CZECH TECHNICAL UNIVERSITY IN PRAGUE

FACULTY OF MECHANICAL ENGINEERING
DEPARTMENT OF PROCESS ENGINEERING

SEPARATION OF MICROALGAE FROM CULTURE MEDIUM USING FLOCCULANT

BACHELOR'S THESIS

2023 LUCIE SCHOLLEOVÁ

BACHELOR‘S THESIS ASSIGNMENT

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Separace mikrořas z kultivačního media pomocí flokulantů

Guidelines:

Harvesting and dewatering have a major impact on operating and investment costs of technology for microalgae production. The aim of the bachelor thesis is to experimentally verify the functionality of the selected flocculants on the separation of microalgae cells from the culture medium. Based on the experimental measurements, to determine the sedimentation velocity of the separated particles and to evaluate the efficiency of the use of flocculants for separation on a semi-operational or industrial scale. Based on the experimental data, define the basic design and operational parameters of the selected technology.

Bibliography / sources:

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III. Assignment receipt

The student acknowledges that the bachelor’s thesis is an individual work. The student must produce her thesis without the assistance of others, with the exception of provided consultations. Within the bachelor’s thesis, the author must state the names of consultants and include a list of references.

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Statement of originality

I hereby declare that this bachelor's thesis was created independently by myself, with guidance from Ing. Mgr. Vojtěch Bělohlav, Ph.D. as the thesis supervisor. All sources of information used have been properly acknowledged and referenced.

In Prague

Lucie Scholleová

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Annotation sheet

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Annotation - Czech: Účelem této práce bylo experimentálně zhodnotit funkčnost použití flokulantů pro separaci mikrořas z kultivačního media. V rámci práce byla spracovaná rešerše na technologie používané ke kultivaci a separaci mikrořas. V návaznosti byl uskutečněn návrh vhodné technologie pro separaci na základě naměřených výsledků.

Annotation - English: The objective of this thesis was to experimentaly evaluate the functionality of flocculant use for the separation of microalgae. A research of technology used for cultivation and separation of microalga was carried out. As a result, a design of suitable technology was done based on the measurement results.

Keywords: Circular economy, Biorefinery, Microalgae cultivation, Microalgal biomass harvesting and dewatering, Bioproducts, Biofuels, Sustainability

Utilization:

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Abbreviations

CO_2 - Carbon dioxide

CCS - Carbon capture and storage

CCU - Carbon capture and utilization

PBR - Photobioreactor

TAG - Triacylglycerol

LED - Light-emitting diode

RE - Recovery efficiency

CF - Concentration factor

DAF - Dissolved air flotation

DIF - Dispersed air flotation

IAF - Induced air flotation

SAF - Suspended air flotation

Physical quantities

u_s settling velocity $[m\ s^{-1}]$

g gravitational acceleration $[m\ s^{-2}]$

r particle radius $[m]$

η viscosity $[m\ s^{-1}]$

ρ_p density of particles $[kg\ m^{-3}]$

ρ_l density of liquid $[kg\ m^{-3}]$

D_p particle diameter $[m]$

C_D drag coefficient $[-]$

μ dynamic viscosity of culture medium $[Pa\ s]$

S cross-sectional area $[m^2]$

R_2 outer radius of the settler $[m]$

V_{su} flow rate $[m^3\ s^{-1}]$

c_{su} concentration $[kg\ m^{-3}]$

R_1 radius $[m]$

c_t concentration $[kg\ m^{-3}]$

i lamellae $[-]$

L length of the lamella $[m]$

α angle of the lamella inclination $[°]$

n speed of the rotations $[rpm]$

u_{sr} radial component of peripheral velocity $[m\ s^{-1}]$

h axial distance between discs $[m]$

φ angle of disc inclination $[°]$

t_s settling time $[s]$

H length of the bowl $[m]$

ρ_s density of solid $[kg\ m^{-3}]$

c mass concentration $[g\ L^{-1}]$

m mass of dried biomass $[g]$

V_S volume of culture medium sample $[L]$

D_{CM} density of the culure medium $[g\ cm^{-3}]$

c_M concentration in the culture medium solution $[vol\ \%]$

V_t total volume of the culture medium $[L]$

Introduction

The inevitable problematic of air pollution is apparent, as the data shows CO_2 emissions are the primary driver of global climate change as well as health-related problems [1,2]. Furthermore, CO_2 is a significant contributor to environmental damage. Given that there is currently no low-emission alternative to many industrial processes, it is necessary to consider technologies such as carbon capture and storage (CCS) or carbon capture and utilization (CCU) in order to reduce emissions [3,4]. Addressing this environmental issue with an engineering outlook is one of the most promising strategies. Hence, the interest in the areas of CCS and CCU technologies have been increasing. Along with the research and further studies extensively being carried out within the biorefinery field, particularly in the context of microalgae cultivation systems [5]. The final products of microalgal biomass is another intriguing aspect of this area with a variety within the pharmaceutical, biofuel and other industries.

Microalgae

To define microalgae, we can specify their function which is based on conversion of water, CO_2 and light. They are so-called unicellular photosynthetic micro-organisms; they can live both in saline and fresh water environments [6]. They belong into the category of eukaryotes under which the definitions of classes are mainly distinguished by their pigmentation, life cycle and basic cellular structure [7]. The most relevant and important classes for CCU applications are: green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*) [8]. The microalgae can also be divided into autotrophic or heterotrophic group. Where the autotrophic, more specifically the photoautotrophic, due to having photosynthesis as the main process, require only inorganic compounds such as CO_2, salts and a light energy source for growth. While the heterotrophic (also called organotrophic) can be, either using the energy from light source, then they are specified as photoheterotrophs, or the chemoheterotrophs carry out the oxidization of organic compounds. Heterotrophs therefore require an external source of organic compounds as a source of energy [9].

For the purpose of the cultivations within photobioreactors (PBR) the microalgae types considered do undergo photosynthetic processes. Based on which the conversion of light, water and CO_2 to algal biomass is taking place. The production of storage lipids

in the form of Triacylglycerols (TAGs) occurs, similarly as in higher plants [6]. Rapid growth and high productivity can be observed in number of species, and an accumulation anywhere from 1% up to 90% of lipids of microalgal dry biomass can be induced [10] . Due to this, the most appealing production is within the biofuel range of products from microalgae cultivations. Yet, the options and possibilities of bioproducts from the microalgal biorefinery are of a greater extent. As Garcia et al. 2018 [11] suggests microalgae systems, in comparison to other technologies, have a significant advantage, due to microalgal biomass being simply valorised as a bioproduct and/or energy, which is particularly intriguing within the circular economy context. As Stegmann, et al. [12] analyzed there are various perspectives and definitions on the relation between circular economy and bioeconomy. The conclusion drawn is that circular bioeconomy entitles both and the fundamental point of it is the *"use of wastes and residues as a resource"*, seen in *figure 1*.

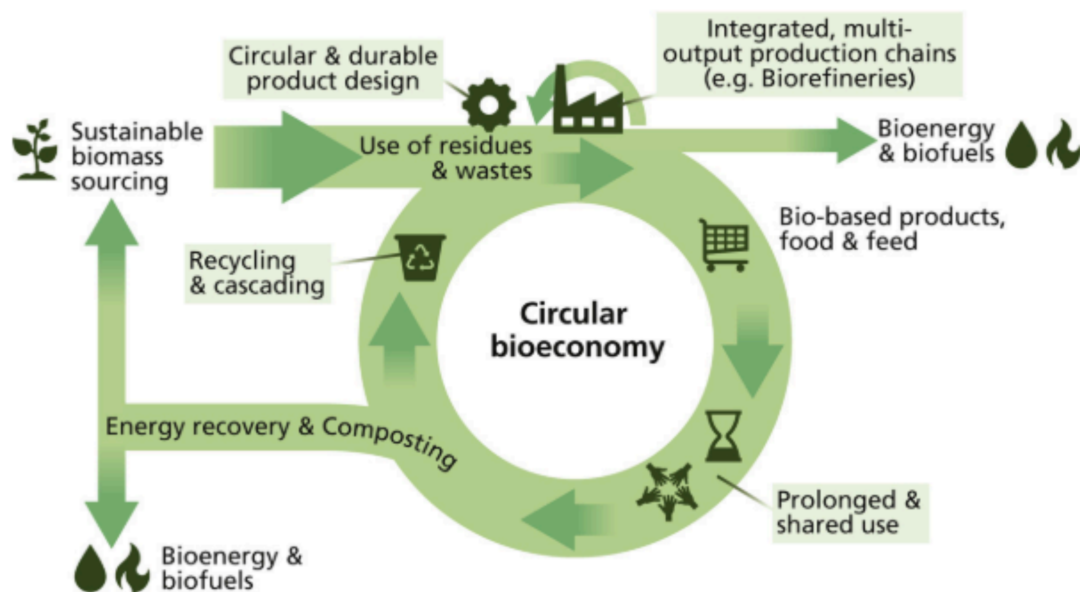

Figure 1- Elements of circular bioeconomy [12]

In terms of quantity of the bioproducts, namely bio oil, 1 acre of microalgal raw material yields approximately 95000 litres of bio oil. This data shows that the amount of production of bio oil is about 100 times higher in comparison with the oil being produced by other crops. It is also due to the time frame in which normal crops are planted and grown, which is in terms of months compared to 14 days for algal based cultivation [13].

Biorefineries

The main concept of biorefining is to be defined as "*the sustainable processing of biomass into a spectrum of marketable food and feed ingredients, bio-based products (chemicals, materials) and bioenergy (biofuels, power and/or heat)*" [14].

In biorefinery as such, independent of used biomass or the final product, the choice of the right processes paths and technologies is of a high importance. In a biorefinery facility the biomass conversion processes and equipment are integrated into production of multitude of products from biomass [15].

Classification of a typical biorefinery systems is further explained. There is a simplification of biorefinery types and then separation of those into groups according to which main characteristics the classification is being carried out upon. The recognized features are namely: platforms, products, feedstocks and processes [16]. For a further specification, a biorefinery would be firstly put into a group based on the platform, which is the link between the feedstock and final products. Then following classification is going to be based on the energetic or non-energetic products that are grouping the biorefinery system into either energy-driven or material-driven. Platform in case of a microalgal biorefinery could be already stated oils in the form of TAGs, the products then might be for instance biodiesel. Feedstock is described as the converted raw material into the products sold [17]. Clearly the one stable variable is going to be the feedstock in all the microalgal biorefineries, which is the microalgal biomass. Processes are closely related to the product wanted. Processes are divided into mechanical/physical, biochemical, chemical and thermochemical. The mechanical processes do not in any way interfere with the biomasses chemical structure, unlike the biochemical processes that use microorganisms or enzymes and chemical processes such as oxidation and combustion which is an example of thermochemical processes [16]. There might usually be more than one processes in a biorefinery, namely in the microalgal biorefinery we will come across the pre-treatment process, the process of cultivation to get the desired amount of biomass, this is followed by harvesting and dewatering processes along with cell lysis to be able to carry out the process of extraction and purification for recovery of the final product [18].

If we focus on microalgal biorefineries, the completely optimised systems are of a great help to the circular bioeconomy, climate change mitigation and support of growth within numerous industrial sectors [19]. The circulation of CO_2 as well as the usage of waste

water, both otherwise polluting the environment, through these systems is one of the reasons why the microalgal biorefineries are of such interest.
The source of water, for some of these microalgal cultivations, is closely dependent on the specific cultivation. The water sources include possible use of fresh, brackish, saline or waste water, depending on the microalgae species. The usage of treated waste water, which is highly advantageous due the nutrient utilization, might not be possible in various biorefineries due to the final products being of a more hygienically supervised area. Regarding these products there might be particular rules and regulations implemented since the use of microalgae in the pharmaceutical and food industry applications follow a different standard of requirements, as it is of high priority for the product to be safe for further human ingestion and application [20]. This may make the process more intricate not only technologically but also economically, which is a large factor to any system within the research and production. Also, the efficiency is greatly linked to the sustainability of the produced biomass.

As shown in *Table 1* alongside the advantages it is also important to address the disadvantages that arise with the processes of microalgae, their cultivation systems and further technological procedures. One of which is that the cultivations and the parameters that are controlled such as the stated usage of water source or the source of light that the cultivation receives. Another disadvantage, especially in regard to the economical aspect of microalgal biorefineries, is the processes of harvesting and dewatering of microalgae from the cultivation system. These processes have been accounting for as much as 30% of the costs that are related to the total production cost and therefore the research for more economically feasible optimization of this process is necessary in order to have an overall cycle of greater sustainability [21].

Table 1- Advantages and disadvantages of Microalgal biorefineries[3,22]

Advantages	**Disadvantages**
Wastewater usage (economic feasibility)	Safety regulations & possible contamination
Variety of products	Specific cultivation condition requirements
Diverse cultivation systems	High energy requirement for mixing
Increased solar conversion	Significant harvesting and dewatering costs
CO_2 utilization	

Cultivation systems

There are a few options when it comes to the cultivation systems and their set up. There are three main types of cultivation systems – open, closed and hybrid. The major difference in these systems is the exposure to the surrounding environment [23].

Open cultivation systems

Usually open pond systems and/or raceway ponds are mostly set up outside in the form of shallow tanks. There are a few types of designs, with various material choice, size, mixing system and other aspects of and open pond system. The design is carried out with the prioritization of parameters such as depth and mixing due to their importance of light exposure and aeration of the microalgal cultivation. Homogeneity of the mixture strongly affects the efficiency of the overall process [10]. Cruz et al. 2018 [24] points out that open systems are the most common in current overall outlook at cultivating of microalgae. These systems are advantageous for a larger production due to the lower cost of manufacturing and operation, including mixing. The outside placement has a great advantage of natural light source. Yet, the exposure to the natural surroundings, such as weather and animals, inflict higher probability of contamination and are not allowing much of a controlled environment. When it comes to controlling of light source, growth and temperature of the medium, they are not suitable for cultivation of microalgal biomass of various species and for certain industries. The area required for open cultivation is larger than for the closed cultivation systems, which is another disadvantage [25].

Closed systems

Specifically, Photobioreactors (PBRs) are widely used mainly because they can be designed and optimized based on the desired parameters for certain species and conditions that are ensuring the right amount of light, temperature and cultivation medium flow. The ability of better condition control in PBRs also allows for cultivation of a higher microalgae cell concentrations than in open cultivation systems [26]. Although the closed systems have the advantage of rarely experiencing contamination, it is important to note potential risk of cleaning issues, bio-fouling of the walls on the PBR tubes/plates and oxygen build-up which results in the limitation of growth of the

microalgal cultivation. The higher costs of manufacturing and operation of a PBR have also been shown to be disadvantageous [27].

Photobioreactors

Further specified, there are numerous models of photobioreactors: bubble columns, air lift, horizontal tube, helicoidal, agitated tanks and others [45]. There have been numerous advances and improvements implemented into the design and configuration of PBRs based on the research done in the past years. The main objectives that the innovation has been focused on is promoting the biomass productivity, light absorption, the yield of light too biomass and photosynthetic efficiency [28]. PBRs are vessels filled with culture medium with controlled environment and the design is mainly focused on an adequate illumination of the vessels to ensure sufficient rate of bioconversion of CO_2 into biomass [29]. To narrow down the classifications, the main types of PBR devices may be divided into flat panel and tubular. With tubular devices being the most promising for production of high-value microalgae biomass on large scale [30].

The tubular PBRs are categorized according to the placement of the tubes. They can be arranged and connected with horizontal, vertical (*figure 2)*, helical or inclined configuration. The two main processes to be considered in tubular PBRs are aeration and illumination [31]. The aerating system contains a pump and a degasser. It is responsible for the CO_2 distribution through the cultivation medium and constant movement of the cultivation in a turbulent flow as well as removal of O_2 produced by photosynthesis. The cultivation and growth of the microalgae takes place in tubes that are clear which allows for the light source to be well distributed [32]. The illuminating system is usually consisting of LED lamps when it comes to indoor PBRs placement and dependent on natural light in outside settings [33].
Flat panel PBRs, as seen in *figure 3,* contain the microalgal cultivation in a transparent material reactor with an aeration system. Even though in this PBR design a dense and thick layer of microalgal cultivation occurs, the photosynthesis is promoted by illumination of the whole layer of cultivation ensured by the dispersion of the cultivation medium between the two flat panels [34]. The mixing is important in these PRBs but as the placement of the aeration system is at the bottom of the panel it is more sufficient in the removal of O_2 formed.

Column PBRs consist typically of cylindrically shaped vessels illuminated from a source outside of the vessel or inside of it. Based on the pattern of the flow the classification of them is then into air lift or bubble column reactors. Regardless, the main objective to focus on when designing any vertical column PBRs is the proper aeration and mixing of the culture medium [31,33].

Figure 2- Vertical tubular photobioreactor [35]

Figure 3- Flat panel photobioreactor [35]

Hybrid systems

These systems are a multiple-stage combination of open and closed cultivation systems. In other words, a microalgal cultivation in the phase of biomass growth is kept in one type of the systems and afterwards the culture medium is transferred into a different system to continue another stage such as stress induction for lipid accumulation [25,33]. The possibly of a hybrid or two-step cultivation system has been adapted showing a possible reduction of environmental impact on the cultivation as well as increase of 10% in biomass yield and 10-40% in lipid accumulation (results taken after both stages of cultivation of N.oculata) and therefore the cultivation might be worth divining into multiple systems in certain stages [32,36,37]. Narala et al. stated in the comparison of cultivation systems the two-stage hybrid system that is has proven to be more advanced [25].

The most suitable choice of cultivation system/s is highly dependent on the microalgae species, production scale, final product, financial budget and other factors. Therefore, these factors along with the whole scheme of the biorefinery including the downstream processes, the operation and maintenance and the efficiency have to be evaluated and considered to be sustainable, affordable and possibly scalable [38].

Products

The applications of microalgae stretch from the industrial to commercial uses with variety of final products obtained. As seen in *figure 4, t*o be able to specify the products, they can be divided into three main groups- direct use, biofuels and bioproducts.

As far as the processes for direct use products go, the extraction is usually followed by drying and a powdered product is the outcome [3].

The biofuels produced from a microalgal biomass, also known as 3rd generation biofuel, have been seen as a plausible alternative to 1st and 2nd generation biofuels [3]. To obtain these 3rd generation biofuels various subsequent processes have to take place. They can be classified into three subgroups according to the phase and the extraction process that is used. To simplify the subgroups, we can clarify that the pre-treatment of algal wall lysis takes place to extract the desired raw materials (carbohydrates or lipids). The first is based on carbohydrates obtained from microalgae then being pre-treated with saccharification followed by fermentation. The products of this subgroup are bioethanol and biogas. Whereas biodiesel is produced from oils/lipids that undergo the process of transesterification [39].

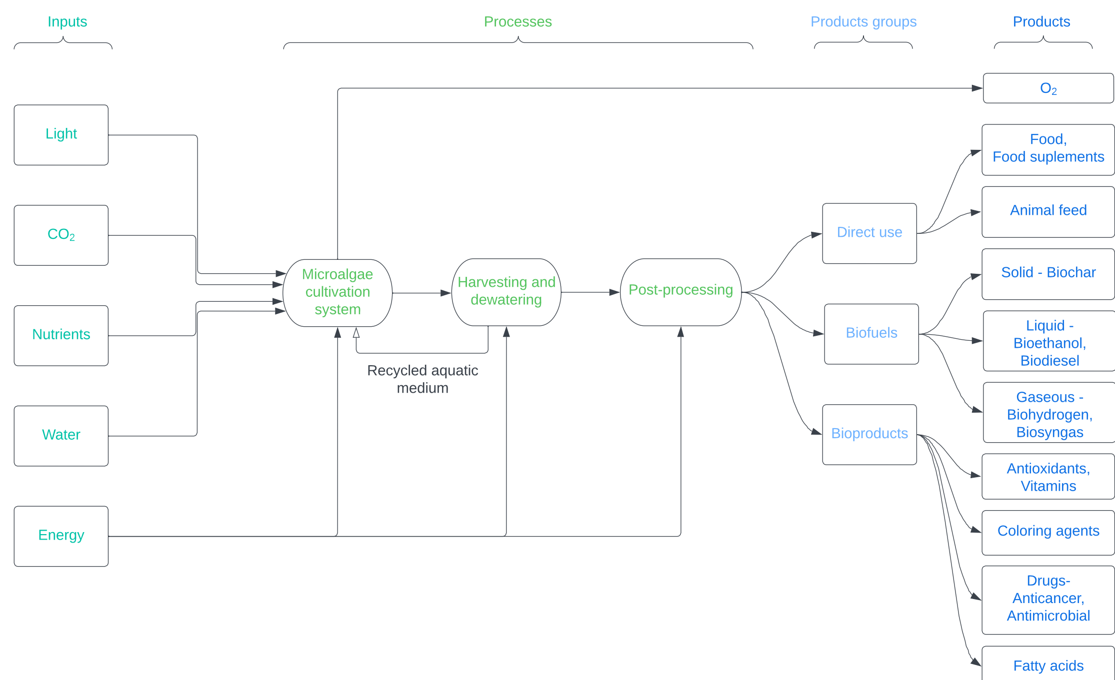

Figure 4-Microalgal biorefinery flowchart [22,39]

As for the bioproducts that microalgae provide, also shown in *figure 4*, there are multiple valuable ones. In the pharmaceutical industry, for instance, the vitamins from microalgae were introduced for human consumption. This is due to the nutrient rich profile and antioxidant contents. Because of these properties some of the microalgal species provide the benefits of acting as a prevention from oxidative stress and protection from free radicals are observed [39]. Other advantages include improved immune response, fertility, skin health and more. Some microalgal species are used as natural colouring in farmed fish or cosmetics also providing added vitamin A into the final products [20].

Objectives and thesis outline

The harvesting process of microalgae form an aquatic cultivation requires efficient techniques especially for certain microalgal groups with low cell density [32]. One of the main factors that are taken in account regarding this process along with the efficiency is the economic feasibility. The process of microalgal harvesting accounts to around 20 to 30% of the total costs related to the microalgal biorefinery production [40]. Which indicates that with more cost-effective process solution the overall production has a better potential to be implemented on a larger scale. Further, a research will be conducted on various processes and technologies used for microalgal harvesting and dewatering.

Thesis objectives:

- Research main harvesting and dewatering processes to be able to navigate the mechanics and technology used in them.
- Determine processes that can be experimentally verified with the available equipment and materials
- Choose suitable technologies to support chosen processes and evaluate their design and operational parameters

Harvesting and separation of microalgae

Harvesting, thickening and separation or so-called dewatering are processes that follow the cultivation stage of microalgal biomass. The techniques used for these processes depend on the facilities and the final product that is to be obtained from the particular biorefinery. There might be more steps used in this process which varies depending on the equipment type [2].

The harvesting technique is also very highly dependent on the species and use of microalgae, since if the main profile is starch for further processes as production of bioethanol or biofuel then the technology subsequently also the harvesting and dewatering is going to be different from the one focused on retrieval of lipids from the microalgal cultivation for biodiesel production. It is also important to consider the microalgal biomass and lipid stability post-cultivation. As K. Napan, et al. suggests temperature, time and processing of microalgae after being harvested from the cultivation medium might also strongly affect the outcome of the amount and quality of the final product [41].

The *figure 5* shows some of the possible operations for harvesting and dewatering that can be used. Either the harvesting technology can be used alone if it has a sufficient efficiency of the biomass recovery rate or the technologies might be combined to create a more effective subsequent process. Either option can also be supported by adding the drying process at the end [21].

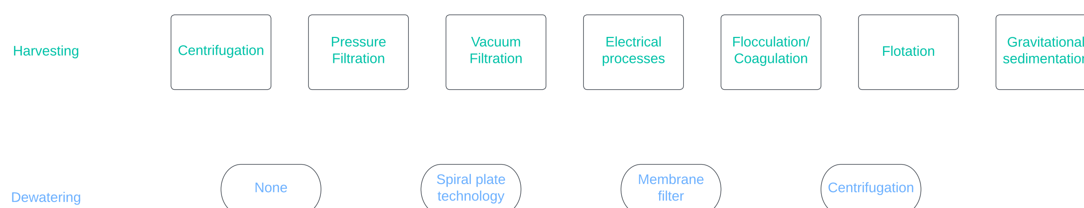

Figure 5- Microalgal harvesting and dewatering techniques [42][21]

Equations 1 and *2* enable us to clearly quantify the effectiveness of separation of solid microalgal part from the liquid cultivation medium the recovery efficiency (RE) term is introduced, which determines *"ratio of the mass of cells recovered in the final product to the total mass of cells in the initial culture"*, along with concentration factor (CF)

describing *“the ratio of the concentration of microalgae biomass in the final product to the initial concentration in the culture mass”* [43,44].

Recovery efficiency

$$RE = \frac{mass\ of\ cells\ recovered\ in\ final\ product}{total\ mass\ of\ cells\ in\ the\ initial\ culture} \quad (1)$$

Concentration factor

$$CF = \frac{concentration\ of\ microalgae\ biomass\ in\ the\ final\ product}{initial\ concentration\ in\ the\ culture\ mass} \quad (2)$$

Due to microalgae cells being extremely small the measurements of the mass of the cells and mass concentration might be affected by as little as the change of a factor in environment that the measurement is taking place in. Accordingly, the results of the *equation 1* and *2* might be influenced by them. Therefore, it is usually stated these quantities are an estimate. Nonetheless, a valid result can be obtained with experimental methods and reasonable approach.

Gravitational sedimentation

This separation process occurs naturally by the means of gravitational force acting on the particles of the microalgae in the cultivation medium. During this particle-liquid separation process, a concentrated slurry of microalgae settles at the bottom of the vessel, with the liquid solution clearly separated on top. This process, known as sedimentation, is determined by the sedimentation velocity of the microalgae, which is influenced by the strain characteristics such as the particle radius and density. It is further determined by Stoke's law [45].

$$u_s = \frac{2}{9} \cdot g \cdot \frac{r^2}{\eta} \cdot (\rho_p - \rho_l) \tag{3}$$

where u_s $[m\,s^{-1}]$ is settling velocity, g $[m\,s^{-2}]$ is gravitational acceleration, r $[m]$ is particle radius, η $[m\,s^{-1}]$ is viscosity of liquid, ρ_p $[kg\,m^{-3}]$ is density of particles, ρ_l $[kg\,m^{-3}]$ is density of liquid.

The *equation 3* calculates the theoretical settling velocity with considering that the particles of microalgae are not of an ideally spherical shape and also that they are in the Newtonian region. The *equation 4* is introduced and is applicable for calculations of settling velocity also considering different drag force regions of the spherical particles that are settling. The consideration of microalgal cells as spherical is mainly due to the exact geometry being impossible to be identified for each of the microscopic particles.
In order to be able to use the *equation 4* for calculation of the settling velocity of a particle in a liquid solution, the region of settling has to be specified. Therefore, the *equation 5* in combination with *equation 4* is used and the new criterion is further determined as a multiple of $C_D Re^2$, as to be seen in *equation 6* [46].
The *equation 4*, which is also from Stoke's law describing the free settling velocity:

$$u_s = \sqrt{\frac{4}{3} \cdot \frac{D_p \cdot (\rho_p - \rho_l) \cdot g}{C_D \cdot \rho_l}} \tag{4}$$

where u_s $[m\,s^{-1}]$ is settling velocity, g $[m\,s^{-2}]$ is gravitational acceleration, D_p $[m]$ is particle diameter, ρ_p $[kg\,m^{-3}]$ is density of particles, ρ_l $[kg\,m^{-3}]$ is density of liquid, C_D $[-]$ is drag coefficient.

$$Re = \frac{u \cdot D_p \cdot \rho_l}{\mu} \tag{5}$$

where $u\ [m\ s^{-1}]$ is settling velocity, $D_p\ [m]$ is particle diameter, $\rho_l\ [kg\ m^{-3}]$ is density of liquid, $\mu\ [Pa\ s]$ is dynamic viscosity of the culture medium.

$$C_D Re^2 = \frac{4}{3} \cdot \frac{{D_p}^3 \cdot (\rho_p - \rho_l) \cdot \rho_l \cdot g}{\mu^2} \tag{6}$$

where $g\ [m\ s^{-2}]$ is gravitational acceleration, $D_p\ [m]$ is particle diameter, $\mu\ [Pa\ s]$ is dynamic viscosity of culture medium, $\rho_p\ [kg\ m^{-3}]$ is density of particle, $\rho_l\ [kg\ m^{-3}]$ is density of liquid.

As the *table 2* suggests this method is a very cheap and simple way of separating microalgae from the liquid medium. There are low requirements when it comes to apparatus, but as seen it the *equations 4-6* the species chosen for this method will have a significant effect on the settling velocity based on the specific size of their particles. Therefore, it has been the most prevailing method for harvesting microalgae species with larger cell diameter such as *Spirulina* [20]. Yet there might be certain limitations such as the extended periods of time that it takes from microalgae to settle from its cultivation medium due to their densities being similar to each other [47].

There are various types of settlers that can be used in regard to the process of gravitational sedimentation. For microalgal separation from cultivation medium we can consider for instance circular settler/thickener and lamella settler. In *figure 6* the schematics of a design of circular settler and lamella settler are shown.

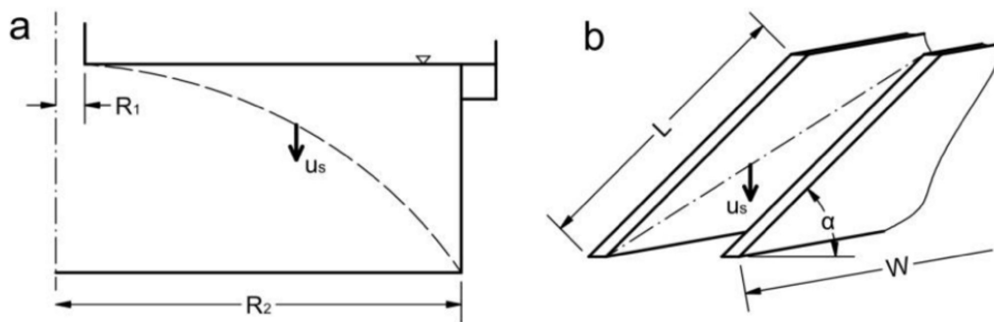

Figure 6- Scheme of a-circular settler, b-lamella settler [47]

The circular settler is designed for primary thickening as well sedimentation of microalgal particles. The dimensions such as cross-sectional area $S\ [m^2]$ and outer radius $R_2\ [m]$ of the settler are determined with respect to the suspension with a certain flow rate $V_{su}\ [m^3\ s^{-1}]$ and concentration $c_{su}\ [kg\ m^{-3}]$ which is fed into the inlet of an inner radius $R_1\ [m]$ and it settles in a parabolic motion with the settling velocity $u_s\ [m\ s^{-1}]$. The sludge continuously removed from the bottom of the settler has a concentration $c_t\ [kg\ m^{-3}]$ and the remaining culture medium is drained with the possibility of recirculation into the cultivation system. The use for untreated microalgae separation might be problematic due to long sedimentation periods and therefore possible degradation of the sludge [44]. The solution to this can be pre-concentration or proper selection of designing dimensions for circular settler, *equations 7* and *8* shown in the formulas.

$$R_2 = \sqrt{{R_1}^2 + \frac{V_{su}}{\pi\ u_s}} \tag{7}$$

$$S = \frac{V_{su}\ c_{su}}{u_s}\left(\frac{1}{c_{su}} - \frac{1}{c_t}\right) \tag{8}$$

In a lamella settler the fed cultivation medium with the microalgal biomass flows up in between and the microalgal cells separate from the medium onto the surface of the lamella. This is due to the settling velocity being in a vertical downward direction as seen in the *figure 6b* and the flow is at the incline angle directing up between the lamellas, which is resulting in the microalgal particles having a sedimentation velocity with the direction towards the lamella surface. Therefore, the particles sediment and the medium continues to flow up and out of the settler. The sedimented sludge falls down and is either continuously emptied or collected at the bottom and emptied in larger batch. The suspension flow rate $V_{su}\ [m^3\ s^{-1}]$ is determined with respect to the lamellae design in the settler. The following parameters are needed to complete the calculation with the use of *equation 9*. Number of lamellae $i\ [-]$, the length of the lamella $L\ [m]$, the width of the lamella and an angle of the lamella inclination $\alpha\ [°]$.

$$V_{su} = i\ u_s\ L\ W\ \cos\alpha \tag{9}$$

The major significance of the sedimentation velocity in the calculations of settlers and thickeners is also clear from the above *equations 7-9*.

Centrifugation

Same as gravitational sedimentation, centrifugation is based on the Stoke's law, meaning that the radius and density of algae cells as well as the sedimentation velocity plays a major role in this harvesting method [47]. The harvesting method of centrifugation is considered the most efficient due to the efficiency reaching over 95% [20,40]. Even though the process is effective and fast the processing time, the residence time of slurry in the centrifuge, settling depth and other aspects have to be taken into consideration to be able to ensure that the retrieved microalgal biomass is up to a viable standard that will be required for further processing and fulfilling the potential of high-value final products [27].

Currently, as *Macfuge* pilot plant has shown, there is a possibility of production of fully automatic centrifuges with the ability to work in a continuous mode without integration of the cell structure. They are effective in reaching concentration over 95% when particle size is $> 2\mu m$ [48].

Centrifugation is very beneficial in terms of application to almost all microalgal species Due to the high shear forces created by the rapid movement, the microalgal cells are at high risk of damage to their cellular structure and therefore this process becomes destructive rather than efficient. Considering that this method is highly economically and energetically consuming, it might need more extensive design and research done before it is applicable onto a larger or industrial scale as harvesting technique for all microalgal product [38]. Another way of making centrifugation a viable part of microalgal harvesting and dewatering, is having it as a second stage of a two-step process. By prior separation of the microalgal suspension in the culture medium the cost of centrifugation is lowered [40,49].

Centrifugal technology comes in various types of devices that have been implemented in the separation processes of microalgae. But the two types of centrifugal devices that have been considered the most promising for microalgae harvesting with regards to multiple aspects such as biomass concentration or cost are disc stack and decanter centrifuges [49].

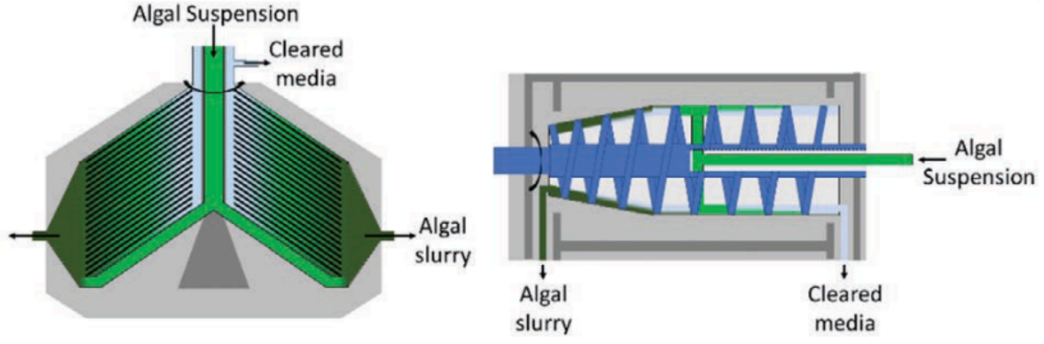

Figure 7- Disc stack (left) and decanter (right) centrifugation methods [49]

Disc centrifuges are commonly used for microalgae particles ranging at $0.02 - 0.05\%$ in concentration [50,51]. The basic structure of such centrifugal device, as seen in *figure 7*, consists of a central inlet, a stator drum, rotating discs and outlets for concentrated discharge of algal slurry and as well for the clarified medium [34,47].
The *equation 10* specifies the settling velocity in this process, according to parameters of the device and microalgal culture, described by Stoke's law.

$$u_s = \frac{D_p^2 \left(\rho_p - \rho_l\right) \left(2\pi \frac{n}{60}\right)^2}{18\,\mu} r_2 \tag{10}$$

where $u_s\ [m\, s^{-1}]$ is settling velocity, $D_p\ [m]$ is diameter of the separated particles, $\rho_p\ [kg\, m^{-3}]$ is density of particles, $\rho_l\ [kg\, m^{-3}]$ is density of liquid, $\mu\ [Pa\, s]$ is the dynamic viscosity of the culture medium, $n\ [rpm]$ is the speed of the rotations and $r_2\ [m]$ is the outer radius of the rotating discs.
The correlation in *equation 11* determines the radial component of peripheral velocity $u_{sr}\ [m\, s^{-1}]$.

$$\frac{u_{sr}}{u_s} = 0.27 \left(\frac{r_1}{r_2}\right)^{-0.397} \left(\frac{h}{r_2\ \tan\varphi}\right)^{-0.957} \tag{11}$$

where $r_1\ [m]$ is the outer radius of the rotating discs, $h\ [m]$ is the axial distance between discs and $\varphi\ [°]$ is the angle of disc inclination.
The specification of the flow rate processed by the culture medium follows the *equation 12*.

$$V_{su} = i\, 2\, \pi\, r_2\, h\, u_{sr} \tag{12}$$

Another frequently used device utilizing the centripetal force for microalgal harvesting is the decanter centrifuge. As seen in the schematic of the design in *figure 8*, the device consists of conical horizontal bowl with screw conveyor, central inlet and outlets for both separated microalgal particles and liquid medium [52].
The processing time for of settling of the microalgal particles with the use of decanter centrifuge can be calculated as seen in the *equation 13*.

$$t_s = \frac{18\,\mu}{D_p^2\left(\rho_p - \rho_l\right)\left(2\pi\frac{n}{60}\right)^2}\ln\frac{r_2}{r_1} \tag{13}$$

where t_s $[s]$ is settling time, D_p $[m]$ is diameter of the separated particles, ρ_p $[kg\ m^{-3}]$ is density of particles, ρ_l $[kg\ m^{-3}]$ is density of liquid, μ $[Pa\ s]$ is the dynamic viscosity of the culture medium, n $[rpm]$ is the speed of the rotations, r_2 $[m]$ is the outer radius of the bowl and r_1 $[m]$ is the inner diameter of rotating screw conveyor.

To be able to design all of the dimensions *equation 14* is providing the length of the bowl H $[m]$.

$$V_{su} = \frac{\pi\,(r_2^2 - r_1^2)\,H}{t_s} \tag{14}$$

The *equations 10-14* were all described by Bělohlav and Jirout [47] in the detailed "*Design methodology of industrial equipment for microalgae biomass primary harvesting and dewatering*".

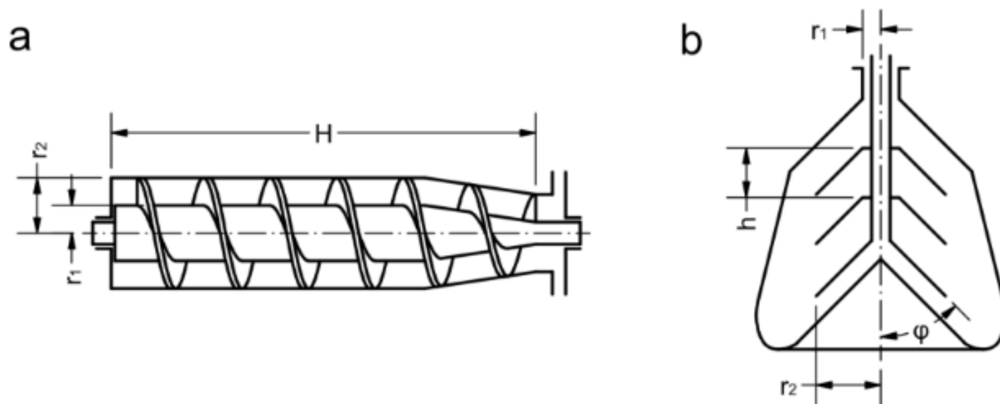

Figure 8- Schematic with geometrical parameters a) decanter centrifuge and b)disc centrifuge [47]

Filtration

There are various filtration techniques relevant for use in the area of microalgal harvesting. These consist of mainly microfiltration, macrofiltration, pressure filtration, vacuum filtration dead-end filtration and tangential flow filtration [21,45]. The filtration process is based on the microalgal particles being separated mechanically from the liquid medium by the means of porous bed/membrane. The microalgal culture medium passes through as so-called filtrate and remaining is the microalgal slurry or so-called concentrate.

To ensure the effective separation, it is usually applied after a primary harvesting operation, such as centrifugation, flotation or coagulation/flocculation [42,53]. The size of pre-separated microalgae, when the filtration process is a second stage of separation following coagulation/flocculation, makes macro-filtration the suitable type due to its membrane having size $> 10\ \mu m$. Microalgae cells that undergo filtration as a single separation process allows for micro-filtration, with membrane pore size $0.1 - 10\ \mu m$ [50].

The required driving force for filtration to occur can be obtain in form of pressure, temperature or concentration drop across the system process so that the fluid is forces to flow through the porous bed/membrane successfully[53]. The thickening of the microalgal deposits on the filtration membrane increase during this process and therefore the resistance is increased which lowers the filtration flux upon a constant pressure drop [54]. This fouling phenomenon increases the cost of operations and as seen in *table 2* would be considered the main filtration process disadvantage, increasing the cleaning and maintenance requirements [42,55]. It has been shown to occur more on more porous membranes. The use of coagulation or flocculation prior to filtration is advantageous due to the microalgae cells increasing size [50]. Therefore, enabling a larger choice of filter types and well as lowering irreversible fouling [56].

The methods of filtration shown in *figure 9* are dead-end filtration and cross-flow or also called tangential-flow filtration. Dead-end filtration which uses the gravitational force to support the flow in the vertical direction is said to be more suitable in terms of recovery of larger cells $> 70\mu m$ and tangential-flow filtrations pose advantage of a higher filtration rate due to the larger tangential velocity of fluid to the membrane, therefore allowing for complete separation of microalgal particles from the culture medium with lower fouling [42].

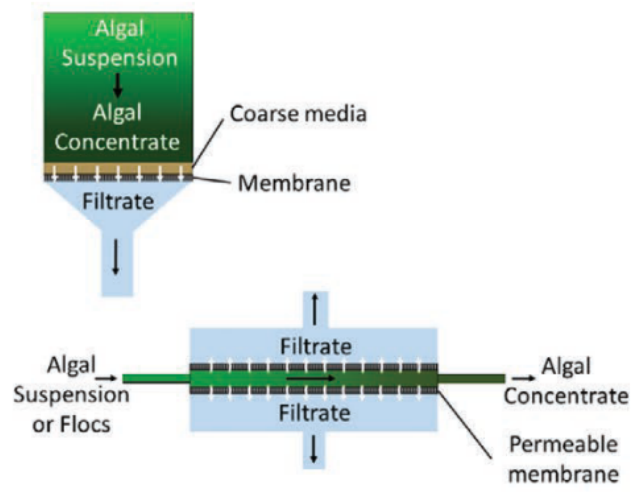

Figure 9- Dead-end (top) and tangential-flow filtration (bottom) [49]

Flotation

This technique may be described as "inverted" sedimentation. Due to the separation of microalgae from the culture medium happening in the opposite direction of natural gravitational sedimentation in this process. Gas or air bubbles are providing a lifting force, therefore instead of the settling of microalgal biomass at the bottom of a container a layer is formed at the top surface. This process is promoted by technologies introduced further but might happen naturally with certain microalgae species, yet even in such species it is suitable to use to increase the speed or effectiveness of flotation. When it comes to the size of the bubbles in the flotation process the aim is to use air bubbles varying from 10-1500 μm in size depending on which of the flotation methods are used. This is due to the size of microalgae ranging 5 µm (Chlorella) to 100 µm (Spirulina) so for the attachment of the bubbles onto the surface of microalgae the size plays a major role [57]. Microalgal characteristics of low density and self-float are suggesting that the process of flotation will have a higher effectiveness in the microalgal removal than sedimentation [42]. The addition of certain chemical or biobased substances (flocculants further researched and explained in the following chapter) before starting flotation also makes a difference when it comes to the bonding of air bubbles to the cells.
The most common area of flotation process is used in wastewater treatment with the processes of coagulation/flocculation usually following it. The combination of flocculants and flotation has been proven to function as a large scale separation technique that is inexpensive and has a relatively low operation time and small space requirements [58]. This can be applied to the microalgae cultivation medium as well to increase the effectiveness of the separation. It does not require large area of space and the time of operation is also relatively short. The classification into dissolved air flotation (DAF), dispersed air flotation(DIF)/induced air flotation (IAF), suspended air flotation (SAF), electrolytic flotation and ozonation-dispersed flotation is according to the bubble size [45].

The set-up of dissolved air flotation (DAF) unit as seen in *figure 10* consists of a saturator, flotation tank and a monitoring unit that monitors the saturation percentage. The formation of bubbles can be attributed to the decrease in pressure of water pre-saturated with air of a higher pressure. This follows Henry's law, describing the air solubility principle. The bubble size is in the range of $10 - 100\ \mu m$ [59].

Due to its feasibility, even though it has certain disadvantages, DAF has been proven to be a simple solution for large-scale use in various areas of applications.

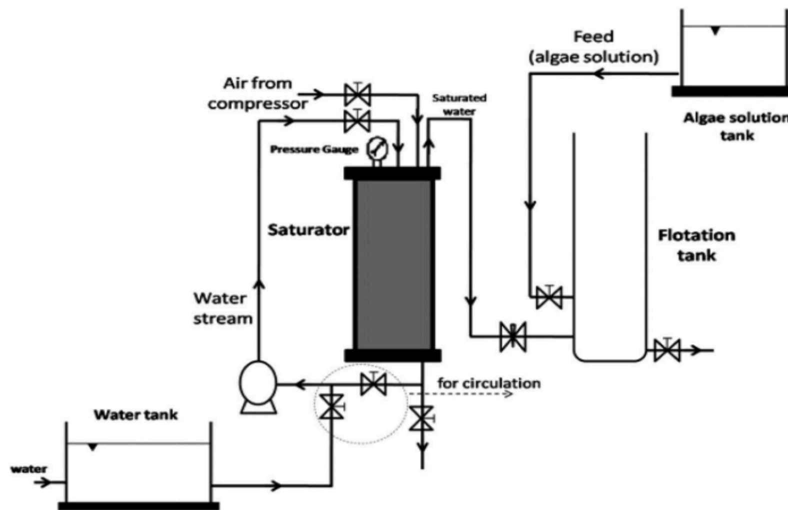

Figure 10-Schematic of dissolved air flotation unit [60]

Dispersed air flotation (DIF) and Induced air flotation (IAF) are both using an agitator working at high-speed and air injection system to mechanically form bubbles. By this a centrifugal force is created and the bubbles are pumped either beneath (DIF) or into (IAF) the agitator and they are mixed with the rest of the liquid in the tank. In these methods of flotation the bubble size is $700 - 1500\ \mu m$ [58].

Suspended air flotation (SAF) is another flotation process with small bubble size, similarly to DAF. Yet, it differs from DAF due to utilizing chemicals, namely cationic surfactants, to create the air bubbles. This mixing is done separately and directly after the release into the microalgal culture medium the bubbles takes place enabling the flotation process to occur. With SAF the main advantage over other flotation techniques is lower energy cost requirement, however the possibility of subsequent recycling of the medium might not always be possible [34,60].

Flocculation and coagulation

The processes of flocculation and coagulation are used for the separation of the microalgae particles from the medium which they are cultivated in, so-called *Bulk harvesting* [3]. Consequentially, in both flocculation and coagulation larger microalgae flocs are formed which means that the bigger size allows for an increase of the sedimented cell recovery [32].

More specifically, the addition of flocculants allows for the aggregation of the particles into the formation of flocs due to polymers (usually cationic) added to the culture medium. Whereas, when using coagulation, either by addition of electrolytes or adjustment of pH, it lowers the electrostatic repulsion in between the negative charged surface of the microalgal cells allowing for their aggregation [42].

By altering the size of the settling particle, the process of sedimentation is going to be faster as is suggested by the *equation 10* [46].

$$u_s = \frac{D^3 \cdot g \cdot (\rho_s - \rho_l)}{18\eta} \tag{10}$$

where u_s $[m\ s^{-1}]$ is settling velocity, g $[m\ s^{-2}]$ is gravitational force, D $[m]$ is diameter of settling particle, η $[m\ s^{-1}]$ is viscosity, ρ_s $[kg\ m^{-3}]$ is density of solid, ρ_l $[kg\ m^{-3}]$ is density of liquid.

The process of flocculation is considered a more proficient harvesting technique compared to others. This is due to flocculation proving to be effective and inexpensive [32]. The advantages of pre-concentration by flocculation or coagulation are not limited just to improvement in harvesting efficiency, they also tend to decrease maintenance and operation costs [61]. Therefore, the methods of harvesting previously mentioned (summarized in *table 2)* which depend on sedimentation velocity and/or on the density of the particles will be potentially intensified and improved by flocculation pre-treatment.

Flocculation can be separated into groups according to its basic mechanisms. As seen in *figure 11- A*, the microalgae cells surface charge is affected by ion, polymer or colloidal absorption, leading to reduction of charge and therefore its neutralization. Similar looking schematic, just with spaces of different charge on the surface, can be applied to the mechanism of electrostatic patching, where the particle surface is reversed by the polyelectrolyte and as a result patches opposite charge.

These allow for the interactions based on the thermodynamic balances of interaction energies (*figure 10- B*).

Another mechanism, so called bridging (*figure 10-C and D*) uses charged polymers to attach themselves to the surface of the cell and then form a bridge between each other, therefore in conclusion aggregating.

Lastly, mechanism seen in *figure 10-E* is when a precipitate is formed around and in-between cell, leaving them closely packed together in an *entrapment* [62].

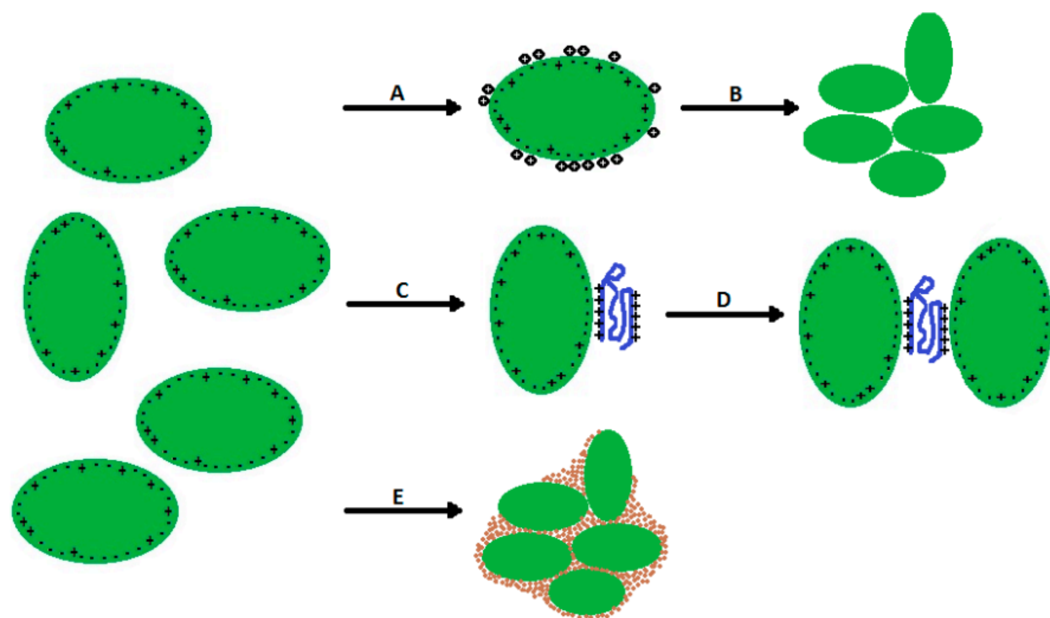

Figure 11- Basic flocculation mechanisms [62]

Flocculants are highly dependent on multiple factors such as pH, cell surface characteristics, environment etc.. The dosage and type of flocculant also play a major role in the resulting outcome. [62].

Types of flocculants are divided into groups based on the flocculant origin or chemical composition.

Bio-flocculation depend on the bacteria and fungi that are coexisting within the microalgal medium in surroundings of the microalgae cells. These microorganisms secret exopolysaccharides (EPS) which consequently may lead to flocculation of the microalgae [45].

Similarly, auto flocculation also does not proceed by the addition of chemicals. Rather the pH might be changed in the culture medium caused by the precipitation of salts naturally occurring by the means of the environment. This type of flocculation namely

takes place in waste water microalgal cultivations due to salts that are naturally present there.
When it comes to chemical flocculation, multiple polymers (both biopolymers and inorganic polymers) and salts have been tested throughout various studies [63].
Polymer flocculants, both natural or synthetic, are long-chain molecules, which consist of monomer sand can be categorized further based on their electrical charge into cationic, anionic or non-ionic. Depending on the charge they can use one of either of the mechanisms in *figure 11* [64].
Flocculants leave traces of chemicals in the separated liquid culture medium. In this case the liquid cannot be reused or anyhow further treated. Also, the microalgal biomass might not be possible to further use for higher value products.
However, there are flocculants that might classify as safe for food and pharmaceutical industry. Yet, this depends on the legislation around the regulation in these industries and in each specific country [65].

Table 2- Advantages and disadvantages of harvesting and dewatering techniques [32,42,66]

HARVESTING TECHNIQUE	ADVANTAGES	DISADVANTAGES
Gravitational Sedimentation	• Inexpensive • Simple technology	• Long process time • Not for all microalgal species
Centrifugation	• High efficiency of biomass recovery • Applicable for almost all microalgae species	• Economically infeasible • Possible cell damage • Not suitable for other than high-value products
Filtration	• Possibility of culture medium recirculation • High cell recovery efficiency	• Not suitable for smaller cell size • Frequent thorough cleaning
Flotation	• Low cost requirements • Short operation time • Possible of application on a large scale	• Usually requires the use of flocculants • Low quality of microalgae
Flocculation and Coagulation	• Flocculant/Coagulant variety • Easy technique • Increase the efficiency of following techniques	• Addition of chemicals • Dependency of function of some flocculants on a specific pH

Experimental part

Based on the research, experiments to determine the sedimentation velocity were carried out. More specifically the measurements of gravitational sedimentation of microalgae particles with and without the use of flocculants were conducted. As suggested the use of flocculants can highly affect the effectivity of almost all the separation methods, therefore lower the cost of the harvesting and dewatering processes in the biorefinery. This could make the microalgae biorefining more cost-effective and the access and possibility of various productions could be increased or scaled-up. Allowing us to use significantly more of the potential that microalgae have to offer.

To obtain the concentration of microalgae in each batch used in the experiments, a preliminary measurement was performed. The used microalgae species was *Chlorella Vulgaris,* the culture was incubated and cultivated in a laboratory reactor. It was chosen based on availability and relevance for the experimental part of the thesis. *Chlorella* is a very commonly produced genus in the microalgal biorefinery industry [67,68]. The characteristics are cell size in the range from $2 - 10\ \mu m$ and density which is similar and therefore compares to the one of the culture medium in the range of $1{,}040 - 1{,}140\ kg\ m^{-3}$ [50,69].

Concentration of microalgal biomass in culture medium

The preliminary measurement to determine concentration of microalgae in the culture medium was carried out by the drying method. The concentration of microalgae in the culture medium is important for further calculations and assumptions about the process of settling. Such as, if particle concentration in the solution is less than $0.2\ vol\ \%$ the interference is less than 1 %, therefore it is considered *free settling* [70]. The range of mass concentration of microalgae in literary sources has been from around $0.5\ [g\ L^{-1}]$ up to $20\ [g\ L^{-1}]$ [26,42].

The culture medium was measured out into two samples of $50mL$ each. Two shallow ceramic bowls were weight on the laboratory scale and the microalgal cultivation was poured into the bowls. Then the bowls were placed into the laboratory drying oven for 105°C. The water evaporated leaving the concentrated microalgae biomass in the bowls. A second weight measurement was carried out to obtain the mass of the concentrations.

Equation 11 was used to calculate the values of the concentration obtained by this measurement.

$$c = \frac{m}{V_S} \qquad (11)$$

where $c\ [g\ L^{-1}]$ is mass concentration of microalgal biomass in culture medium, $m\ [g]$ is mass of dried biomass, $V_S\ [L]$ is volume of culture medium sample.
These measurements were done for both experiments separately, therefore the results of the initial mass concentrations are to be seen in *table 3* (for first cultivation batch collected) and *table 4* (for second cultivation batch collected).

Table 3- Initial mass concentration of microalgae for experiment "gravitational sedimentation without the use of flocculants

	Bowl weight g	**Bowl with microalgae g**	**After evaporation g**	**Dried biomass g**	**Resulting concentration g/L**
Bowl 1	53.1950	97.6122	53.2363	0.0413	0.826
Bowl 2	50.9961	96.3629	51.0354	0.0393	0.786
Average					**0.806**

The average mass concentration of the microalgae cultivation used for the gravitational sedimentation experiment without the use of flocculants was 0.806 $g\ L^{-1}$.

Table 4- Initial mass concentration of microalgae for experiment gravitational sedimentation with the addition of flocculants

	Bowl weight g	**Bowl with microalgae g**	**After evaporation g**	**Dried biomass g**	**Resulting concentration g/L**
Bowl 1	57.1202	99.7170	57.1735	0.0533	1.066
Bowl 2	56.9788	97.5669	57.0351	0.0563	1.126
Average					**1.096**

The average mass concentration of the microalgae cultivation used for the gravitational sedimentation experiment with the use of flocculants was 1.096 $g\ L^{-1}$.

The average values were used to verify that the sedimentation process was a type of free settling. The values of mass concentration of the microalgae along with average density of the medium being $1090\ g\ cm^{-3}$, as seen in *equations 12* and *13*, were used to calculate the volume percent of microalgae particles in the culture medium.

$$V_M = \frac{c}{D_{CM}} \tag{12}$$

where $V_M\ [L]$ is volume of microalgae in the culture medium, $C\ [g\ L^{-1}]$ is mass concentration of microalgal biomass in the culture medium and $D_{CM}\ [g\ cm^{-3}]$ is the density of the culture medium.

The *equation 12* provided result for the volume of microalgae $V_M\ [L]$ in the culture medium and *equation 13* the result for microalgae particle concentration in the culture medium solution $c_M\ [vol\ \%]$. The total volume of the culture medium $V_t\ [L]$ was $1\ L$.

$$c_M = \left(\frac{V_M}{V_t}\right) \cdot 100\% \tag{13}$$

Table 5- Numerical results of microalgae volume and concentration in each microalgal cultivation batch

BATCH	Volume of microalgae $[\boldsymbol{L}]$	Concentration of microalgae $[\boldsymbol{vol}\ \%]$
sedimentation without flocculants	0.000739	0.074
sedimentation with flocculants	0.001006	0.101

In this case the dry weight was measured to obtain results of initial microalgal biomass concentration in the culture medium used for the experiments and providing the results in *tables 3*-5. There are various other measuring methods estimating the mass concentration. Such as measuring the cell count of microalgae, Chlorophyll-content or absorbance (so-called optical density) [61].

As results in *table 5* indicate the concentrations of microalgal particles in the culture medium are bellow $0.2\ vol\ \%$, therefore the sedimentation that has taken place in either of the experiments is without larger interference than 1 % and classifies as *free settling*.

Gravitational sedimentation experiment

The first experimental part was based on the research done. As already stated, the sedimentation velocity plays a major role in designing, parametrization and function of most microalgal harvesting methods and technologies. Therefore, presenting the results of a gravitational sedimentation experiment allows us to determine the sedimentation velocity of the separated particles and evaluate the potential possibilities for designing of the gravitational sedimentation technologies.

Materials and method

This experiment was carried out with two samples collected on 16.5.2022 and 24.5.2022 from a cultivation batch. The cultivation prior to collection of first sample 16.5.2022 (used for measurement *trial 1*) took place over the course of 20 days. The second sample collected on 24.5.2022 (used for measurement *trial 2 and 3*) had a cultivation period prior to collection of 27 days. The samples were stored since collection in a closed container in a refrigerator at 8 ℃ until the measurements took place at a room temperature of 21 ℃ for the first two trails and for the third trial the measurement took place in the refrigerator at 8 ℃. The conditions, except the temperature, were kept constant for all measurements. The third trial being measured in a colder environment was decided due to the interest if the temperature difference when the microalgae is settling will affect it in any significant way.

The measuring equipment used was a standard plastic measuring cylinder, with volume of 500 mL and scale markings by 5 mL. To ensure the accuracy of readings a version of visual absorbance method was chosen to be a suitable method for this experiment of gravitational sedimentation. This method is based on Beer's law which suggest that *„the absorbance of light in a substance is directly proportional to the concentration of the absorbing substance"* [71]. A light was shined through vertically along the back wall of the measuring cylinder and therefore the ability to see the line separating the clearer medium zone from the sedimented zone was easier to distinguish. There were still expectations of certain inaccuracies occurring due to human error. Those were prevented to the best ability by doing multiple readings for each measurement, as well as comparing every collected value to a photograph taken right after each reading.

From the moment when culture medium was measured out to be 500mL the measuring cylinder was covered with a thick paper bag; this was constant during the time period between each reading. It was done to prevent the microalgae that are settling to be

exposed to longer periods of light in addition to allowing the conditions for measurements to stay the same during all of the trials no matter where exactly the measuring cylinder was placed on the table.
The reading of the results of the first trial took place every 2 hours for the total amount of 16 hours. The following two trails had readings taken every hour for 10 consecutive hours.

Results

The values obtained during the measurements are represented in a graphical form in the *figure 12* bellow. They were then used to calculate the average sedimentation velocity *table 6*.

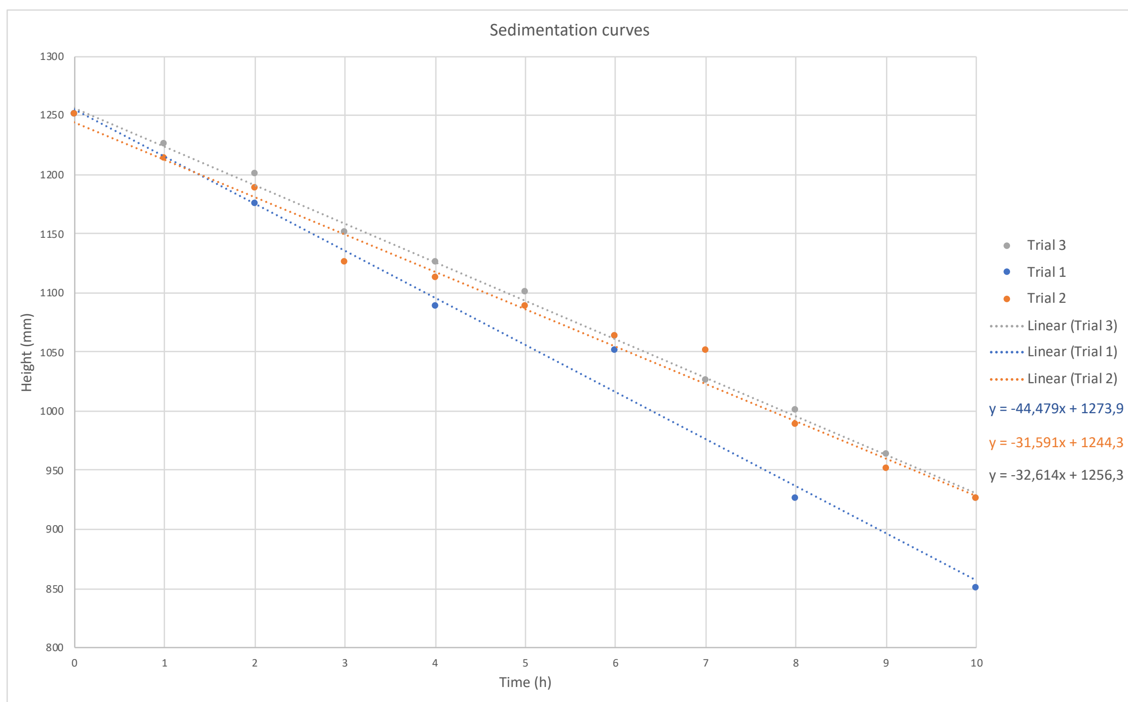

Figure 12- Graphical representation of data collected from measurements of sedimentation

Table 6- Results of sedimentation velocity

Trial	Average sedimentation velocity $\left[\frac{mm}{h}\right]$
1	22.7
2	32.5
3	32.5

In the *figure 12* graph the 3 extra readings during the trial 1 measurement were left out to be able to compare graphically the sedimentation in all three trials during the same measured time period.

As Jirout T. [72] suggests the determination of settling velocity can be done with the use of graphical representation of interface height potted against time, which is the sedimentation curve. The first part of this curve is constant until a critical point, where the sedimentation starts to slow down until fully completed.

In the experiment carried out only the first part of the sedimentation was measured out. Therefore, as seen in *figure 12*, the rate at the which the settling takes place is nearly constant. Meaning the for the full process of sedimentation of microalgae to be completed the time requirement would be more than 16 hours.

The results shown in *table 6* show us that the first trial had a slower average sedimentation velocity of $22{,}7\ mm\,h^{-1}$, whereas despite the data set for the other two trials differing. They have unintentionally worked out to have the same average sedimentation velocity of $32{,}5\ mm\,h^{-1}$.

Despite the results not differing drastically, there is still a clear indication that the first collected batch was possibly either in a more active growth phase which could mean that the produced oxygen might have promoted flotation of the microalgae cells to the water surface.

There has also been some observation of microalgal cells fixation of the biofilm on the walls of the measuring cylinder (*figure 14*). To solve this problem a smother surface of the measuring cylinder used might help. Therefore, for further experimental methods the suggestion would be to use glass measuring cylinders and make sure to carry out a thorough cleaning of the walls.

Figure 13- Sedimentation experiment

Figure 14- Sedimentation measurement close up

Gravitational sedimentation experiment with addition of flocculants

The objective for the second part of the experimental part is the verification of the functionality of used flocculants for the separation of microalgae cells from the culture medium and evaluation of the efficiency of the flocculants for separation on a semi-operational or industrial scale.

Preparation of flocculants

The flocculants chosen were prepared prior to the sedimentation experiment. The choice of flocculants was based on functionality proven by experimental measurements in a thesis by Dudek [65]. The two following flocculants have given satisfactory results regarding flocculation on a laboratory scale.

The flocculant used was primarily the Sokoflok 54 PWG (PWG 54), which is a cationic polyacrylamide flocculant in powder form. Second used flocculant was also cationic polyacrylamide flocculant designated as Amcon Yesfloc CWE35 (CWE 35), which was in a liquid form.

Each of the flocculants were prepared into a stock polymer solution by dissolution in a beaker with pre-measured $250\,mL$ of demineralized water. The amount of $2\,gL^{-1}$ of each raw flocculant was mixed into the liquid. All of the quantities were measured on a laboratory scale. The flocculant was mixed for a 2 hour period with the use of a magnetic mixing station. Then the beakers were visually checked. The flocculants were completely diluted and therefore ready for further use. The flocculants were used within 12 hours from mixing to ensure that the polymer hydrolysis cause by longer storage is avoided.

The amount of flocculant added into the cultivation medium was measured out with a pressure pipette to ensure an addition of accurate amount of flocculant solution to the microalgal cultivation medium each time. For this experiment the flocculation and subsequent sedimentation of flocs was expected to be fast, therefore a larger scale was introduced to allow for an accurate reading.

The mixed stock polymer solution will further be referred to as *amount of flocculant added* in mL.

Materials and method

This experiment was carried out with a sample collected on 24.11.2022 from a cultivation batch. The cultivation prior to collection of the sample took place over the course of 15 days. The sample was collected and measured out directly into the measuring cylinder right before each of the measurements then took place. The conditions were kept constant for all measurements. The measuring equipment used was a standard glass measuring cylinder, with the volume of $2\ L$. The height of the full $2\ L$ volume filled by medium was measured to be $400\ mm$. To ensure the accuracy of readings a video of each measurement was taken, and the results of sedimentation time were collected. After the amount of $2\ L$ of cultivation medium was collected and measured out, it was placed in a mixing station and a specific amount of flocculant was added as the agitator was turned on to mix in the prepared flocculant. This agitator was turned on at for 2 minutes each time, to ensure the homogenization of the flocculant in the culture medium. After that the medium with already created flocs was poured into the measuring cylinder and the time that it took for it to settle was collected.
The occurrence of inaccuracies due to human error were still possible. However, photographs were taken directly after each reading to be able to look into further comparison of floc size and clearness of water medium. After each measurement the equipment was properly cleaned and prepared for the next measurement to prevent inaccuracies due to the biofilm build up or other equipment inconsistencies.

The amount of PWG 54 flocculant addition was determined based on literature research and therefore a trial measurement was done with the amount that it was supposed to be sufficient and increased until the floc size and/or sedimentation velocity was in a sufficient range up to an optimal amount of water clarity. The addition of larger than amount necessary was trying to be avoided. Therefore, when the optimal value was satisfied the next flocculant available CWE 35 was measured.

Results

The sedimentation velocities were calculated and resulted as seen in *table 7* for PWG 54 and in *table 8* for CWE 35. The amount of flocculant added is given in mL per 2 L of cultivation medium and the sedimentation velocity was recalculated into $m\,h^{-1}$ for an easily understandable data set.

Flocculant PWG 54

Table 7-Results for average sedimentation with addition of PWG 54

Amount of flocculant added $[mL]$	Average sedimentation velocity $\left[\frac{m}{h}\right]$
16.5	3.0
17.0	4.8
17.5	10.3
20.0	18.0

The *figures 15-18* show the resulting solutions after the sedimentation with pre-added flocculant.

As seen the floc size when *16.5 mL* added was smaller compared to the following measurements, therefore the dosage pf flocculant was still not sufficient for in terms of water clearness. The flocks were mostly settled but there were still a lot of them dispersed around the medium without motion. This sample could be possibly easily already suitable for subsequent process of filtration to obtain clear water and microalgal sludge.

The *17 mL* flocculant addition has already shown increase in the floc size and the sedimentation as well. The water medium was significantly clearer, but the sedimentation velocity was measured at under *5* $m\,h^{-1}$.

Figure 15-PWG 54- 16.5 mL

Figure 16-PWG 54- 17 mL

A larger difference in terms of sedimentation velocity occurred in the following measurement when $17.5\ mL$ of PWG 54 was added. The floc has size increased yet the clearness of the water seemed to be cloudier than with the two previous measurements. The increase in amount of flocculant would suggest the improvement in medium clarity along with larger floc size. The optical impurity of the medium did not correlate with the sedimentation velocity. The sedimentation velocity was increased by an over 5 $m\ h^{-1}$.

The measurement taken with the addition of $20\ mL$ of flocculant was considered as sufficiently clear with larger flocks, some even of sizes over 1 mm. The liquid medium was also clearer, and the sedimentation has reached velocity of $18\ m\ h^{-1}$.

Figure 17-PWG 54- 17.5 mL

Figure 18-PWG 54- 20 mL

Flocculant CWE 35

The amount of CWE 35 flocculant added was starting at *10 mL* flocculation occurred slightly at *20 mL* but the clarity of the medium was not sufficient as was not the settling time.

Therefore, only two further measurements of *25 mL* and *30 mL* of added CWE 35 were done.

Table 8-Results for average sedimentation with addition of CWE 35

Amount of flocculant added $[mL]$	**Average sedimentation velocity** $\left[\frac{m}{h}\right]$
20	Not sufficient time frame
25	1.6
30	2.7

Figure 19- CWE- 30 mL

Figure 20- CWE 35- 25 mL

Figure 21- Microalgae at the surface and walls of the measuring cylinder

Comparison of results

The addition of 16.5 mL PWG 54 and 30 mL CWE 35 have had comparable results in terms of sedimentation velocity of the flocs. Yet, the appearance of them is varying when the clarity of the water is taken into consideration. If the medium treated by CWE 35 was to be filtered out, even the smaller flocs might have been captured but the water medium would not be clear as when using the 16,5mL of PWG 54.

The CWE 35 of 30 mL and PWG 54 of 16,5 mL have had similar/comparable sedimentation velocities. Therefore, the assumption of a better effectiveness of PWG 54 can be expressed if the amount of added flocculant is of concern.

In the experiments a slight microalgae flotation and has occurred to at least a certain extent in all of the trials and it has not changed even after fully finishing the sedimentation process. It can be seen in *figure 21* how some of the flocs have moved upwards to the surface of the water medium or has attached to the walls of the measuring cylinder.

Design of technology

Based on the research and experiments done in the previous parts of this thesis the following design of the sedimentation technologies was calculated. The parameters chosen in the designing process follow an industrial scale. The suspension flow rate was chosen to be 10 $m^3\ h^{-1}$. Other parameters that were to be chosen for the calculations were based on either the existing technology parameters or the research papers published containing suitable or possible parameters.

Table 9-Design of separation technology for sedimentation velocity (without flocculants)

Separation technology	**Parameter**	Sedimentation velocity $[\boldsymbol{mm\ h^{-1}}]$	
		22.7	32.5
Circular settler	R_1 [m]	0.05	0.05
	R_2 [m]	12	10
Lamella settler	L [m]	2	2
	W [m]	2.5	2.5
	i [-]	180	130
Decanter centrifuge	r_1 [m]	0.1	
	r_2 [m]	0.2	
	H [m]	0.6	
	n [rpm]	5000	

Table 10-Design of separation technology for sedimentation velocity (with flocculants)

Separation technology	**Parameter**	Sedimentation velocity $[\boldsymbol{m\ h^{-1}}]$					
		1.6	2.7	3.0	4.8	10.3	18.0
Circular settler	R_1 [m]	0.05	0.05	0.05	0.05	0.05	0.05
	R_2 [m]	1.5	1.1	1.1	0.9	0.6	0.5
Lamella settler	L [m]	0.25	0.25	0.25	0.25	0.25	0.25
	W [m]	0.25	0.25	0.25	0.25	0.25	0.25
	i [-]	200	120	110	70	34	20
Decanter centrifuge	r_1 [m]	0.1	0.1	0.1	0.1	0.1	0.1
	r_2 [m]	0.2	0.2	0.2	0.2	0.2	0.2
	H [m]	0.04	0.06	0.03	0.06	0.03	0.02
	n [rpm]	2500	2500	2500	1000	1000	1000

Conclusion

The research has shown that the use of flocculants has been an important area of further experimental procedure in the microalgal harvesting and dewatering.

The results of settling by the means of natural gravitation sedimentation and gravitational sedimentation with addition of flocculants were clearly showing the enhanced effectiveness of flocculation on the settling velocity.

The use of two types of flocculants were proven to be functioning. In the case of dosage not exceeding 15 mL of flocculant added per litre of culture medium, it can be stated that the PWG 54 has a more effective function in both the size of flocs formed as well as clarity of the separated liquid medium.

The parameters considered in the parameter design of the technology, namely a circular and lamella settler and a decanter centrifuge, followed an industrial scale, with suspension flow rate $10\ m^3\ h^{-1}$.

The circular settler design show that the calculated parameter of outer radius is significantly smaller for the flocculated algae. Because the only deciding parameter that changes the design is the sedimentation velocity.

The design of lamella settler is using almost hundredfold larger lamella area for unflocculated microalgae settling. The drawback in case of separating the flocculated microalgae with the lamella settler comes in the form of the fouling of the lamella plates. Nevertheless, the lamella settler could potentially be seen as the best option of technology for separation of flocculated microalgae if the main objective is to keep it simple and inexpensive but also increase the efficiency of the separation process.

The decanter centrifuge parametrization has shown that due to the significant increase in settling in the flocculated particles, this can be a positive aspect. The parameters such as speed of rotation and length of the bowl can be adjusted to ensure sufficient function.

Further work on the economic aspects and the effect of flocculation on the microalgal harvesting could be carried out to provide a better overview regarding the feasibility.

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