *PAN10 73 %* *PAN11 36 %* *PAN12 28 %* *PAN13 32 %* *Porosity %*	0.5/0.45 0.35/0.27 0.23/0.33 0.23/0.32 0.28/0.32 0.2/0.33 Normal membrane /NaOH coating	-	-	-	Pore size around 20 nm, porosity and electronegativity test, Submerged filtration	[39]
0.19 µm Nylon 6,6	0.48	0	1.5	-		[42]

IONPs – iron oxide nanoparticles, MCE – mixed cellulose esters, PES – polyether-sulfone, PTFE – Polytetrafluoroethylene, PVDF – Polyvinylidene fluoride, PAN – Polyacrylonitrile, UF – ultrafilter, [1] – value at the beginning and the end, [2] – mass fraction

For clarity purposes, a comparison chart (Tab. 6.) is presented with a few pros and cons for each of the previously mentioned methods. Some of the mentioned cons may be solvable. For example, flotation would greatly benefit from a cheap, human approved flocculant which would solve all its main problems. In the case of filtration, the invention of an antifouling system that would be easy to operate and would not hinder the production process would be beneficial.

Tab. 6. *Pros and cons of separation methods*

	PROS	**CONS**
Sedimentation	• Low operation costs • Simple maintenance • Small energy demand • High productivity [1]	• Low productivity • Large space requirement • Low microalgae quality • High operation costs[1] • Low output concentration
Flotation	• High productivity • Consistent • Small flocculant dose • Small energy demand • Used in water treatment	• The necessity of a flocculant • Low microalgae quality • Often hard to scale-up
Centrifugation	• High productivity • Used in industry • Uncontaminated by chemicals • Consistent and highly efficient • Recyclable culture medium • Easy to scale up	• High energy demand • Unsuitable for thin suspensions • High purchase price • High maintenance costs
Filtration	• High productivity • Highly efficient • Uncontaminated by chemicals • Recyclable culture medium • Easy to scale up	• Medium energy demand • Unsuitable for thick suspensions • Expensive membranes • High maintenance[2] • Low microalgae quality[1]

True when: [1] with flocculant, [2] till fouling solved

2.4.1 Patent review

Around forty-five different patents regarding microalgal harvesting were found by two different patent searche engines. Even though the same keyword, namely "algae harvesting" was used each of the search engines provided slightly different results. Their comparison can be seen in Fig. 23. The difference might be caused by an error in counting because sometimes several versions of the same patent were displayed but the difference in the number of filter patents indicates that it might not be the only cause.

The patents were divided into eight categories based on the used method. Due to the similarities between designs in each category an example will be presented for some of the methods.

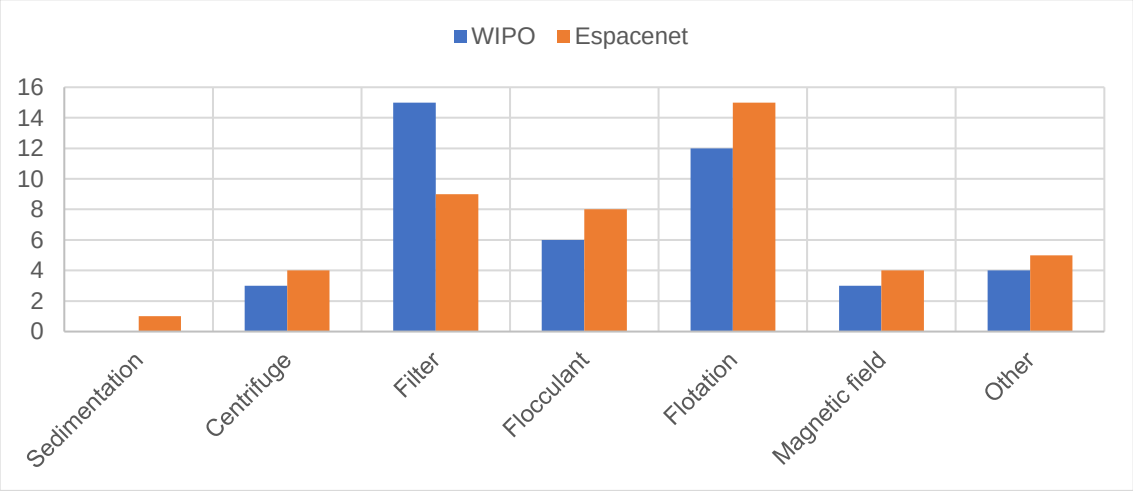

Fig. 23. *Distribution of used patents*

a) Centrifuge

Almost all the presented patents were based on the disk centrifuge design (Fig. 24.). The benefits described by the patent are: "…high in separation speed, capable of recycling separated culture solution, good in practicability and convenient to operate. [56]"

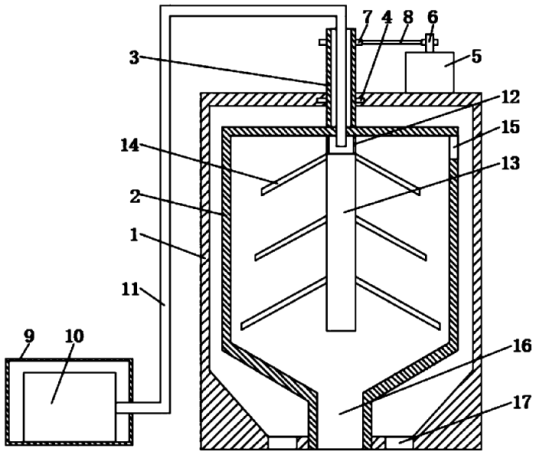

Fig. 24. *Disk centrifuge scheme [56]*

b) Filter

The two most commonly found methods that use filtration as the main mechanism of particle separation were either a vacuum belt filter (Fig. 25.) or a cartridge type of a filter. The benefits of using a filter type harvesting method are low cost, low energy input, re-usable filtrated medium, and functionality without flocculant [58].

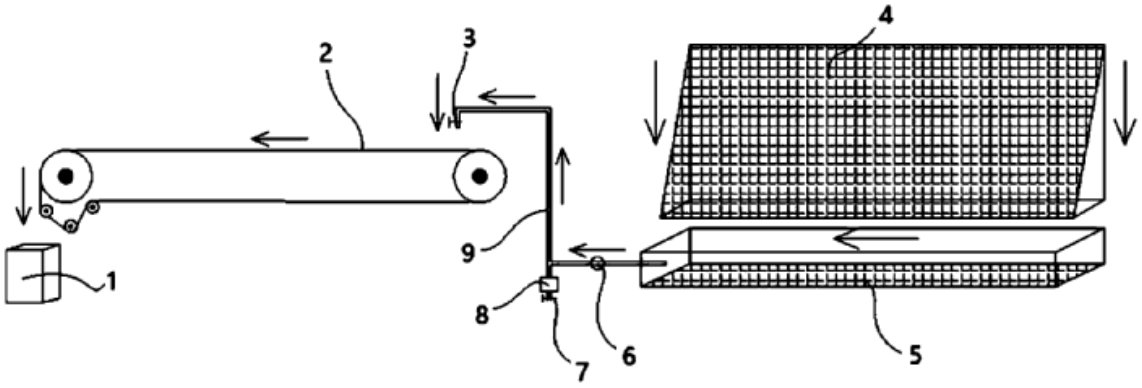

Fig. 25. *Vacuum belt filter scheme [57]*

c) Flocculant

Several patents did not specify what should be the subsequent process after the flocculation of microalgal cells. Included in this category are also those patents which mention that several processing methods are viable after the use of flocculant. The used flocculant was often Chitosan or another organic polymer.

d) Flotation

As was mentioned in 2.1.2 there are several mechanisms through which flotation may be achieved. From those mentioned DAF was the most common in the patents. Apart from working parameters or used flocculant, there was no significant difference in the design part between the devices hence Fig. 8. well represents most of the patented devices.

e) Magnetic field

Several patents were based on the addition of magnetic nanoparticles which would bind to the surface of microalgal cells. When an external magnetic field was applied to the culture, the cells should be attracted to one of the poles. However, apart from lab-scale devices, no functional industrial scale device was found.

f) Other

Methods in this category did not fit in any of the previously mentioned categories. These methods often require an immobilized microalgal species which is bound to a fabric. An example of such a device would be a machine that utilizes brushes, vacuum, or a press which then separates the cells from the fabric outside of a pond [59].

2.4.2 Methods used in the industry

To verify the gathered information an effort was made to find out what are the harvesting methods that are commonly used by the major microalgal producers. A list of ten producers was assembled which had their production volume or cultivation surface area presented on their websites (Tab. 7.). However, only one of them has provided the information regarding the harvesting method on their website and three others have a patent connected with their name. An assumption can be made that most of the other producers will use one of the technologies mentioned in Tab. 7 as their production is mostly aimed at the food industry. Due to this fact, methods that use flocculant are due to their toxicity out of the question hence only centrifugation or filtration remains. From these two, centrifugation seems like the more reasonable method as it is easier to operate, swift, and more efficient [72] but arguments in favor of filtration can be also found. To fully evaluate which of these methods is better more data from the industry is necessary. Unfortunately, no company was willing to share their knowhow.

Tab. 7. *List of major microalgae producers*

Producers	***Prod. (tons)***	***Surf. (ha)***	***Technology***	***Species***
Yunnan Green A Biological Project Co., Ltd [60]	3000	-	Screen vacuum belt filter, inclined harvesting, centrifugal or a cyclone separator [2] [61]	spirulina, chlorella, and Haematococcus Pluvialis
Inner Mongolia Rejuve Biotech Co. Ltd. [62]	1200	63	Vacuum belt [2] [57]	spirulina
Earthrise Nutritionals LLC [63]	820[1]	43,2	Filters	spirulina
Fuqing King Dnarmsa Spirulina Co. Ltd. [64]	1600 400	- -	Centrifuge [2] [56]	spirulina chlorella
Jiangshan Comp Spirulina Co [65]	800 50	-	- -	spirulina chlorella
Dongtai City Spirulina Bio-engineering Co. Ltd. [66]	1100[1]	60	-	spirulina, chlorella
Cyanotech Corporation [67]	650[1]	36	-	spirulina, Hawaiian Astaxanthin
E.I.D. – Parry (India) Limited [68]	990[1]	52	-	spirulina, chlorella
Febico [69]	1000	-	-	Chlorella, Spirulina
Algomed [70]	45	-	-	
Taiwan Chlorella Manufacturing Company [71]	400	-	-	chlorella

[1] *Expected values,* [2] *patented technology*

3 Experimental basics in harvesting and separation of microalgae *Chlorella* from the culture medium

As part of this thesis, several experiments were conducted. These experiments were aimed at testing whether it is possible to separate and concentrate microalgae produced in the laboratory photobioreactors (PBR) using the appliance available in the laboratory. The methods at disposal were sedimentation with and without flocculant, filtration, and centrifugation. While all mentioned methods were tried, the path that uses flocculant to sediment the cells that are subsequently filtered out seems to be most promising as other methods produced no significant results. This does not mean that centrifugation is not a suitable method only that available appliance was not designed for bulk microalgal cell separation.

3.1 Characteristics of *Chlorella*

3.1.1 The concentration of *Chlorella* in the culture medium

To obtain a reference values a concentration of the culture medium and size of the cells was measured. The microalgae had been cultivated in PBR for two weeks at a temperature of around 35 °C. The batch was harvested on 6.2.2020 and the experiment was carried out the same day. 4 times 50 ml samples were gathered from the PBR. Each sample was weighed with a laboratory scale. Swinging bucket centrifuge set to 3500 RPM for 5 minutes was used to separate water from the samples. After each centrifugation demi water was added to rid the sample of dissolved salts and other inorganic materials. The centrifugation cycle was performed 4 times. Subsequently, the samples were dried at 105 °C till their weight was constant. Finally, the samples were weighted with a laboratory scale. The results of this experiment can be seen in Tab. 8. Our average mass concentration was 0.30±0.07 g L^{-1} and the average mass fraction was 0.31±0.07 g kg^{-1}.

Tab. 8. *Algae concentration in culture medium*

Sample	***Volume (mL)***	***Weight (g)***	***Dried (g)***	***φ_i (g L^{-1})***	***ω_i (g kg^{-1})***
1	50	48.7422	0.0114	0.228	0.234
2	50	48.8374	0.0129	0.258	0.264
3	50	48.7604	0.0182	0.364	0.373
4	50	48.8856	0.0171	0.342	0.349
Average				**0.30**	**0.31**

The value of mass concentration is slightly lower than an average of 0.4 g L^{-1} which is common in the literature.

The mass concentration φ_i and mass fraction ω_i were calculated according to Eq. (9-10) where m_{si} is the weight of the dried biomass, V_i the volume of the given sample, and m_i its weight at the beginning of the experiment.

$$\varphi_i = \frac{m_{si}}{V_i} \tag{9}$$

$$\omega_i = \frac{m_{si}}{m_i} \tag{10}$$

3.1.2 Size of chlorella cells

To separate *Chlorella* cells from the suspension using a filter plate it is necessary to determine the size of the cells so the proper filter can be selected. For this purpose, a microscope with an added digital camera and plate with a 10 µm scale was used. As a test sample a slightly diluted culture medium which was cultivated 2 weeks was used. Fig. 26. is a photo taken during the experiment.

As can be seen in this figure the diameter of the cells can vary dramatically. The biggest cells are close to 9 µm wide while the smallest of them are around 2 µm. Moreover, the number of small cells is fare larger than that of the big ones, therefore a microfilter with a smaller pore size around 1-2 µm may be the most suitable for this culture medium but further experiments are necessary to validate this notion.

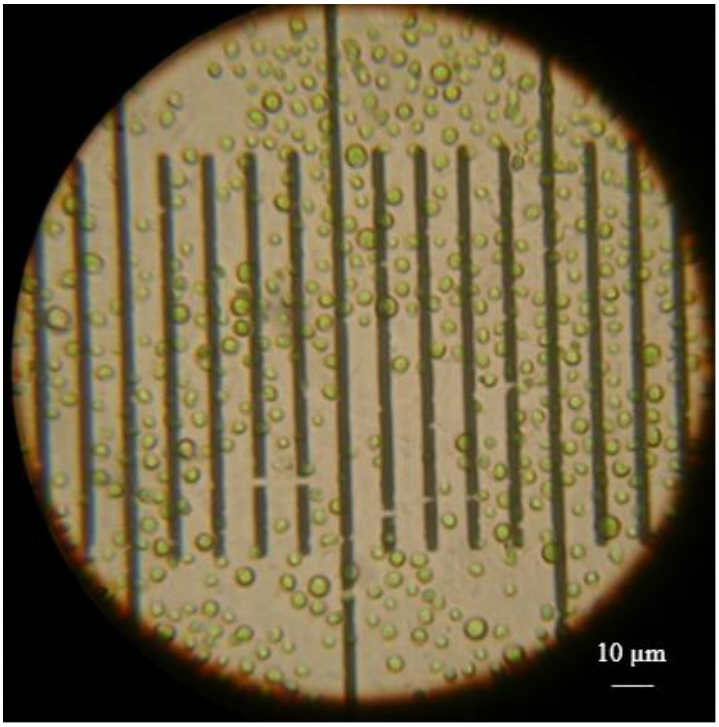

Fig. 26. *Chlorella size measurement. 1 unit =10 µm*

3.2 Flocculation of Chlorella

3.2.1 Flocculant selection

As part of other experiments, flocculated microalgae will be required. Therefore, a test of available flocculants was conducted to determine which of them is the most suitable.

Three organic synthetic flocculants, namely Sokoflok V16, V54, and V56 from company Sokoflok s.r.o., were available. Of those V16 being anionic and V54 and V56 cationic. A 500 ml solution with a mass concentration of 2 g L^{-1} was prepared from every flocculant. To fully dissolve the flocculant the solution was placed on a magnetic stirrer and stirred for 2 hours. The volumes of microalgal suspensions on which the flocculants were tested were 50 and 200 mL. To each stirred microalgal suspension, a small amount (0.1 mL) of different flocculant was incrementally added. After the addition, the suspension was stirred for a minute and then let to settle. The suspensions were then visually compared, the size of flocks and turbidity of the liquid were the main parameters. There was not much of a difference between the effect of V54 and V56, both performing very well. On the contrary, when V16 was added no flocculation occurred even after the addition of a larger amount of flocculant. This may be due to the anionic nature of the flocculant which was shown not to be suitable for microalgae flocculation. V56 was selected for additional testing as it should have higher ζ potential than V54.

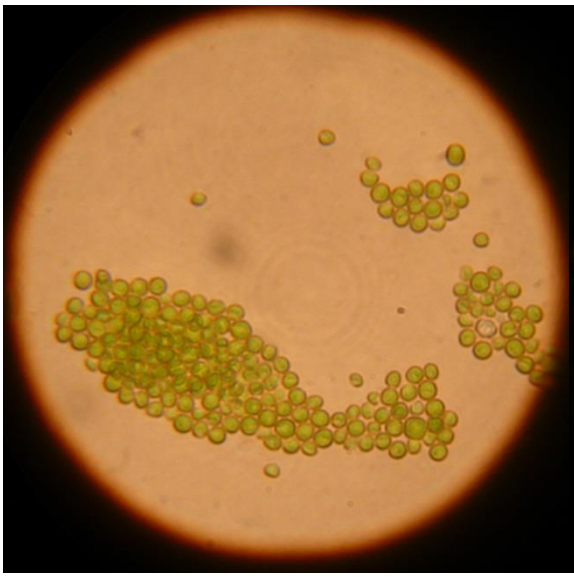

Fig. 27. *Small flocks under a microscope*

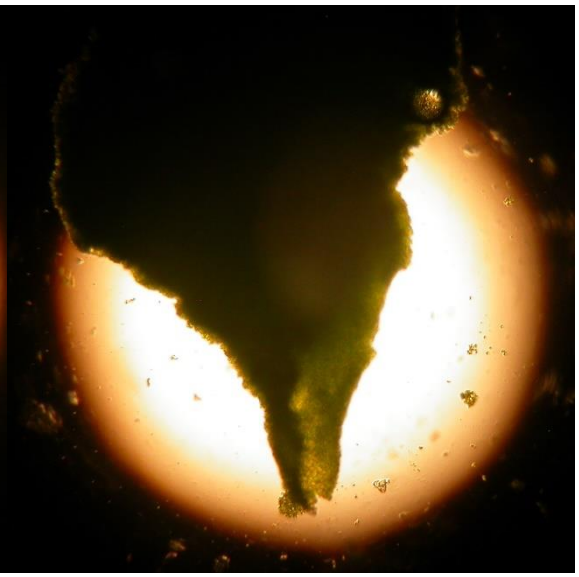

Fig. 28. *Clear state under the microscope*

3.2.2 Flocculant optimization

To determine a suitable amount of flocculant for a given volume of suspension and the desired flock size a second experiment was performed. A 500 ml solution of V56 with a mass concentration of 0.2 g L^{-1} was prepared. This solution was then stirred for 2 hours on magnetic stirrer to ensure that the flocculant is fully dissolved. The experiment consisted of 2 beakers each with the same volume of microalgal suspension. The volume varied from 100 mL to 500 mL with 50 mL increments. To verify the data 1 L and 3.5 L experiments were also conducted. A small volume (1 mL) of flocculant was incrementally added to the given volume of microalgae. The suspension was then stirred for a minute and then let to settle. For a comparison purpose, there was always a difference of 0.5 mL of flocculant between the beakers. The whole setup can be seen in Fig. 29. At the beginning of the experiment, three states of the suspension were selected as a reference. Small flocks, big flocks and clear (see Fig. 30.-Fig. 32.). The state was achieved when there was not a significant visual difference between the beakers and the state of the suspension was similar to the reference picture. The data from the experiment can be seen in supplement 1. For better results turbidity meter should be used to exclude the human factor but unfortunately, such a device was not yet at disposal.

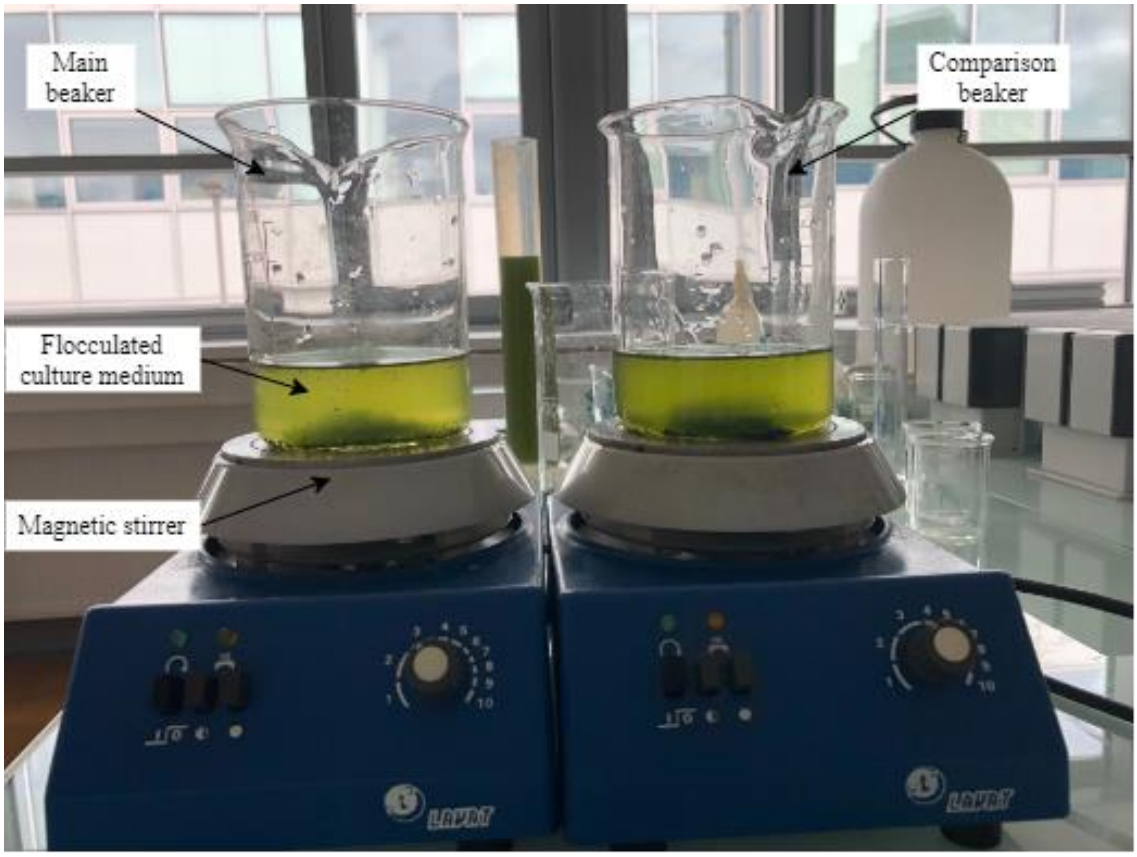

Fig. 29. Setup of the flocculant optimization experiment

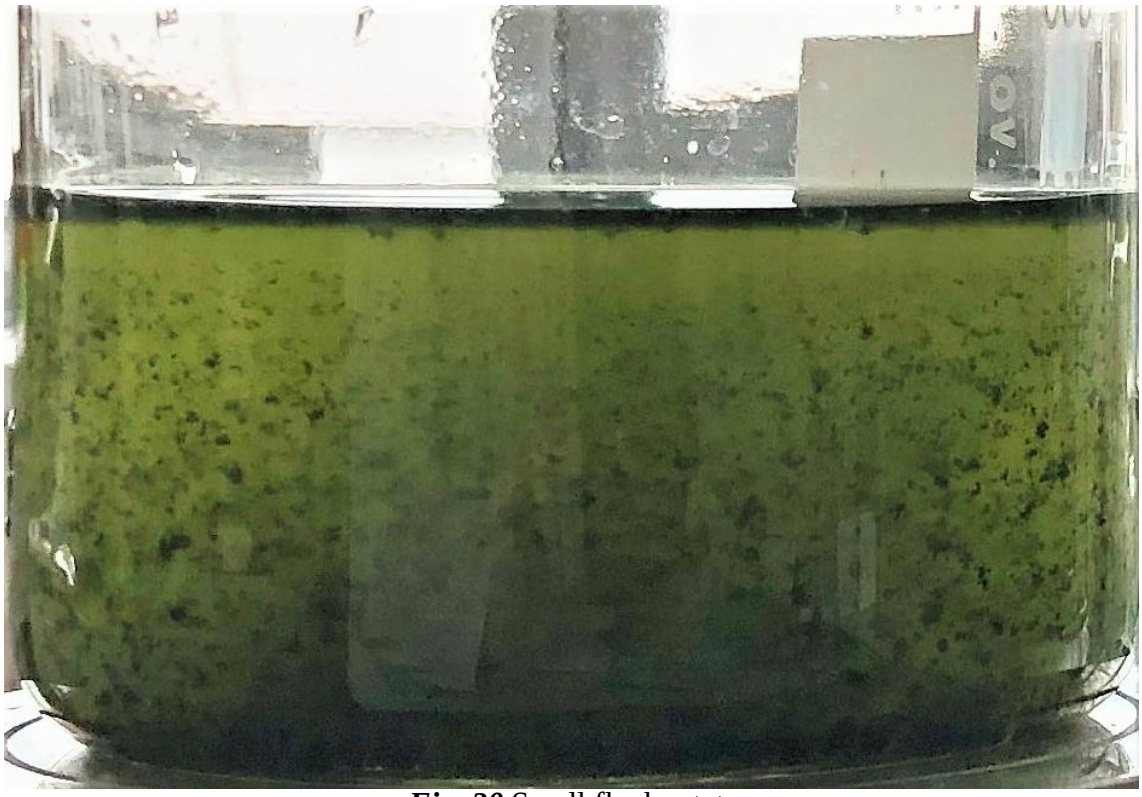

Fig. 30.Small flocks state

Fig. 31. *Big flocks state*

Fig. 32. *Clear state*

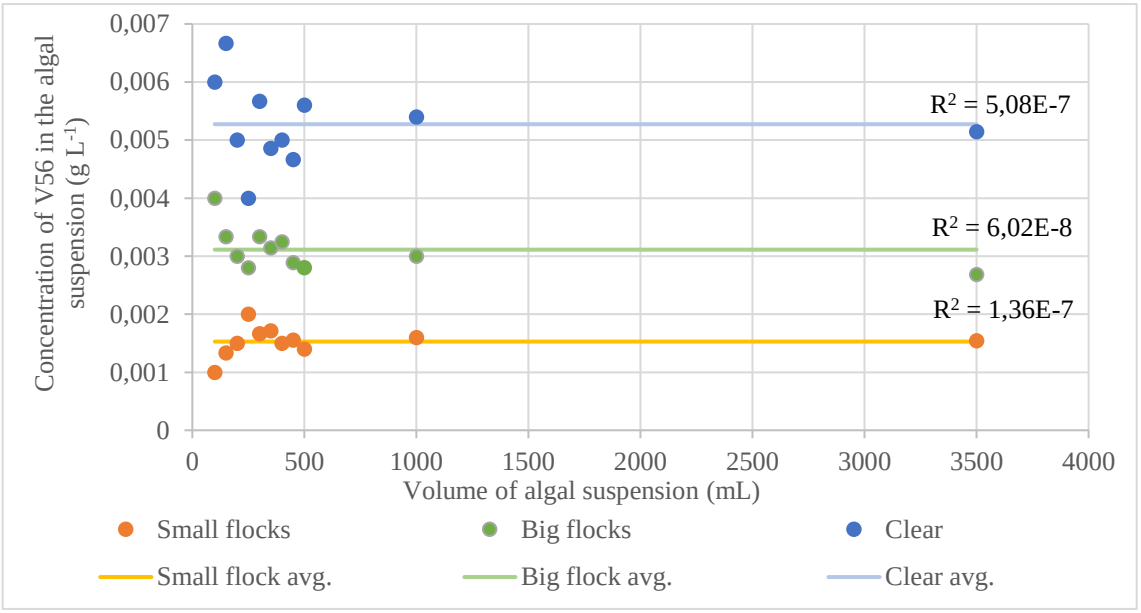

Fig. 33. *Amount of V56 per volume of algal suspension*

Small flocks state is a state where throughout the whole volume of the suspension, small visible flocks are formed. This state could be used during filtration with macrofilters, which cannot catch the small microalgal cells. The big flocks state is similar to the former one but the size of the flocks is around 3-5 mm. Finally, a clear state is when most of the cells are gathered in flocks at the bottom of the vessel.

Every state has its range of values and an average. For small flocks, the concentration of flocculant should be between 1-2 mg L^{-1} and it has an average value of 1.6 mg L^{-1}. The big flocks were formed between 2.5-3.5 mg L^{-1} with an average of 3.3 mg L^{-1}. And lastly, the clear state was managed when the concentration of flocculant in the medium was 4.5 mg L^{-1} and higher. The average value of the clear state was 5.2 mg L^{-1} but the concentration of 6 mg L^{-1} is more suitable for a large volume of suspension. The fluctuation in values was caused by the unprecise measurement method that was heavily influenced by the human factor.

During this experiment, 86 ml of concentrated algal suspension was created containing 2.98 g of dried algal biomass. This results in φ_i = 34,81 g L^{-1}, meaning that we have 117 times more concentrated suspension than that at the beginning. However, the efficiency of the method is rather low as some of the algal flocks were lost when the water was poured out.

3.3 Filtration

As part of this thesis, most of the filters that were available at the faculty were tested including a basket centrifuge filter (Tab. 9.). While all of the filters had pores larger than the average cell the hypothesis was that after a while a sufficient number of cells would bind to the membrane surface. These cells would then aid in the formation of a cake layer on which a larger number of cells would be retained. Even though not ideal it would be the first step towards the production of flocculant free microalgal concentrate. Unfortunately, none of the filters was capable to sufficiently retain unflocculated microalgal cells for the cake layer to form. Hence flocculation prior to filtration was necessary.

A prove of concept test with flocculated suspension was also conducted. 5 L of clearly flocculated microalgal suspension was poured into a transparent tube with a filter at its end. Due to the experiment configuration, low filter area, and small transmembrane pressure a thick 1 mm high microalgal cake (Fig. 22.) formed nearly instantly on the surface of the filter clogging up the whole system.

Even though the result was not ideal the experiment proved two key points to us. Firstly, we have a filter that is capable of retaining the cells, and secondly that the systems using dead-end filtration can clog really fast even with a small amount of algal suspension. Further experiments with different flock sizes and transmembrane pressures will be necessary to fully evaluate this method.

Tab. 9. *List of used filters*

Filter type Manufacturer	***Maximal temperature (°C)***	***Permeability 200 Pa air (L dm^{-2} min^{-1})***	***Minimal particle size (µm)***	***Thickness (mm)***	***Area density (g m^{-2})***
JF4230 JUNKER FILTER	90	200	$d_{10}/d_{50}/d_{90}$ 7.5/34.2/69.8	0,4	210
PP325M10 ČESKÉ FILTRY	95	390	10	2,2	325
FINET KAF5 MITOP	90	60	20	2,1	500
VACTEX 20P CZECH FABRICS	90	80	20	1,1	665
ECE144294 ECE GROUP	80	120	20	1,0	330
JF4300 JUNKERFILTER	90	15	$d_{10}/d_{50}/d_{90}$ 7.0/31.0/54.0	0,5	320
SEFAR 340W SEFAR	80	12	20	0,7	340
JF4140 JUNKERFILTER	90	11	$d_{10}/d_{50}/d_{90}$ 2.5/10.7/51	1,0	580

Fig. 34. *Filter test*

4 Conclusion

The literature review was conducted and three main mechanisms of separation were found. The first came gravitational separation under which sedimentation and flotation fall. Sedimentation is the simplest of the mentioned methods but was found to be unsuitable for microalgal separation as it is slow and generally inferior to flotation, which is more reliable and has higher output concentration. Apart from electrolytic floatation, all gravitational methods require a flocculant for swift and reliable performance. The used flocculant determines the cost of the process, quality of microalgae, and may affect subsequent processes. Even though many flocculants were reviewed none was found to be suitable for human use. Moreover, the cost of many flocculants is substantial. Due to this fact, gravitational methods may be more expensive than the other.

The second method is centrifugation. Only a few research papers review this method as it is often considered to be too expensive both in terms of acquisition and maintenance costs. This conclusion seems strange as no other method was found to reliably produce uncontaminated microalgae. It would be advisable to further test the efficiency and reliability of this method to verify these statements. Design and testing of a suitable centrifuge could be part of a magister thesis as such a device is currently not available at the laboratory.

Lastly filtration. The method is reported to be promising as it is expected to have the benefits of centrifugation without its disadvantages. As it stands, this method suffers from low throughput, high maintenance issues caused by substantial fouling of the filter. Contrary to centrifugation filtration is supposed to be less effective with higher concentrations. Thus, a combination of these two processes may be a viable way to produce an uncontaminated high concentration microalgal suspension with lower running costs.

During the experiments, it was found that it was impossible to retain unflocculated microalgae as the diameter of an average cell (5 μm) is too small for macro filters. As filters with a smaller pore size were unavailable a higher particle size was necessary. Consequently, a suitable quantity of flocculant V56 for the desired flock size was determined to 3.3 mg L^{-1}. A macro filter was then able to retain the flocculated cells but a thick cake layer prevented water from passing through. Such a drawback might be overcome by applying a higher pressure difference but such an experiment was not carried out. It was also found that flocculated microalgal cells tend to adhere more to the surface of the appliance and are also difficult to remove.

In the future, a more sophisticated filtration unit will be necessary to fully test the potential of this technology. Such a unit could produce a suspension with higher microalgal concentration that would be more suitable for disintegration processes. For this purpose, a vacuum filtration may be used as it should not interfere with flocculated cells and therefore cannot destroy the flocks. An improvement in measurement capabilities will be also necessary to better understand the properties of the filtrated cells. The average, maximum, and minimum particle size of flocculated cells are the parameters that were not currently known, and thus its effect could not be determined.

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