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Harvesting of microalgae: overview of process options and their strengths and drawbacks

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(NON-PRINTS ITEMS)

Abstract– Microalgae harvesting is a major challenge because microalgal cells are small and carry a negative surface charge, and biomass concentration in cultures is relatively low. The microalgal biomass (0.05% w/w) needs to be concentrated to a paste with 15-25% water content. This dewatering process is ideally performed in two stages, including a first pre-concentration step in combination with a second dewatering step. Microalgae are a very heterogenous group of organisms differing in size and shape and culture conditions. Applications of microalgal biomass range from low-value (biofuels) to high-value applications (nutraceuticals). It is therefore likely that the optimal harvesting technology differs between species, culture conditions or the final application of the biomass. Harvesting should not cause contamination of the biomass or influence biomass quality. Finally, water recycling to reduce the water footprint is an important aspect to include into the harvesting process. This chapter gives an overview of several harvesting process options with the focus on their strengths and highlighting the aforementioned aspects.

Keywords - dewatering, coagulation, flocculation, filtration, membrane, centrifugation, flotation

(CHAPTER STARTS HERE)

6.1 INTRODUCTION

Microalgae have attracted in the past decade a lot of interest in various industrial applications ranging from biofuels over wastewater treatment to the production of high-value natural products such as pigments or nutritional supplements (e.g. Chisti, 2008; Mata et al., 2010; Park et al., 2011). Nevertheless, production of

microalgae is still limited to about 10 to 20 thousand ton dry matter per year and the cost of production remains too high for applications such as energy production, feed production or wastewater treatment (Benemann, 2013). One of the main factors that limits the large-scale production of microalgae is the challenge of low-cost biomass harvesting (Brennan and Owende, 2010; Molina Grima et al., 2003; Uduman et al., 2010; Vandamme et al., 2013).

Because microalgae are cultivated as suspension in a liquid medium at a concentration of about 0.5 g L^{-1} or 0.05 %, harvesting microalgal biomass requires efficient solid-liquid separation technologies. Because solid-liquid separation is an important unit operation in many production processes, a wide range of technologies are available. These can be divided into two broad categories: methods where particles are separated from the liquid phase based on gravity or buoyancy (sedimentation, flotation, centrifugation) versus methods where the particles are removed from the liquid mechanically by means of a filter or screen. The use of both gravity-based and filtration-based methods is challenging because of the small size of microalgae cells (5 - 20 µm). Flocculation might be combined with gravity- or filtration-based harvesting technologies to aggregate individual microalgal cells into larger particles and thus facilitate separation. Centrifugation is generally the preferred harvesting method in commercial microalgae facilities that target high-value products (Molina Grima et al. 2003). This is a very convenient technology, but it is very costly and energy-demanding, requiring up to 1 MJ kg^{-1} of dry biomass (Milledge and Heaven, 2012). Several other technologies have been proposed for harvesting microalgae that have a lower cost and energy demand. The aim of this book chapter is to give an overview of all technologies available for harvesting microalgae, and to discuss their advantages and drawbacks.

6.2 REQUIREMENTS FOR AN EFFECTIVE MICROALGAE HARVESTING TECHNOLOGY

Production of microalgae has similarities to fermentation as well as agriculture. As in fermentation, microalgae are microorganisms that are cultivated in a liquid medium. Similar to agricultural crops, microalgae require light to grow. The dependence of microalgae on light results in lower biomass concentrations in microalgae production than in fermentation: as microalgal biomass concentration increases in the culture, growth is reduced because of mutual self-shading of the cells. In the open raceway ponds that are today typically used for microalgae production, biomass concentrations are about 0.5 g L^{-1} (Benemann, 2013). This is much lower than in cultures of bacteria, yeasts or even heterotrophic microalgae (e.g. *Schizochytrium* or *Crypthecodinium*), where a biomass concentration of 100 g L^{-1} can be achieved (Bunch, 1994; Ganuza et al., 2008). As a consequence, in phototrophic production of microalgae, a much larger volume of culture broth needs to be processed to generate the same amount of biomass as in

heterotrophic production of microorganisms. Any technology for harvesting microalgae should therefore be capable of processing large volumes of culture at a minimal cost and with a minimal energy demand. During harvesting, the culture broth needs to be thickened into a paste with a dry matter content of about 20% or 200 g L^{-1}. As the biomass concentration in the culture broth is generally only about 0.5 g L^{-1}, this requires a 400 times up-concentration. The best way to achieve this is by using a combination of two or even more technologies rather than using a single technology (Pahl et al., 2013; Uduman et al., 2010). A distinction can be made between harvesting and dewatering, where harvesting refers to concentration of a culture with a 0.05 % dry matter content to a slurry with a 1 - 5 % dry matter content, and dewatering to the further concentration of that slurry to an algal cake with a 15 - 25 % dry matter content (Shelef et al., 1984; Uduman et al., 2010).

Microalgae are a heterogenous group of microorganisms that comprises representatives from different evolutionary lines of eukaryotes. As a result, they strongly differ in size, shape, cell rigidity and cell surface properties (e.g. charge, hydrophobicity) (Eldridge et al., 2012). Moreover, microalgae excrete organic matter in the culture medium and the quantity of organic matter that is excreted and its properties also differ between species (R. K. Henderson et al., 2008). The cellular properties of microalgae and the organic matter they excrete in the medium have an important influence on the harvesting of the biomass (e.g. Y.-S. Cheng et al., 2011; Vandamme et al., 2016). These properties of the microalgal cells and the organic matter they excrete in their medium are often influenced by culture conditions. Stationary culture conditions induced by nutrient stress, for instance, can influence the size of microalgal cells (e.g. Fabregas et al., 1985), cell surface properties (e.g. Zhang et al., 2012) or the excretion of organic matter (e.g. Myklestad, 1995), properties that influence harvesting. As a result, it may be necessary to adjust the harvesting strategy to the species of microalgae that is produced, or even to the conditions under which a species is cultured.

The technologies used for harvesting microalgae should not interfere with the final use of the biomass. The microalgal biomass may be used without further processing for a single application (e.g. animal feed, food or a nutritional supplement), or a single product may be extracted from the biomass (e.g. lipids, pigments) while the remaining fraction is a waste product. Opinions today, however, converge on the biorefinery concept, in which the value of the biomass is maximised and waste is minimized by refining the biomass into different components that are used for various applications ranging from energy to animal feed, food or fine chemicals (Gouveia, 2014; Wijffels and Barbosa, 2010). Particularly when the biomass or biomass fractions are used for human or animal consumption, it is important that it is not modified or become contaminated during the harvesting process. Any form of contamination by chemical additives used for harvesting should be avoided, particularly if the chemical additives would trigger alteration of the biomass.

Production of microalgae also requires very large volumes of water to prepare the culture medium. Most of this water, however, can be recycled after harvesting (Yang et al., 2011). To reduce the water footprint for microalgae biomass production, it is important that the water or spent culture broth is recycled after harvesting. The harvesting technologies that are used should therefore not interfere with the recycling of the culture medium, e.g. by causing contamination of the culture medium. Microalgal cells that escape harvesting may end up in the recycle flow of the culture medium and may be returned to the culture broth. This might lead to selection of those cells that cannot be harvested, e.g. cells that are small enough to pass through a filter or screen, or cells with specific surface properties that avoid flocculation. This selective pressure may result in a gradual decrease in the harvesting efficiency, particularly when microalgae are produced using a continuous culture method (Bull and Collins, 2012). Therefore, it is important that a harvesting method achieves a high recovery efficiency, or that the spent medium is treated after harvesting to remove residual cells before recycling.

6.3 FLOCCULATION

6.3.1 THE POTENTIAL OF FLOCCULATION TO FACILITATE HARVESTING OF MICROALGAE

During flocculation, numerous individual microalgal cells are aggregated into larger particles called flocs. These flocs can be more easily separated from the culture broth than the individual cells, and this is the case for both gravity-based and filtration-based separation. Spontaneous aggregation of microalgal cells in suspension is prevented by the negative surface charge of the cells. This surface charge is generated by the presence of charged groups on the cell surface, mainly carboxylic acid groups. Because the pK_a of carboxylic acids is about 4 - 5, microalgal cell surfaces are negatively charged down to a pH of about 4 - 5 (Brady et al., 2014; Hadjoudja et al., 2010). The negative surface charge attracts positive ions dissolved in the medium and this results in the formation of a cloud of counterions around the cells. These counterions cause an electrostatic repulsion between the cells. Flocculation can be induced by removing or overcoming this repulsive force. This can be done by addition of chemicals that either neutralize the negative charge on the cell surface (either entirely or in patches), or bind to the surface of multiple cells and form bridges between these cells (Bratby, 2006). Once the electrostatic repulsion between the particles has been offset and the cells can approach each other, they are mutually attracted and held together by Van der Waals forces. An overview of the most common flocculation methods that have been used for harvesting microalgae is given below.

Flocculation is a widely used technology in different fields of industry such as drinking water production and wastewater treatment from mining. Despite the fact that many off-the-shelf technologies are available, methods that have been successfully used in other industries cannot simply be transferred to microalgae harvesting. The disadvantage of flocculation is that it usually requires the addition of chemicals. In most industrial applications of flocculation, these chemicals end up in the waste sludge that is produced and they are disposed of with this waste. In microalgae harvesting, on the contrary, most of these chemicals end up in the harvested biomass and contaminate the biomass. Therefore, the potential toxicity of the chemicals used for flocculation is critical in microalgae harvesting, much more so than in other industries. The economics are also very different. When flocculation is used to remove impurities from water, the cost is related to the volume of water that is processed rather than the quantity of impurities that is removed. In the case of microalgae harvesting, on the contrary, the cost is related to the amount of biomass that is harvested, and not to the volume of culture broth that is processed.

6.3.2 METAL SALTS

Metal salts like aluminum sulphate or ferric chloride are widely used flocculants in different industries. When dissolved in water, the iron or aluminum ions hydrolyse to form positively charged hydroxides that cause flocculation by neutralizing the negative surface charge of particles (Bratby, 2006). At higher dosages, the metal hydroxides form a precipitate that enmeshes particles in suspension and causes them to settle. Flocculation by metal hydroxides has been intensively studied (e.g. Duan and Gregory, 2003). The use of metal salt flocculants in practice, however, has as a disadvantage because it results in contamination of the harvested biomass with relatively high concentrations of metals (Şirin et al., 2012). The counterions of the metals remain in the medium and can interfere with the recycling of the culture medium. Moreover, the dosages that are required are often quite high and pH adjustment is often needed for the coagulants to work properly, which could also involve a significant cost (Garzon-Sanabria et al., 2012). Nevertheless, metal salts are useful as a model system to understand the fundamental mechanisms of flocculation in microalgae (e.g. Wyatt et al., 2012). Autoflocculation and electro-flocculation are two flocculation methods that function in a somewhat similar way as metal salt flocculants but that have fewer disadvantages (see below).

6.3.3 AUTOFLOCCULATION

Autoflocculation is a spontaneous flocculation of microalgae that occurs when the pH of the culture medium increases. Autoflocculation is a somewhat misleading

terminology as microalgae do not flocculate by themselves at high pH, but flocculation is rather induced by the precipitation of chemicals which is pH-dependent (González-Fernández and Ballesteros, 2013). In microalgal cultures, the pH often rises above 8-9 as a result of photosynthetic depletion of CO_2. At such alkaline pH levels, Ca can precipitate as calcium phosphates or calcium carbonates and Mg can precipitate as magnesium hydroxide or brucite (Brady et al., 2014). This mechanism does not only depend on the pH but also on the concentration of calcium, magnesium and other ions in solution (e.g. Smith and Davis, 2012). In some conditions, flocculation can occur spontaneously as a result of photosynthetically induced increase in pH (Spilling et al., 2011), in other cases an artificial increase in pH by addition of bases is required (Vandamme et al., 2012). These precipitates can carry positive charges and cause flocculation by neutralizing the surface charge of microalgal cells or by a sweeping flocculation mechanism. At least in the case of magnesium hydroxide, the flocculation mechanism is probably very similar to that induced by Fe or Al hydroxides (García-Pérez et al., 2014). These calcium or magnesium precipitates are not as toxic as metals and thus cause fewer problems with contamination of the biomass. They can even be removed from the biomass after harvesting by dissolving them by mild acidification (Beuckels et al., 2013; Vandamme et al., 2015).

It is interesting to mention that flocculation of microalgae can also be induced by a decrease in pH. When the pH of the culture medium is reduced down about 4, the carboxylic acids on the microalgal cell surface are protonated, the surface charge of microalgal cells becomes neutral and flocculation occurs (Liu et al., 2013).

6.3.4 ELECTRO-COAGULATION

Electro-coagulation (EC) has been referred as one of the most effective method for reducing harvesting costs, as it avoids the use of flocculants, uses small electricity amounts, is very fast and efficient (Poelman et al., 1997; Matos et al., 2013; Pacheco et al., 2015). EC has been widely used for the treatment of wastewater (Camcioglu et al., 2014; Bukhari, 2008) and to improve the quality of the drinking water (Alfafara et al., 2002; Poelman et al., 1997). Publications show its efficiency for the removal of small colloidal particles, dyes (Alinsafi et al., 2005), total suspended solids, chemical oxygen demand and turbidity (Inan et al., 2004). For microalgae biomass harvesting from its culture medium, only a few studies have been published (e.g., Xu et al., 2010; Vandamme et al., 2011; Uduman et al., 2011; Matos et al., 2013; Pacheco et al., 2015). In all studies an efficient separation technology, requiring low energy, no flocculant addition and resulting in nor (or a little) secondary contamination of the biomass recovered has been validated.

The EC process is safe, selective, versatile, environmentally sound and cost effective (e.g., Alinsafi et al., 2005).

During EC, an electrical current is applied through two reactive electrodes (e.g. aluminium or iron electrodes) or through non sacrificial electrodes (Misra et al., 2015; Guldhe et al., 2015), submerged in the microalgae suspension. The anode electrode suffers an electrolytic oxidation producing metal ions that will serve as coagulant agents for the formation of microalga flocs. Furthermore, oxygen and hydrogen microbubbles are generated due to the water oxidation and reduction (Vandamme et al., 2011; Uduman et al., 2011). Both processes combined, allow for the aggregation of cells and easy separation from the culture medium by flotation to the top. However after a certain amount of time, the aggregated cells will drop to the bottom because of their weight.

The recovery efficiency of EC and the saving of energy of EC vs Centrifugation can be depicted from Table 6.1 for some microalgae species.

‘TABLE 6.1 HERE’

Table 6.1. Recovery efficiency and energy savings of electro-coagulation versus centrifugation reported for various algae species

Algae	**Recovery efficiency (%)**	**Energy save (%) (vs centrifugation)**
Nannochloropsis (marine)	97	92
Spirulina	90 (pH<6)	N.A.*
	88 (pH=6)	N.A.
Phaeodactylum tricornutum	78	98
Chlorella vulgaris (fresh water)	91	87
Scenedesmus	99	94.2
Spirogyra	97	90
Synechochystis	85	N.A.
Neochloris oleabundans	96	N.A.

*N.A.= Not available

Nonetheless, the aluminium/iron content could generate some toxic effects on the biomass, depending on the current density and operation time. Vandamme et al. (2011) reported 1.5% of aluminium in the biomass after EC treatment with a current density of 3 mA cm^{-2} over 10 min, while Matos et al. (2013) found a release of 0.56% and 1.39% when current density was 3.3 and 8.3 mA cm^{-2}, respectively, over 10 min. The electrode depletion not only increases the cost of harvesting but also affects the quality of the recovered biomass. To overcome this limitation, the application of non sacrificial carbon electrodes was suggested by Misra et al. (2015) and Guldhe et al. (2015), by adjusting applied current, pH and the addition of an electrolyte (sodium chloride).

The energy consumption of EC using non-sacrificial electrodes, claimed by Misra et al. (2015) was 3.384 kWh kg^{-1}, and by Guldhe et al. (2015) 1.76 kWh kg^{-1}, which is lower than other conventional harvesting processes like centrifugation (65.35 kWh kg^{-1}) (Guldhe et al., 2015), chemical flocculation (36.81 kWh kg^{-1}), and filtration (3.58 kWh kg^{-1}) (Danquah et al., 2009; Vandamme et al., 2011).

6.3.5 BIOPOLYMER FLOCCULANTS

Polymer flocculants are polymers with charged functional groups. Polymer flocculants can induce flocculation by neutralising the surface charge of particles or by forming bridges between individual particles. The functional groups should ideally be positively charged to allow for interactions with the negatively charged microalgal cells. Polymers are generally very effective at low dosages. In wastewater treatment, polyacrylamide-based flocculants are commonly used. Because they can contain potentially toxic acrylamide residues, flocculants based on natural biopolymers are preferred over synthetic polymers. An effective biopolymer flocculant for harvesting microalgae is chitosan, which is prepared by deacetylation of chitin. However, the cost of chitosan is relatively high due to its use in medical applications. Cheaper alternatives are cationic starch or tanfloc, which are respectively starch and tannins functionalized with quaternary ammonium groups (Roselet et al., 2016; Vandamme et al., 2010). Important factors that influence the effectiveness of polymer flocculants are the molecular weight of the polymer, the number of functional groups (the charge density) and the charge of the functional groups (Garzon-Sanabria et al., 2012; Roselet et al., 2015).

A disadvantage of polymer flocculants is that they often perform poorly when used for harvesting marine microalgae (Bilanovic et al., 1988). This is due to the high ionic strength of the seawater medium, which causes coiling of the polymers and a decrease in their effective size. This problem does not always occur and sometimes polymer flocculants can be effective in seawater (‘t Lam et al., 2014). An alternative may be to use more rigid molecules such as tannin-based flocculants (Roselet et al., 2016) or flocculants based on functionalized nanoparticles, such as nanocellulose (Eyley et al., 2015).

Polymers can be combined with magnetoresponsive Fe_3O_4 nanoparticles to separate the flocculated microalgae from the medium in a magnetic field (Lee et al., 2013; Lim et al., 2012; Xu et al., 2011). This magnetic separation is a faster method to separate the flocs from the medium than gravity sedimentation or flotation. When polymers are used to reversibly interact with the microalgal cells (e.g. through pH-responsive charges), the nanoparticles can be recovered from the harvested biomass and re-used (Xu et al., 2011).

6.3.6 BIOFLOCCULATION

Bioflocculation is the phenomenon where microalgae flocculate spontaneously or where flocculation is induced by the presence of other microorganisms. Some microalgae tend to flocculate spontaneously, such as for example *Ettlia texensis* or *Pediastrum* species (Park et al., 2015; Salim et al., 2014). The mechanisms that are responsible for this phenomenon are often not clearly understood. Mixing of such

bioflocculating microalgae with non-flocculating microalgae can be used to harvest these other microalgae (Salim et al., 2012). Bio-flocculation can also be induced by addition of other microorganisms. Some bacteria produce extracellular polymers that can induce flocculation of microalgae (e.g. Oh et al., 2001). Filamentous fungi appear to be quite effective in inducing flocculation of microalgae (e.g. Zhou et al., 2013). Bioflocculation often occurs spontaneously in high rate algal ponds used for wastewater treatment. Such ponds are colonized by complex communities of microalgae and bacteria and interactions between species of microalgae or between microalgae and bacteria result in bioflocculation, although the mechanisms are often not clearly understood (Posadas et al., 2014; Van Den Hende et al., 2011). Bioflocculation is therefore a promising simple flocculation method in microalgae-bacteria wastewater treatment systems (Craggs et al., 2012).

6.4 GRAVITY-BASED TECHNOLOGIES

6.4.1 GRAVITY SETTLING

Harvesting by gravity sedimentation is a very attractive option because it requires very little energy and relatively low-cost infrastructure. Sedimentation can be carried out in simple settling tanks, but these usually have a relatively large footprint (Figure 6.1, A). Inclined settlers or lamella separators consist of a series of stacked plates, which increases the effective area available for settling and reduces the footprint (Figure 6.1, B). Gravity sedimentation is a relatively slow process and this may result in deterioration of the biomass quality during harvesting. Gravity sedimentation also generates a rather dilute slurry and should therefore be primarily used as an initial concentration mechanism to pre-concentrate the biomass prior to complete dewatering using another technology such as centrifugation or filtration.

The settling rate is a critical parameter to harvest microalgae using gravity sedimentation. Stokes' law dictates that the settling rate increases with the square of the size of the microalgae and their difference in density with the medium. Because most microalgae are small (< 20 µm) and have a density that is very close to that of water, they have a very low intrinsic settling velocity of about 1 cm h^{-1}. This is too low to concentrate the biomass using conventional gravitational settlers. The exception are relatively large microalgae with a high specific density, or microalgae that form aggregates. For instance, Algatech (Israel) used gravity sedimentation to harvest astaxanthin-rich *Haematococcus* cysts (oral communication, Algatech). Because the process was too slow and resulted in a deterioration of biomass quality, however, they later switched to centrifugation as a harvesting method. Gravity sedimentation can also be used to harvest *Arthrospira* filaments that have accumulated glycogen. Because glycogen has a high specific density (about 1.5 g g^{-1}) and *Arthrospira* can accumulate large

amounts of it (> 50% under nitrogen limited conditions), such filaments can have a settling rate of 64 cm h^{-1} and can theoretically be harvested by gravity sedimentation (Depraetere et al., 2015). Because the microalgae *Scenedesmus* forms natural aggregates, it can also be harvested by gravity sedimentation (Wang et al., 2014). Smith and Davis (2013) showed that it may also be feasible to concentrate microalgae with a low settling rate using inclined settlers if these have a low angle and a high aspect ratio.

Gravity sedimentation is the straightforward method of solid-liquid separation to be used in combination with flocculation. Flocculation should ideally result in large flocs that settle fast generate a compact sludge (Smith and Davis, 2012; Vandamme et al., 2014). A high settling rate is essential to have a fast harvesting procedure and to avoid deterioration of the biomass. A compact sludge allows for a significant up-concentration of the biomass to a dense slurry that can be further dewatered using centrifugation or filtration. Although there have been many studies on flocculation of microalgae, relatively few have evaluated to the potential to concentrate the flocculated microalgae using gravity sedimentation, either in simple settling tanks or using inclined settlers. Most research so far has been carried out using bio-flocculated microalgae harvested from high rate algal ponds (Craggs et al., 2012; Nurdogan and Oswald, 1996).

'FIGURE 6.1 HERE'

Figure 6.1. Principles of separation of flocculated microalgae from the clarified culture medium using a settling basin (A), a lamella separator (B) and dissolved air flotation (C).

6.4.2 CENTRIFUGATION

Centrifugation can be considered as a method for enhanced settling. It is the most widely used harvesting technology in production facilities that produce microalgae for high-value products. Different types of industrial centrifuges are available. A disc bowl centrifuge is used for suspension with a low solids content (from 0.01% to 20 % algal dry weight) while a decanter centrifuge is used for suspension with a higher solids content (from 10% to 50% algal dry weight) (Milledge and Heaven, 2012). Both can be operated continuously. Harvesting by centrifugation has many advantages: there is no need to add chemicals during the process, a high dry matter content can be achieved in a single-step process and it is fast and thus avoids deterioration of biomass quality. The disadvantage is that the investment cost for large centrifuges is very high and that centrifuges have a high energy demand. Some approaches have been proposed to reduce the energy demand of centrifugation. Evodos (The Netherlands) have designed a centrifuge with spiral plates which reduces the distance travelled by a settling microalgae and increases the surface area. Significant energy savings can be achieved by increasing the flow rate through the centrifuge. Although this results in a lower harvesting efficiency, the energy consumption per unit of biomass harvested is significantly reduced (Dassey and Theegala, 2013).

Although centrifugation should be replaced by more energy-efficient harvesting methods, particularly when microalgae are produced for low-value applications, it will probably continue to play an important role in the final dewatering of microalgal slurries that are produced after primary concentration using flocculation/sedimentation, flocculation/flotation or membrane filtration because centrifugation can provide a thick paste with a high dry matter content (Shelef et al., 1984). Preconcentration of the biomass results in a reduction in the volume of culture that needs to be processed by at least an order of magnitude and, as a result, the energy demand for centrifugation will also be much lower. Moreover, because flocculation results in an increase in particle size, the centrifugal forces required to separate the biomass from the medium are much lower than for individual cells.

6.4.3 FLOTATION

In flotation, small air bubbles interact with microalgal and carry the cells to the water surface, where they form a scum that can be skimmed off. In dissolved air flotation, the air bubbles are formed by mixing water that is supersaturated with air into the culture (Figure 6.1, C). It uses a return flow of clarified water that is mixed with air under high pressure. In dispersed air flotation, air bubbles are formed by releasing pressurised air through a nozzle or through porous media. Dissolved air flotation tends to perform better than dispersed air flotation because the bubbles that are formed are smaller, but the energy demand is also higher. Electrolysis can be used to generate hydrogen bubbles for flotation. This latter approach works better for marine than freshwater microalgae due to the higher electrical conductivity of seawater (Sandbank and Shelef, 1987).

Flotation depends on the interaction between microalgal cells and air bubbles. Because both air bubbles and microalgal cells are negatively charged, they do not interact and flotation generally does not work well for harvesting microalgae unless additives are used (Garg et al., 2012). Flotation can be improved by the addition of surfactants (Coward et al., 2013; Rita K. Henderson et al., 2008). In dispersed ozone flotation, ozone is used as a carrier gas rather than air. It is believed that ozone modifies the microalgal cell wall and reduces the charge, resulting in better adhesion of microalgal cells to gas bubbles (Y.-L. Cheng et al., 2011). Flotation is often used in combination with flocculation (Besson and Guiraud, 2013; Kwon et al., 2014). The use of flotation to concentrate flocculated microalgal suspensions has some advantages over gravity sedimentation. High biomass concentrations can be achieved in the froth that is skimmed from the surface. Moreover, flotation is a much faster separation method than gravity sedimentation, and therefore there is a lower risk of deterioration of biomass quality during harvesting.

6.5 FILTRATION-BASED SEPARATION TECHNOLOGIES

Filtration refers to a mechanical or physical process to separate solids from water or gases (fluids) by interposing a permeable separator, like screens, filter cloths or permeable membranes, which retains solids. The driving force of active filtration is a pressure drop across the barrier created via vacuum, pressure or gravity. Shelef et al. (1984) described multiple filtration devices including pressure filters (like plate-and-frame press) and vacuum filters (like leaf or Moor filters, Nutsche filters, belt filters, rotary filters), cartridge filters, deep-bed filtration as well as cross-flow filtration. Other approaches include submerged membrane filtration, rotating disks, vibrating/rotating membranes and passive filtration via osmosis (Mo et al., 2015). By altering the characteristics of the permeable separator different types of solids are retained, while others pass with the fluid through the permeable barrier into the filtrate. Most commonly, the pore sizes of the separator are used to create a cut-off based on particle size or molecular weight (Drexler and Yeh, 2014). For membrane filters a distinction can be made between macro-filtration (pore size > 10 µm), micro-filtration (0.1-10 µm) and ultra-filtration (0.001-0.10 µm). Further, functionalized separators have been reported (Mustafa et al., 2014) that also enable fractionation based on for instance surface charge and hydrophobicity.

Filtration is attractive for algae harvesting because of its recovery efficiencies, its ability to separate shear sensitive species, and separation without addition of chemicals avoiding contamination of the biomass and allowing reuse of the permeate (Al Hattab et al., 2015; Barros et al., 2015; Mo et al., 2015). On the other hand, points of attention can be clogging and fouling, cleaning processes for the membranes and investment costs for membranes and pumps. A lack of knowledge of the most relevant operating conditions has been reported. Concerning algae harvesting, filtration can be used for different purposes including algae harvesting, dewatering, and water recirculation.

3.5.1. SCREENING OF LARGER SIZED MICRO-ALGAE

Despite the fact that screening is a solid-solid separation method (Shelef et al. 1984), it can be used for harvesting larger sized algae like Cyanobacteria *Spirulina* (1-10 µm) that form filaments (100-200 µm in length; 10-20 µm in diameter). Especially the intertwined filaments can be harvested on the spot using screens. *Aphanizomenon flos-aqua* is another member of the Cyanobacteria that grows in natural lakes. The principle as screening is introducing particles of a given size. Carmichael et al. (2000) reported the use of screens made of nylon for harvesting

these algae from surface water. Vertical debris screens (0.6 to 1.2 cm in mesh size) were used upstream of the harvesting area to retain fish and flotsam, followed by multiple sets (up to 48) of alga collecting screens. The latter were also made of nylon (20m^2) and were position nearly horizontally. Water passed through the screens while the algae remained on top of the screens. Algae were removed from the screens using water sprays.

3.5.2. HARVESTING OF ALGAE VIA MEMBRANE FILTRATION

Although macro-filtration is suitable for large microalgae cells or flocculated algal biomass (Al Hattab et al., 2015), micro- and ultrafiltration are the most commonly studied membrane types for microalgae filtration. Both are able to retain the microalgae by nearly 100% (Drexler and Yeh, 2014). While microfiltration generally allows higher fluxes at short term, ultrafiltration has been reported to perform better at longer term due to higher fouling resistance (Baerdemaeker et al., 2013; Danquah et al., 2009; Rossignol et al., 1999). The capacity of a filtration unit is mainly determined by the fluxes that can be achieved and the amount of algae biomass that can be retained. Fluxes are expressed as volume of permeate per unit of time per unit membrane surface (L h^{-1} m^{-2}) and are influenced by algae species, cell density of algae, cell integrity, transmembrane pressure, cross-flow velocity, membrane type, pore size, and composition of the medium (Al Hattab et al., 2015; Mo et al., 2015). Fouling negatively influences flux-values and is due to membrane/solutes interactions and cake formation. Parameters like membrane pore size, particles size, surface charge, hydrophobicity of the membrane and the composition of the medium highly influence fouling (Drexler and Yeh, 2014; Rossignol et al., 1999). Extracellular polymeric substances (EPS) like polysaccharides and intracellular products released after cell disruption contribute to fouling. Rickman et al. (2012) reported the importance of submicron particles as primary foulants. Fouling can be partially controlled by generating turbulent flow near the membrane surface, bubbling, backwashing or chemical cleaning (Drexler and Yeh, 2014), but irreversible fouling may occur especially with hydrophobic membranes and/or hydrophobic EPS (Mustafa et al., 2014; Rossignol et al., 1999). In terms of energy use, Mo et al (2015) concluded that membrane filtration (0.17-2 kWh m^{-3}) can be very competitive compared to alternative technologies.

‘FIGURE 6.2 HERE’

Figure 6.2 - Different membrane filtration configurations.

Figure 6.2 depicts a number of active filtration configurations that have different fluid and particles flows across/along the membrane. In case of **dead-end** or **conventional filtration** (Figure 6.2, A), the water flow is perpendicular to the filter and all particles are forced to settle on the filter surface. Particles can only be

removed by backwashing and/or replacement of the filter medium. Because of fouling, dead-end filtrations is mainly applied for filtering low concentrated solutions. Although effective for large microalgae cells (> 70µm) like *Coelastrum* and *Spirulina* species (Barros et al., 2015; Shelef et al., 1984), dead-end filtration is considered not economically viable for most microalgae harvesting purposes. **Cross-flow filtration** or **tangential flow filtration** (Figure 6.2, B) is less susceptible to fouling as, while the permeate passes through the filter, the feed solution flows parallel to the filter surface generating shear stress that reduces the filter cake thickness and keeps the algal biomass more in suspension. The cells in the retentate are kept in the system by recirculating the retentate across the membrane. According to data collected by Mo et al. (2015) from multiple studies with different algae species and diverse membranes types, for cross-flow filtration with an initial algae concentration of 0.04-2 g L^{-1}, fluxes varied between 13 and 150 L hm^{-2} and volume reduction factors of 5-154 were reported. Achievable final algae contents are 8.8-15.5%. By increasing the cross-flow velocity, fouling can be reduced enabling higher fluxes (Rossignol et al., 1999). On the other hand, shear forces induce stress in algae biomass and may result in a release of algogenic compounds and even cell disruption that increase fouling and economic losses. **Submerged membrane filtration** (Figure 6.2, C) refers to a more recent approach where membrane bags connected to a vacuum pump are placed directly in the algal culture (Mo et al., 2015). Backwashable flat screen membrane envelope loops, an integrated permeate channel (IPC) concept (Doyen et al., 2008), have been used as well as non-backwashable membranes (Baerdemaeker et al., 2013; Bilad et al., 2012) and magnetic vibrating of the membranes (Bilad et al., 2013). Depending on the initial algae density, volume reduction factors of 5 to 20 are reported (Mo et al. 2015) with a final algal concentration of 5-150 g L^{-1}. Critical fluxes range from 10 to 50 L h^{-1} m^{-2}. Passive filtration techniques like **forward osmosis** have also been evaluated for harvesting freshwater algae and are associated with a low energy cost. Water is drawn from the algae suspension by concentration gradients using seawater (Buckwalter et al., 2013).

Although considerable final algal concentrations can be achieved technically at small scale, membrane-based technologies may have their main merits as pre-concentration step at larger scale. As fluxes drastically decrease with higher cell densities (Rossignol et al. 1999), high energy input, long processing times and/or large membrane surface areas may be required to reach these cell densities. Alternatively, the major part of the water can be removed via membrane filtration as pre-concentration step (up to 2-7 %) followed by further concentration of the algae biomass (up to 20-25%) using other technologies including centrifugation (De Baerdemaker et al. 2013; Buckwalter et al. 2013; Bilad et al. 2012; 2013) and other filtration approaches. The latter includes the discontinuous but very reliable chamber press filtration (up to 22%) (Mo et al. 2015; Grima et al. 2003).

Microalgae cultivation is a water intensive process requiring in raceways 1000 L of water per kg of biomass (Guieysse et al., 2013) and up to 3360 L of water per L biodiesel (Farooq et al., 2015). Reduction of evaporation and implementation of water recycling can drastically reduce the water use (up to > 85%). Membrane filtration has not only been proven to be competitive for surface water and wastewater treatment before its use in algae cultivation systems, but also offers potential for recycling water after algae growth for reuse in the cultivation system (Drexler and Yeh, 2014). Membranes can remove turbidity and algal or bacterial contamination from the water, while leaving dissolved nutrients in solution for reuse. Different water recycling approaches can be distinguished. After the harvesting step, the algae-free medium can be polished and disinfected via microfiltration or ultrafiltration. Another approach is the integration of the water recycling step into a membrane based (pre-) harvesting step. During such a pre-concentration step, 80% and 90% of the water can be recycled with a volume reduction factor of 5 and 10, respectively. A techno-economic assessment study indicated that recycling of water in a raceway scenario reduced the salt and water use five-fold, and reduced the amount of wastewater, required energy and heat, leading to an overall reduction of CAPEX and OPEX by 4% and 41%, respectively (Thomassen et al., 2016).

6.6 CONCLUSIONS/SUMMARY/COMPARISON OF METHODS

Developing a low-cost and energy-efficient harvesting method remains one of the major challenges to achieve large-scale production of microalgae. During microalgal harvesting, the biomass needs to be upconcentrated 400 times from a dilute culture with a biomass concentration of 0.05% to a microalgal paste with a dry matter content of 15-25%. This is probably best done in a two stage process. One example could be membrane filtration to pre-concentrate the biomass combined with centrifugation to obtain an algal paste. Another example is flocculation combined with a lamella settler followed by dewatering of the sludge using a filter press. Because microalgae are a very heterogeneous group of organisms, it is likely that different species require a different approach for harvesting. Care should be taken that harvesting does not result in contamination (e.g. as a result of addition of a chemical flocculant) or damage (e.g. as a result of shear forces) to the biomass. The amount of contamination or damage that is acceptable depends on the final use of the biomass, and therefore the choice of the harvesting method will depend on the biomass application. Finally, to reduce the water footprint, it is also important that the harvesting method allows re-use of the spent culture medium.

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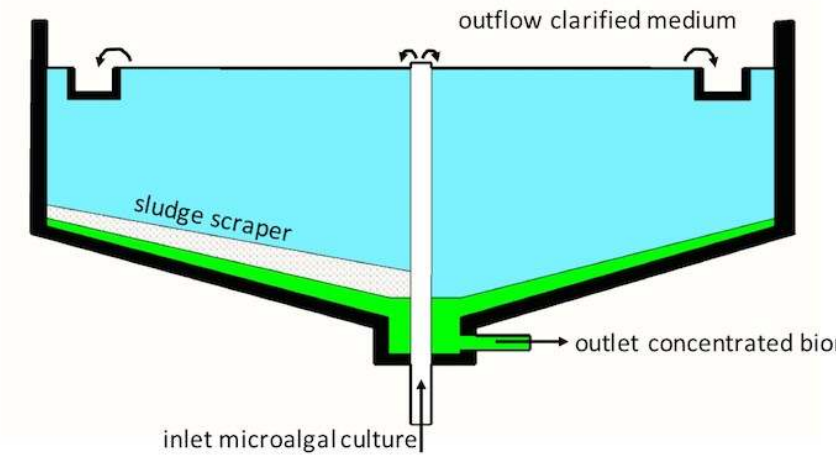
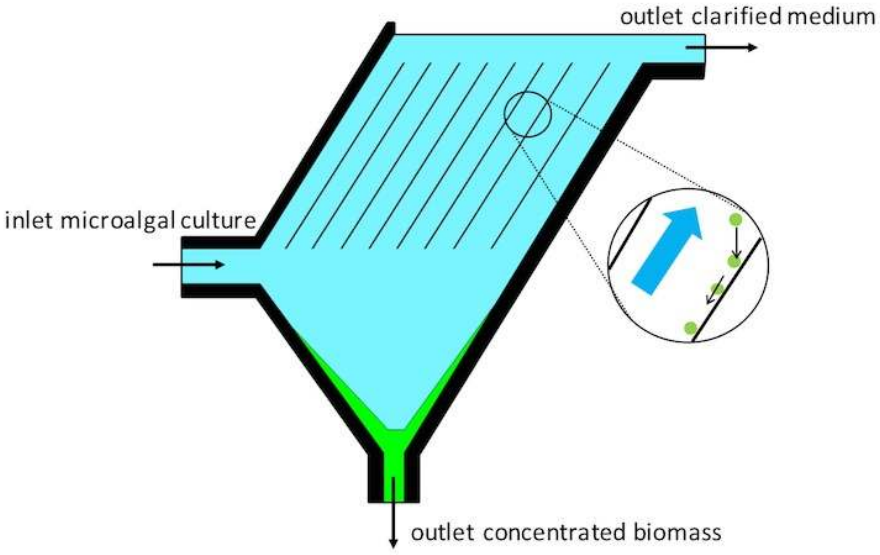
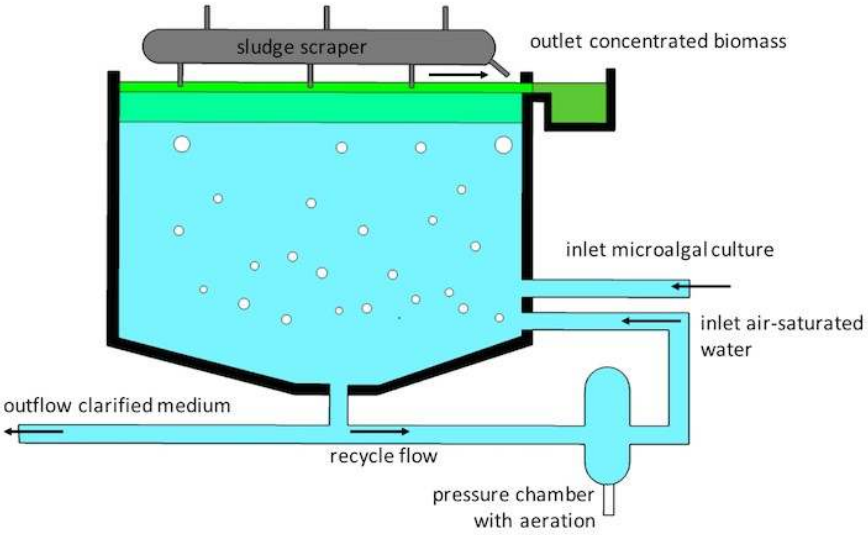
A

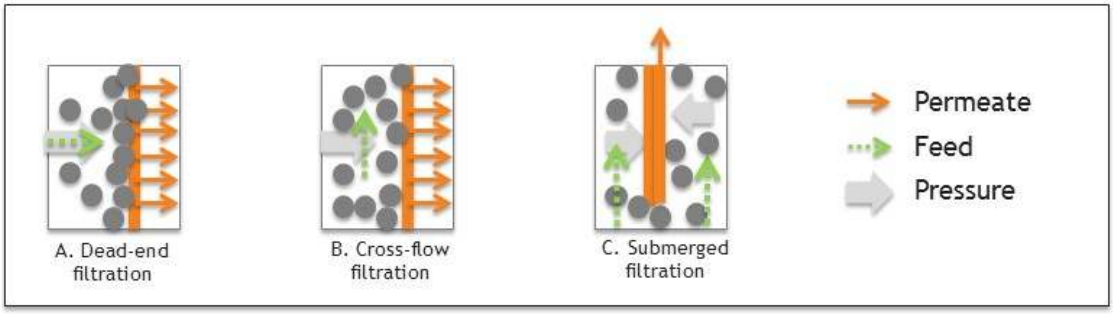

outflow clarified medium
sludge scraper
outlet concentrated biomass
inlet microalgal culture

B


outlet clarified medium
inlet microalgal culture
outlet concentrated biomass

C


sludge scraper
outlet concentrated biomass
inlet microalgal culture
inlet air-saturated water
outflow clarified medium
recycle flow
pressure chamber with aeration


A. Dead-end filtration
B. Cross-flow filtration
C. Submerged filtration
Permeate
Feed
Pressure