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Microalgae Harvesting Methods for Industrial Production of Biodiesel: Critical Review and Comparative Analysis

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Abstract

Microalgae biomass can be used to produce numerous value added products such as biodiesel, bioethanol, biogas and bio hydrogen, fish feed, animal feed, human food supplements and skin care products. Production of value added products from microalgae biomass requires growing and recovery of the algae biomass and extraction and downstream processing of the desired product. However, the major obstacle for using microalgae biomass on an industrial-scale for the production of biodiesel and other value added products is the dewatering step which accounts for 20-30% of the total costs associated with microalgae production and processing. The aim of this study was to review the current methods used for harvesting and concentrating microalgae and to perform a comparative analysis in order to determine the most efficient and economically viable dewatering methods for large scale processing of microalgae biomass. The harvesting techniques investigated included sedimentation, vacuum filtration, pressure filtration, cross flow filtration, disc stack centrifugation, decanter centrifuge, dispersed air floatation, dissolved air flotation, fluidic oscillation, inorganic flocculation, organic flocculation, auto-flocculation, bio-flocculation electrolytic coagulation, electrolytic flocculation and electrolytic floatation. Eight criteria were used for evaluation of these microalgae harvesting techniques: (a) dewatering efficiency (b) cost (c) toxicity (d) suitability for industrial scale (e) time (f) species specificity (g) reusability of media and (h) maintenance. Each criterion was assigned a score between 7 and 15 based on its degree of importance. Higher values were given to the criteria that were deemed most important for development of an efficient and economic large scale dewatering method for microalgae whereas lower values were given to criteria that were deemed necessary for determining a suitable method but were considered less important. The results indicated that of the 16 methods evaluated, 4 scored values of 80/100 and above and were deemed suitable for harvesting microalgae on an industrial scale. Three were physical techniques (disc stack centrifuge (87/100), cross flow filtration (84/100), decanter centrifugation (82/100)) and the forth was the organic flocculation (80) method. These techniques were deemed suitable for large scale use because of their effectiveness, low operational costs, suitability for numerous species, rapidness, minimal maintenance requirement and being environmentally friendly. The other methods were deemed unsuitable because they are not effective in dewatering a wide array of microalgae species, not suited for large volumes, costly and require high maintenance. Although each of the optimum techniques was deemed suitable for harvesting of microalgae on its merit, a combination of methods can also be used to enhance the recovery efficiency and improve the economics. The use of organic flocculation as an initial harvesting step to concentrate the algae suspension and the centrifugation (or filtration) as a secondary dewatering step will reduce the time and costs associated with dewatering. Flocculation allows for effective removal of algae from large amounts of liquid media and as such the costs associated with energy intensive centrifugation and filtration techniques (used individually) can be reduced by using them as secondary techniques since less volumes of microalgae suspension will undergo the secondary treatment.

Keywords: Microalgae; Harvesting; Dewatering; Physical treatment; Chemical treatment; Electrophoresis processes

Introduction

Microalgae are photosynthetic microorganisms that are abundant in nature and capable of growing in various environments [1]. Microalgae biomass can be used to produce numerous value added products such as biofuels (biodiesel, bioethanol, biogas and biohydrogen) [2], fish feed [3], animal feed [4], human food supplements such as vitamins A, B1, B2, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid), Omega 3 fatty acids (Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA)) and chlorophyll [5,6] and skin care products such as anti-aging creams, anti-irritant creams and skin regenerate creams [3,7,8]. Various microalgae strains contain high amounts of proteins (43-71% of dry matter) compared to meat (43%), soybeans (37%), milk (26%) and rice (8%). They synthesize a wide range of amino acids essential for humans and animals which make them great for use in food supplements [9,10]. Microalgae carbohydrates (10-30% of dry matter) are synthesized in the forms of sugars, starch and polysaccharides which are easy to digest [9]. The oil content in the cells can make up 25-77% of the dried biomass weight [11]. Microalgae are regarded as the best candidate for the production of biodiesel as they do not compete with edible crops [1,12] and can produce between 20,000 to 80,000 L of oil per acre per year which is 7-31 times greater than that produced by the best terrestrial crop (palm tree) [13]. Application of biorefinery concept to produce biodiesel and other value added products will enhance the economics of biodiesel production.

However, processing microalgae into biodiesel and other value added products requires culturing of the microalgae, recovery of the microalgae biomass and the extraction and downstream processing of the oil and other value added products [14]. However, the major

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obstacle for using microalgae biomass on an industrial-scale for the production of value added products is the dewatering step [15,16]. Microalgae cultures need to be concentrated because they exist as a dilute suspension containing 0.1-2.0 g of dried biomass per litre [15,17]. Dewatering microalgae accounts for 20-30% of the total costs associated with microalgae production and processing [17,18]. The cost of the extraction, purification and extraction processes decrease with increased biomass concentration [15-17].

Therefore, in order to achieve economically viable biodiesel production, microalgae recovery needs to be made less costly. Different methods for solid-liquid separation can be employed to dewater/concentrate the microalgae culture to 10-450 g/L. Such methods include sedimentation, vacuum filtration, cross flow filtration, pressure filtration, decanter centrifugation, disc stack centrifugation, dissolved air flotation, dispersed air flotation, micro bubble generation organic flocculation, inorganic flocculation, bio-flocculation, auto-flocculation) and electrolytic coagulation, electrolytic flocculation and electrolytic flotation.

The aim of this study was to review the current methods used for harvesting and concentrating microalgae and perform a comparative analysis in order to determine the most efficient economically dewatering methods for large scale processing of microalgae biomass for production of biodiesel and value added products.

Physical Harvesting Methods

Numerous physical methods for microalgae dewatering processes have been used to retrieve the microalgae cells from their liquid suspension. These can be divided into four categories: sedimentation, filtration, centrifugation and flotation.

Sedimentation

In this technique, the solids and liquids are separated from one another by gravitational forces as shown in Figure 1 [19]. Different materials are separated from one another based on the density of the material and/or particle size. A larger difference in density would result in faster sedimentation rates while a smaller difference in densities and/or smaller particle size would require longer time to settle out by gravitational forces [20].

Type of Settling Tanks

Lamella separator (Figure 2a) and sedimentation tanks (Figure 2b) are used to separate solids from liquid [21,22]. Lamella separators offer a greater settling area than conventional thickeners as a result of plate orientation [23]. Lamella tanks work by inserting the microalgae biomass through the inlet. The liquid floats to the surface (effluent) and the biomass is caught onto the slanted plates. With time, the biomass settles down to the bottom of the tank and can be collected through the harvest outflow. Sedimentation tanks are cylindrical with a funnel shaped bottom so that the settled microalgae are concentrated near the outlet. The outlet is placed at the bottom of the tank so that the collection of the settled microalgae can easily be recovered. The tank is equipped with a pump that carries the microalgae biomass from the cultivation tank into the sedimentation tank through the inlet. These tanks work by allowing the denser solids to settle to the bottom of the tank, leaving the clear water at the surface. Once the settling process is complete, the algae can be retrieved from the tank through the outlet.

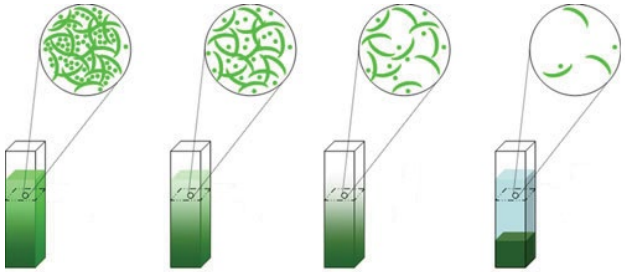


Figure 1: Sedimentation process of microalgae over time [19].

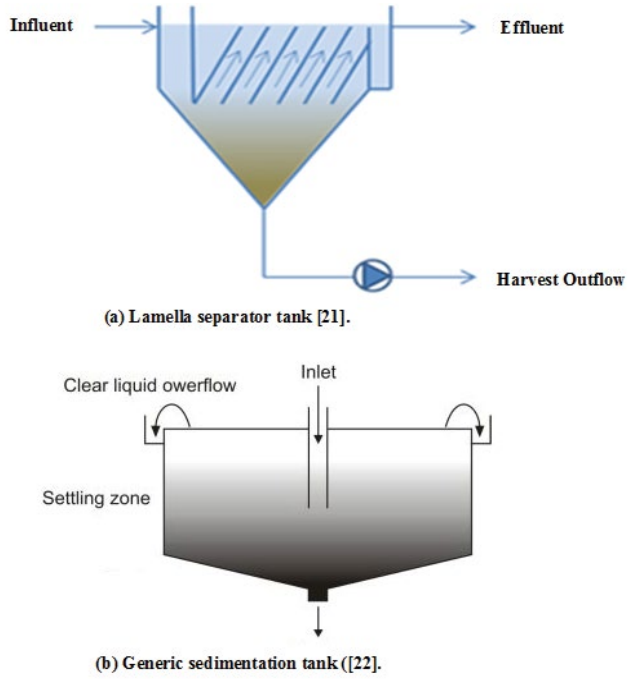


Figure 2: Sedimentation tanks.

Factors Affecting Sedimentation

The factors influencing the settlement rates of microalgae include: density and particle size, temperature, aging of the cells, light intensity and time [24-26].

Density and particle size: The density of marine microalgae varies from 1030 to 1100 kg/m^3 and the density of freshwater microalgae varies from 1040 to 1140 kg/m^3 [27-29]. Granados et al. [30] reported that the densities of fresh water (1000 kg/m^3) and salt water (1025 kg/m^3) are similar to that of microalgae and as a result the rate of settlement of algae is low. Murphy and Allen [31] stated that it is a challenge to remove microalgae biomass from the liquids because of the identical densities of the cells and media.

Cole and Buchak [32] indicated that the rate of settlement is dependent on the type of microalgae present and found the green microalgae to have an average settling rate of 0.1 m/d. Peperzak et al. [33] noted that the sedimentation rate of 24 different microalgae species (ranging in size from 10-1000 μm) varied from 0.4 to 2.2 m/d and there was no correlation between the size of the cells and the sinking rates. Milledge and Heaven [20] reported a settlement rate of 0.1 m/d for *Chlorella* species in freshwater. Yang et al. [34] reported an algae settling rate in the range of 0.1-0.3 m/d. Choi et al. [35] noted that the sedimentation rate of large and small sized algae were 2.6 cm/h and less than 1.0 cm/h, respectively.

Temperature: Knuckey et al. [25] noted that the temperature of 4°C settled a wide array of microalgae species after 24 h when the pH was adjusted in the range of 8-8.5. Davis et al. [36] noted slower settling rates of microalgae in colder waters as a result of increased viscosity. Harith et al. [24] tested the effect of varying temperature (4-27°C) and the presence and absence of light on sedimentation rates of *Chaetoceros calcitrans* at a pH of 8. The highest efficiency (9%) was obtained at day 8 at a temperature of 27°C in the dark. Greenwell et al. [37] noted that harvesting microalgae by sedimentation in high temperature areas will deteriorate the cells.

Cell age: Danquah et al. [15] noted that the settling rate for microalgae harvested during the high growth phase (4-10 days) was lower than that harvested during low growth phase (10-12 days). Choi et al. [35] reported that the settling rate of algae significantly increased in the stationary growth phase of microalgae. Manheim and Nelson [38] noted that in the exponential growth phase (day 6) there was little to no settling in *Scenedesmus sp*. observed over 2 h period, but the greatest removal efficiency was noted in the stationary phase (day 15). They also noted that the settling rate for C. vulgaris species in the exponential phase was 6 times greater than the late stationary phase. Peperzak et al. [33] reported that the settling rate at 15 and 20 weeks for *Phaeocystis globosa* and *Eucampia zodiacus* were 0.5 and 1.0 m/day and 0.7 and 1.0 m/day, respectively.

Light: Danquah et al. [15] noted that the absence of light increased the settling rate during high growth and low growth phases. The supernatant obtained during the high growth phase contained 0.57 g/L of biomass in the presence of light and 0.39 g/L in the absence of light, while the supernatant obtained during the low growth phase contained 0.28 g/L in the presence of light and 0.17 g/L in the absence of light. Schlenk et al. [39] noted that the concentration of microalgae in the light and dark conditions were 1075 cells/mL and 775 cells/mL, respectively. On the other hand, Harith et al. [24] reported that the settling rates observed in the presence and absence of light in *Chaetoceros calcitrans* were similar.

Time: The concentration of microalgae by sedimentation requires long settling times that are greater than 24 h. Park et al. [40] noted long retention times of 1-2 days for algae recovery in large-scale settling tanks. Harith et al. [24] reported that increasing the settling time to 15 days increased the settling efficacy to 94%. Griffiths et al. [41] noted that the percentage of biomass recovery after 24 h of settling for *S. platensis, C. fusiformis, T. suecica, Nannochloropsis* and *Scenedesmus* were 95, 96, 80, 59 and 86%, respectively. Wang et al. [42] noted that the biomass recovery for the species *S. dimorphus* and *C. vulgaris* after 2 h of gravitational settling was 80 and 55%, respectively.

Advantages and Disadvantages

Although sedimentation tanks are effective in concentrating microalgae suspensions to 1.5% total suspended solids (TSS), they are not widely use in the industry. The costs associated with gravitational settling are low, but the reliability of this method without the use of flocculating agents is also low [16,43]. The settling time required is much longer than other processes [44] and energy is required for pumping the slurry [16]. Gonzalez-Fernandez and Ballesteros [45] stated that this method is time consuming and the composition of the cells can change. Mata et al. [46] stated that the cell concentrations obtained by sedimentation are low. Ras et al. [47] indicated that harvesting microalgae biomass by sedimentation alone is not the most efficient method since the cell recovery rates of 60-65% are low.

Filtration

This type of algae harvesting method uses a medium that is permeable so that it can retain the algae biomass while allowing the liquid to pass through. This technique requires a pressure difference across the filter which can be driven by vacuum, pressure or gravity. The membrane filters can be classified based on the size of the pores into macro filtration (greater than 10 μm), micro-filtration (0.1-10 μm), ultrafiltration (0.02-0.20 μm) and reverse osmosis (less than 0.001 μm) [48]. The pressure required to force the fluid across the membrane decreases as the pore size of the membrane is increased. Filtration

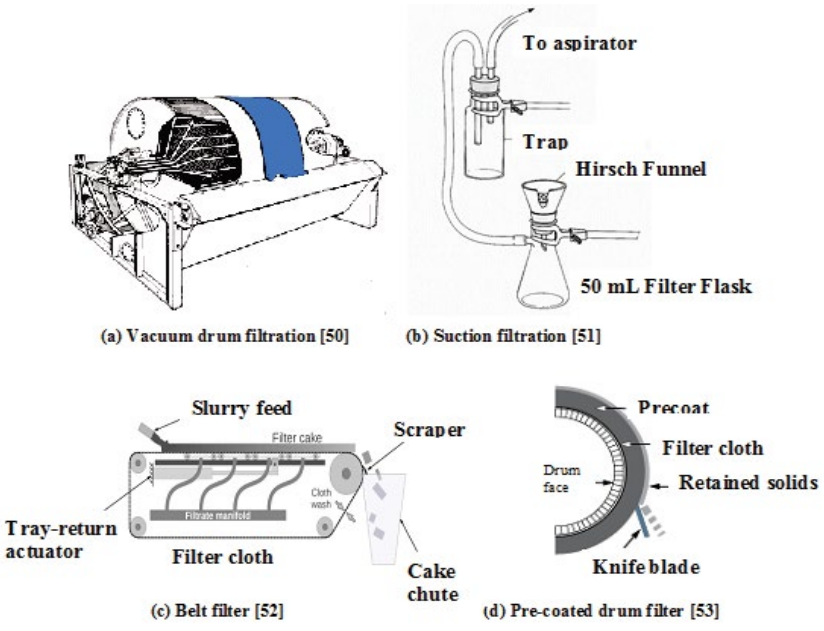


Figure 3: Types of vacuum filtration.

techniques can concentrate microalgae cells in the suspension upto 5-18% and the operating costs vary from $10 to $20/gal. The harvesting efficiency using filtration methods ranges from 20% to 90% [49].

Vacuum Filtration

Vacuum filtration separates solids from liquid media by capturing the solid particles onto a filter while pulling the liquid through by suction from the filter Figure 3 [50-53]. Microalgae range in size from 2 to 30 µm indicating that a micro-filtration membrane is suitable for vacuum filtration [17,48]. Milledge and Heaven [20] stated that the macro-filtration membranes can be used for large microalgae cells or if the algae cells are flocculated together. Uduman et al. [16] reported that the vacuum filtration harvesting technique is most suited for large microalgae cells (greater than 10 µm). Stucki et al. [54] separated *Spirulina platensis* species using vacuum filtration equipped with regenerated cellulose membrane with a pore size of 0.45µm.

Type of vacuum filters: There are five different filter membranes that can be used in vacuum filtration. They are vacuum drum filter, suction filter, filter thickener, belt filter and starch precoated drum filter. Mohn [23] noted that the suction filter, starch precoated drum filter and belt filter were suitable for concentrating the *Coelastrum* microalgae species to a range of 5-37%. The author found the drum filters were not effective harvesting techniques as a result of clogging. Filter thickeners were not recommended as a result of low solid contents (3-7%) and high energy requirements. Ferrentino et al. [55] noted that vacuum filtration equipped with a Buchner funnel and cellulose fiber filters were effective in the recovery of microalgae from solution.

Successful recovery of microalgae cells has been noted using filtration equipped with diatomaceous earth as a filter aid to avoid the clogging of the filter [17]. Gudin and Chaumont [56] reported that precoated drum filter with filter aid (diatomaceous earth) is effective in harvesting the microalgae *Chlamydomonas reinhardtii*. Molina Grima et al. [17] evaluated filters made of cellulose fibers and sand filters and obtained unsatisfactory results but found that diatomaceous earth filter effectively recovered the micro sized *Dunaliella* species. Uduman et al. [16] noted an exceptional recovery of *Dunaliella* species using diatomaceous earth aided filter. Brennan and Owende [48] stated that the use of diatomaceous earth as a filter aid can effectively remove microalgae cells from medium.

Energy consumption: Shelef et al. [43] reported energy consumption in the range of 0.1-5.9 kWh/m^3 depending on the type of filter used. Mohn [23] noted that vacuum filtration consumed 5.9 kWh/m^3 of energy in order to concentrate the suspended solids in solution to 18-27%. They also reported that the energy required to dewater the *C. proboscideum* using suction filter (8% SS), belt filter (9.5% SS) and filter thickener (5-7% SS) was 0.1, 0.45 and 1.6 kWh/m^3, respectively. Umesh [57] noted that harvesting the microalgae strain *Spirulina fusiformis* under vacuum filtration with a coarse pores medium was low in cost

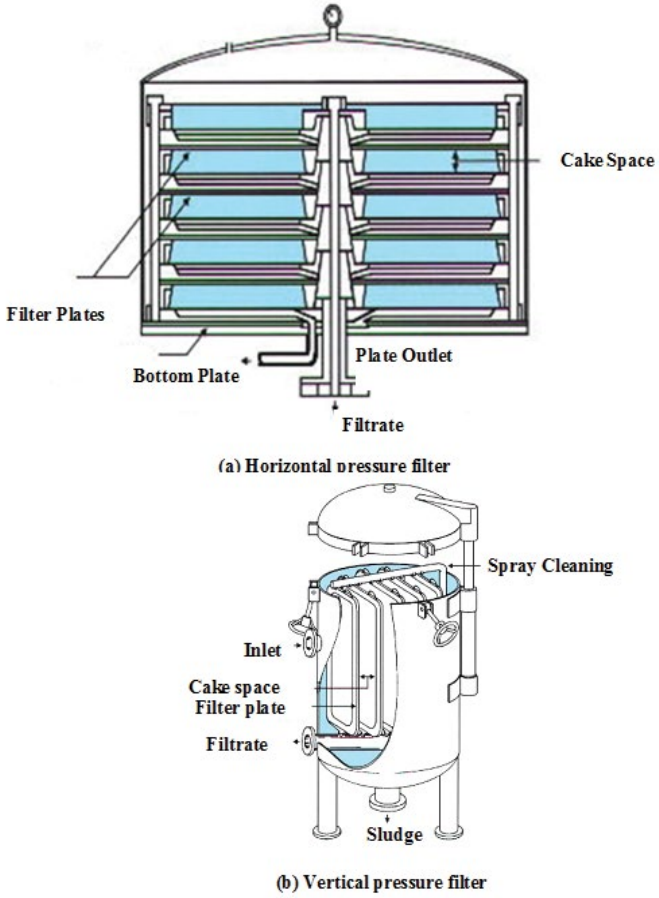


Figure 4: Pressure filter [50].

($83.3/ m^2) and capable of harvesting 23 kg/m^2 kwh. Shelef et al. [43] reported that the pressure drop required for vacuum operations is in the range of 70-80 kPa. Milledge and Heaven [20] reported a power consumption of 0.25 kWh/m^3 for microalgae harvest using vacuum belt filter. Mohn [58] reported an energy consumption value of 3 kWh/m^3 for microalgae harvesting, using vacuum drum filtration.

Advantages and disadvantages: The advantages of using vacuum filtration technique for harvesting microalgae are the preservation of the cells after the recovery process [59]. The effectiveness of the filtration process is dependent on the membrane size and the microalgae cell size. Harvesting microalgae by filtration is more efficient than the sedimentation technique, but drawbacks of this method include membrane replacement and/or periodical washing of the membrane to avoid clogging the membrane pores [45]. However, drawbacks are associated with large energy requirements and costs associated with periodic replacement of membrane as a result of clogging [17,46,60-62]. Arar and Collins [63] recovered microalgae for chlorophyll extraction using vacuum filtration at 6 in. Hg (20 kPa) and noted that higher pressures and prolonged filtration (beyond 10 min) may damage the cells. Uduman et al. [16] noted that filtration technique is suitable for larger microalgae cells, but inadequate for recovery of microalgal species. Rossi et al. [64] noted that rapid clogging of the membrane resulted using the ultrafiltration membrane technique. Flocculation assisted filtration processes would lower the energy requirements, but additional costs for the flocculent would be encountered [20]. Molina Grima et al. [17] recovered microalgae biomass using filtration method and concluded that this harvesting method is not economically viable for large scale production.

Pressure Filtration

Pressure filtration is a technique used for separating particles form a liquid suspension into a compacted form. It works by separating the liquid from the particles (that are collected onto the filter) by means of pressure [65]. The flow of fluids through the filter is created by raising the pressure above the atmospheric pressure to create a pressure differential across the filter [66]. This process is operated in batches that are most often fed from and discharged to a continuous process. A surge tank is required upstream to the filter and one is required for the collection of the filtrate [67].

Type of pressure filtration: Pressure filtration harvesting can be achieved by plate-and-frame filter presses or by using a pressure vessel that is equipped with filters as shown in Figure 4 [50]. The plate-and-frame filter presses works by forcing the liquid in the microalgae suspension through the filter using high pressure. A series of rectangular plates that are mounted in a vertical position, face to face, make up the press system. A fitted filter cloth is applied to each of the plates and they are held together with one another by force under pressure. The fluid that contains the algae is pumped into the gaps between the plates and the pressure is applied in order to force the liquid through the plate outlets and filter cloths. After separation, the dewatered microalgae cake is recovered [68].

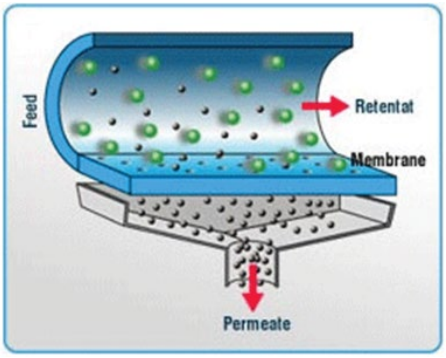


Figure 5: Cross flow filtration diagram [71].

Energy consumption: This method can be considered energy efficient since a minimal amount of energy is required upon assessment of the output product and the amount of initial feedstock added [15]. However, the effectiveness of the method is dependent on the type of algae species.

Molina Grima et al. [17] noted that the amount of energy required to harvest 22-27% (w/v) of C. *paroboscideum* species using pressure filters is 0.88 kWh/m^3. Nagle and Lemke [69] noted an 8% concentration of microalgae (up to 0.5%) using a filter press that has 20 plates and frame (30 cm in diameter), equipped with filter paper that has a pore size of 5 µm. Harun et al. [8] noted that the microalgae *Dunaliella* and *Chlorella* species were too small to be recovered by pressure filtration. Mohn [23] reported that the energy consumed for harvesting *C. proboscideum* using cylindrical sieve (7.5% suspended solid concentration) and filter basket (5% suspended solid concentration) was 0.3 kWh/m^3 and 0.2 kWh/m^3, respectively. He also found that pressure filtration was not suitable for the species *Scenedesmus, Dunaliella* and *Chlorella*, but was satisfactory for other larger microalgae species such as *Coelastrum proboscideum* and *Spirulina platensis*.

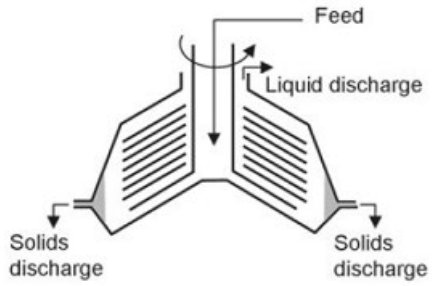
Advantages and disadvantages: Some of the advantages of using pressure filtration are: the cakes collected (composed of the particles in the liquid suspension) have low moisture content, the soluble recovery from the cake is high, re-circulating the filtrate for 1-2 min will clean the filter, high degree of clarity in solutions can be achieved and alloy and synthetic materials can be used to construct the filters and the internal parts [66,70]. The disadvantages of using this technique include: the difficulty in washing the filter medium which increases when the solid is sticky, the internals are difficult to clean in food-grade applications and the difficulty in viewing the condition of the filter due to vessel encapsulation [70].

Cross Flow Filtration

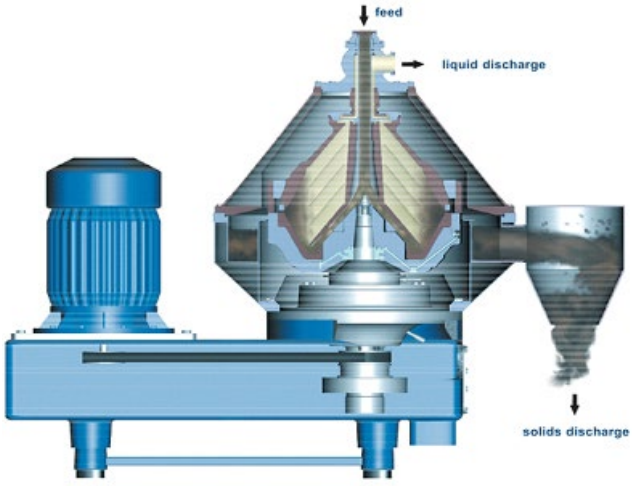
Harvesting microalgae cells in large volumes can be effectively done using cross flow filtration a shown in Figure 5 [71]. In this technique, the sample flows tangentially across a membrane. The particles larger in size than the membrane pores are retained and referred to as the retentate. Smaller particles pass through the membrane with the liquid solution and are referred to as the permeate.

Membrane type: Ultrafiltration or microporous membranes are the type of filter membranes used in this technique. These membranes are available with a wide range of pore sizes and molecular weight retentions. Polymer membranes have a long operating life when used at suitable cross flow velocity conditions and low transmembrane pressures. Petrusevski et al. [59] used a cross filtration with a membrane pore size of 0.45 µm and achieved a biomass recovery efficiency of 70-89%. Rossignol et al. [72] found that polymer membranes were effective in recovering the marine microalgae species *Haslea ostraria* and *Skeletonema costatum*, but the performance depended on the hydrodynamic conditions,properties of the microalgae and the concentration of the microalgae cells.

Uduman et al. [16] reported that the initial flux for microfiltration membranes were much higher than those of ultrafiltration, but they



(a) Borowitzka and Moheimani [79]



(b) Enviropro [80]

Figure 6: Disc stack centrifuge.

clogged more easily. Zhang et al. [73] used a cross-flow ultrafiltration membrane with a cross-flow velocity of 0.17 m/s and noted an increase in algae concentration from 0.104% to 92.5% at the membrane surface with a harvesting efficiency value of 46.01 g/m^2/h. Rossi et al. [74] tested 14 various inorganic membranes and noted that the ultrafiltration membrane ATZ-50 kDa illustrated the best performance and concentrated the *Arthrospira platens* species by a factor of 20. Rossi et al. [64] used a cross-flow filtration technique equipped with an organic ultrafiltration membrane (polyacrylonitrile, 40kDa) and concentrated *Arthrospira platens* by a factor of 10. Rossignol et al. [72] concentrated the species *Skeletonema costatum* using cross-flow ultrafiltration with a flux of 30 l/h for 12 h. Rose et al. [75] effectively concentrated the species *Dunaliella salina* by cross-flow ultrafiltration with flux rates of 30-40 l/h. Walsh et al. [76] concentrated the species *Thalassiosira pseudonana* to 2.3 L from 2840 L which was composed of 2.33x10^{12} cell/L using microfiltration membrane system. Ahmed et al. [77] noted that the resistance of the cross-flow microfiltration decreased as the cross-flow velocity increased from 0.13 to 4 m/s while harvesting *Chlorella* sp. species.

Energy consumption: Rossignol et al. [72] reported that the energy consumption using cross-flow filtration techniques can range from 3kWh/m^3 to 10kWh/m^3 depending on the feed characteristics, the system design and the pressure used. Danquah et al. [15] noted that cross-flow filtration can also be used in sensitive suspensions and is a cheap method for concentrating suspended solids in the range of 2.5-8.9 % with an energy consumption in the range of 0.38-2.06 kWh/m^3. Crittenden et al. [78] reported that the energy consumption for a cross-flow filtration technique was 5kWh/m^3. Danquah et al. [15] reported that the amount of energy required for dewatering microalgae to a concentration of 8.88% (w/v) was 2.06kWh/m^3.

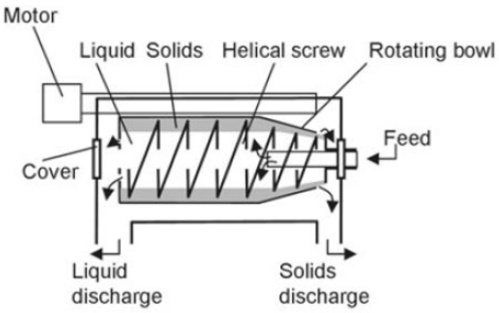


Figure 7: Decanter centrifuge [79].

Advantages and disadvantages: Cross flow microalgae filtration is advantageous over other conventional harvesting methods such as sedimentation, flocculation and centrifugation because it results a in complete removal of debris and microalgae cells [16]. The equipment are considered to be cheap because the costs are only associated with pumping and replacement of membranes [72]. The structure and properties of the recovered microalgae are preserved using this filtration technique [59]. However, large scale recovery of algae cells using this method can be limited due to fouling and frequent replacement of the membrane [16].

Centrifugation

This type of removal mechanism is widely used in beverage, food and pharmaceutical industries. Centrifugation is a process in which a centrifugal force is used to enhance the separation of solids. Spinning the suspension creates the pressure differential necessary for particle separation from the liquid suspension. Thus, the efficiency of the recovery process is dependent on the centrifugal force [17].

Types of Centrifuges

The two types of centrifugation used for harvesting microalgae are: disc stack and decanter centrifuges.

Disc stack centrifuge: The most common industrial centrifuge used today in commercial plants producing high value products and algal biofuel is the disc stack type centrifuge. It consists of a shallow cylindrical bowl that has numerous stacks of metal cones (discs) which are closely spaced together as shown in Figures 6 [79,80]. Separation of the materials is based on densities. The mixture is placed on the centre of a stack of discs and the lighter phase of the mixture remains on the inside towards the centre while the denser phase is displaced outwards to the underside of the discs. This technique separates materials of different densities by layering them [81]. It is most suited for separating materials with particle sizes in the range of 3-30 µm and for concentrations of suspensions that has solid content ranging from 2 to 25% [20].

Heasman et al. [82] evaluated the cell recovery efficiency of nine different microalgae species using a disk stack centrifuge and noted a recovery efficiency greater than 95% at a force of 13,000g. They also noted that the recovery efficiency declined with a decrease in the

gravitational force to 60% and 40% at gravitational forces of 6,000g and 1,300g, respectively. Sim et al. [83] noted a 90 % microalgae removal efficiency using a disc stake type of centrifuge. Vasudevan et al. [84] achieved an 18% microalgae concentration using a disc centrifuge. Mackay [85] used a disc centrifuge operating at a force of 4,000-15,000g for a biomass suspension containing 0.2-20% v/v algae cells. Chojnacka et al. [86] harvested the *Spirulina* sp. using a disc type centrifuge operating at 6,000 rpm for 5 min.

Decanter centrifuge: Decanting centrifugation is based on the concept of using a special settling tank in which the solids in suspension are forced to fall down due to the gravitational forces [87]. The decanter centrifuge (Figure 7) operates continuously by pumping the cultivated microalgae biomass into the centrifuge bowl whereby the suspended particles in solution are forced to the bottom of the bowl. The liquid left after the particles have been extracted is passed through the overflow pipe [88].

Molina-Grima et al. [17] noted that concentration of microalgae biomass using a decanter centrifuge is better than other harvesting methods. Dassey and Theegala [89] achieved a harvesting efficiency of 28.5% for microalgae at a flow rate of 18 L/min using continuous flow decanter centrifuge. Smith and Charter [87] reported that the clarity of the liquid produced after separation was not as great as that achieved using disc-stack centrifugation. Vasudevan et al. [84] reported that a 12% microalgae concentration was achieved using a decanter centrifuge. Mackay [85] reported that the decanter centrifuge operates using a force of 4000-10000 g and is effective for slurries with a biomass content of 5-80%. Vasudevan et al. ([84] stated that microalgae biomass needs to undergo an initial thickening step such as dissolved air flotation in order to concentrate microalgae suspensions (0.02-0.05 weight %) to 2-3% before using decanter centrifugation.

Energy Consumption

Disc-stack centrifuge: The energy consumption reported in the literature for the disc stack centrifuge varied from 0.53 kWh/m^3 to 5.5 kWh/m^3. Alfa Laval [90] used a disc type centrifuge for dewatering microalgae and achieved a 16% with a power consumption of 0.53kWh/m^3. Mohn [23] noted a 12% suspended solids concentrate of the microalgae species *Scendesmus* using a disc-stack centrifuge with an energy consumption of 1 kWh/m^3. Goh [91] harvested microalgae grown in pig waste using disc centrifugation with an energy consumption of 1.4kWh/m^3. Sharma et al. [92] noted that the disc stack centrifuge consumed 5.5kWh/m^3 for *Chlorella sp*. harvesting.

Decanter centrifuge: The energy consumption reported in the literature for decanter centrifuge varied from 1.3kWh/m^3 to 8kWh/m^3. Sim et al. [83] noted that an energy consumption of 1.3kWh/m^3 was required for concentrating microalgae biomass from 0.04% to 4.00% using a decanter type centrifuge. Molina Grima et al. [17] achieved a microalgae concentration of 22% (w/v) using decanter centrifuge with an energy consumption of 8kWh/m^3. Mohn [58] reported that the energy consumption for harvesting microalgae (20% DS) using decanter centrifugation was 4 kWh/m^3.

Advantages and Disadvantages

Disc-stack centrifuge: The advantages of using disc-stack centrifuge for harvesting microalgae is their high removal efficiency compared to other industrial centrifuges. The concentration of the feed for these units is typically in the range of 0.5-10% w/w. This type centrifuge handles high flow rates and is capable of separating fine (0.1-100 μm) particles [93]. This device can be used to separate solid from liquid in continuous, semi-continuous and batch operation. Some of the disadvantages of this type of centrifuge include: low dry substance content in the discharge system, mechanically complex, costly and the small space between the closely stacked discs makes it harder to clean and may require chemicals for cleaning [92,93].

Decanter centrifuge: The dewatered biomass using the decanter centrifuge is much more concentrated than that achieved using the disc centrifuge. However, the decanter centrifuge is more suited for suspensions with higher solid particles and is unsuitable for microalgae suspensions [58]. This type of centrifuge is most suited for separating materials with particle sizes greater than 15 μm and solid suspensions containing higher than 15% [20]. It operates at inertial forces that are less than 6000 g. The disadvantages of using this method for microalgae harvesting are: highly concentrated feeds (typically in the range of 4-40% w/w) is required, the liquid leaving the system may not be clear due to the presence of fines, processing finer particles may result in poor flow properties of the thickened solids and cause mechanical difficulties, much more energy intensive than disc centrifuges and the high costs associated with the equipment required for processing large volume [17,20,93-95]. This type of centrifuge has been estimated to consume 3000 kWh/ton of dry alga biomass.

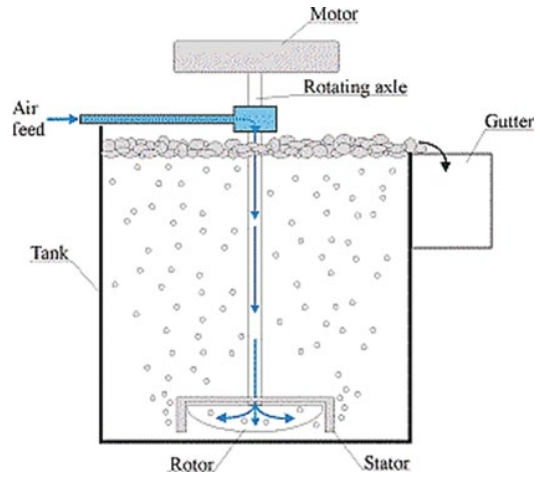


Figure 8: Dispersed air flotation technique [99].

Flotation

The flotation technique for microalgae harvesting takes advantage of the low density of microalgae [28]. This technique is classified as a physiochemical gravity separation process in which gas bubbles pass

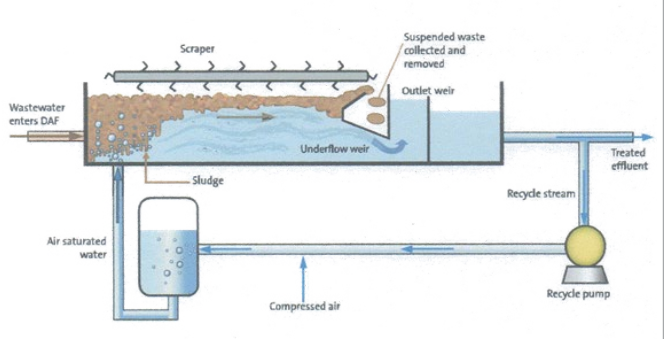


Figure 9: Dissolved air flotation apparatus [21].

through a liquid-solid suspension causing the microalgae to float to the surface by adhering to the gaseous bubbles [43,96]. The aeration also assists in removing the volatile organic compounds that are in the solution which provide cleaner residual water [43]. The efficiency of this method depends on the suspended particles instability, higher air-particle contact corresponds to a lower instability [97]. In flotation technique, the size of the particle is of importance, the smaller the particle size the more likely it will be lifted to the top of the medium by the bubbles. Solutions with particle sizes of less than 500 μm can be used in flotation [98].

Types of Air Flotation

The flotation processes are grouped by the method that is used for the bubble formation into: dispersed air flotation, dissolved air flotation, microbubble generation and electrolytic flotation.

Dispersed air flotation: This technique requires the use of a high speed mechanical agitator for bubble formation and an air injection system as shown in Figure 8 [99]. The gas mixes with the liquid as it is introduced at the top of the vessel and is allowed to pass a disperser that creates bubbles ranging in diameter from 700 to 1500 μm [100]. These bubbles are a magnitude larger (1000 μm in diameter) than those produced using dissolved air flotation technique.

Chen et al. [101] used dispersed air flotation for dewatering of microalgae *Scenedesmus quadricauda*. They used three varying surfactants in order to remove the microalgal cell: non-ionic X-100, cationic *N*-Cetyl-*N*-*N*-*N*-trimethulammonium bromide and anionic sodium dodecylsulfate. They also found that surfactants played a role in increasing the integrity of the bubble avoiding rupturing, and noted that this removal method was most successful with the use of cationic *N*-Cetyl-*N*-*N*-*N*-trimethulammonium bromide surfactant.

Yan and Jameson [102] noted that the dispersed air flotation technology resulted in 98% microalgae removal efficiency. Xu et al. [103] reported a 93.6% recovery efficiency of *B. braunii* using dispersed air flotation technique. Kurniawati et al. [104] harvested *Chlorella vulgaris* and *Scenedesmus obliquus* using dispersed air flotation assisted with chitosan and achieved a recovery efficiency greater than 93%.

Dissolved air flotation: The dissolved air flotation technique is the most widely used flotation technique in the treatment of industrial effluent [98,100]. This method requires a reduction in water pressure that is presaturated with air. The liquid is then injected into the flotation tank at atmospheric pressure. Bubbles are generated from the diffuser nozzles and rise through the liquid carrying microalgae cells in the suspended media to the surface of the tanks as shown in Figure 9

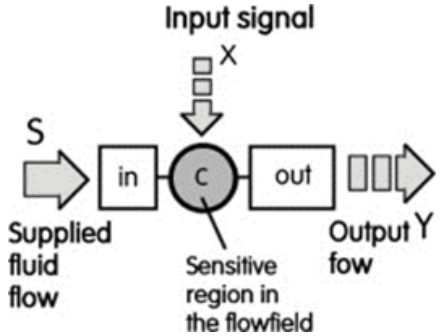


Figure 10: Principle of fluidic oscillation - The continuous flow of fluid (S) is integrated with a weak input signal (X) which causes the change in output flow (Y) [107].

[21]. The cumulated biomass at the surface can be skimmed off and collected. The clarified liquid portion is saturated with air and recycled back into the flotation tank [100]. The supply of air into the system can be controlled by changing the saturator pressure or by changing the ratio that is recycled back into the tank. The size of the bubbles formed can be controlled by the saturator, operated above atmospheric pressure and by the injection flow rate [16,105]. The flow rate must be great enough to prevent backflow, provide a pressure drop and allow for bubble growth on the pipes surface [16]. Small bubbles ranging in size from 10 to 100 μm (with a mean size of 40 μm) are desirable [105].

The dissolved air floatation microalgae separation is usually coupled with the use of a chemical flocculation process. Edzwald [28] investigated the use of dissolved air flotation for microalgae recovery and noted that this method required pretreatment by flocculation, but was more successful than the settling technique. Wiley et al. [106] used dissolved air flotation for microalgae harvest and noted a suspended solids concentrate of 5% with an energy consumption of 7.6 kWh/m^3. Goh [91] noted that this method was effective in harvesting microalgae from pig slurry when coupled with the alum flocculent with a high dosage of 0.3 g/L.

Fluidic oscillation: The recovery of microalgae can also be achieved by micro-bubble generation through fluidic oscillation as shown in Figure 10 [107]. This method works by converting a continuous air supply into oscillatory flow with a regular frequency, generating bubbles that are the size of the exit pores [108]. The miniature bubbles are formed by fitting a diffuser to the bi-stable valve which ensures that the bubbles formed are approximately 10 times smaller than those originally dispersed in flotation methods [107]. Fine bubbles that are the size of the exit pores are generated by use of a fluidic oscillator [108]. The bubbles formed attach to the hydrophobic cells suspended in solution and carry them to the surface. At the surface the bubble raptures leaving the cells behind [109].

Hanotu et al. [108] reported a microalgae recovery efficiency of 99.2% using microbubble generation at a pH of 5 with the aid of ferric chloride coagulant (150 mg/L). Elder [110] noted a removal efficiency greater than 95% using micro-bubble flotation. Yap et al. [111] noted a removal efficiency greater than 95% for removal of *Microcystis* and filamentous *Cylindrospermospsis* using micro-bubble flotation. However, not much research has been performed using this technique for microalgae recovery to deem it economically suitable for large scale recovery of microalgae cells [20].

Factors Affecting Air Flotation

The factors affecting the efficiency of harvesting microalgae using the air flotation technique include: pH, air flow rate, alkalinity, recycle rate, hydraulic loading and time. The velocity of the skimmer determines the concentration of the slurry formed and the height above the surface of the water [16].

pH: One of the most important parameters that affects the flotation processes is the pH. Lin and Liu [112] stated that the pH affects the reaction routes and the interfacial properties. Chen et al. [101] noted that maintaining the pH in the range of 5-8 using anionic sodium dodecylsulfate had little to no effect on the removal efficiency (95%) of microalgae *Scendesmus quadricauda*. They attributed this to the electrostatic interactions between the positively charged microalgae surface and the surfactant that are facilitated by pH values less than 8. However, at pH values above 8 the interaction between the algae surface and surfactant was weak, which did not allow for efficient removal. Kwon et al. [113] reported that flocculent assisted air flotation

technique required a pH of 7-8 for organic and 5-6 for inorganic flocculants in order to reach a removal efficiencies greater than 90%. Zhang et al. [114] reported a harvesting efficiency greater than 90% for *Chlorella zofingiensis* using an air flotation technique by adjusting the pH to 6.2. Hanotu et al. [108] reported that acidic conditions were optimal for effective removal of microalgae.

Alkalinity: Besson and Guiraud [115] noted that the microalgae flotation recovery efficiency was improved by using sodium hydroxide at a concentration of 0.0085 mol/L (over the tested range of 0.000 to 0.025 mol/L). Schlesinger et al. [116] noted that calcium hydroxide is most effective in flocculating microalgae that can be recovered using flotation techniques. Thangavel and Sridevi [117] reported that carbonate salts can be used for microalgae flocculation to enhance the recovery efficiency of the microalgae. Chen et al. [101] reported that the change in alkalinity from 0 to 50 mg/L using $NaHCO_3$ did not have any effect on the removal efficiency (95%) of *Scenedesmus quadricauda* species.

Air flow rate: Reports in the literature show that varying the air flow rate from 68 to 206 ml/min did not have any effect on the removal efficiency (92%) of the *Chlorella* sp. species (Liu et al. [118]. Coward et al. ([119] harvested *Chlorella* sp. using flotation technique at a flow rate of 100 L/h. Dassey and Theegala ([120] reported that an increase in the air flow rate beyond 160 ml/min did not significantly increase the microbubble production and as such no increase in harvesting efficiency was noted. Hanotu et al. [108] noted that the air flow rate of 85 L/min was effective in the separation of microalgae form the media.

Medium: Zhang et al. [121] found that the algal media influences the rate of harvesting efficiency. They noted that the algal cells that were nitrogen deprived had lower concentrations of surface functional groups as they went from the exponential to the stationary and declining growth phases. They noted that these functional groups are made up of proteins and polysaccharides that are important for stabilizing the surface charge which affects the adhesion onto the bubbles in dissolved air flotation technique. They also noted that more Alum coagulant was required to achieve higher harvesting efficiency (90%) in the exponential phase compared to the stationary and declining phases as more alum is required to destabilize the surface of the generated cells in the exponential phase. There was a linear correlation between the concentration of surface functional groups and alum dosage for a given harvesting efficiency. Coward et al. [119] stated th cells at the highest flotation harvesting efficiency was observed during high growth phase as a result of higher zeta potential. Schenk et al. [11] noted that nutrient limitation in the media can improve the harvesting efficiency of microalgae flotation methods.

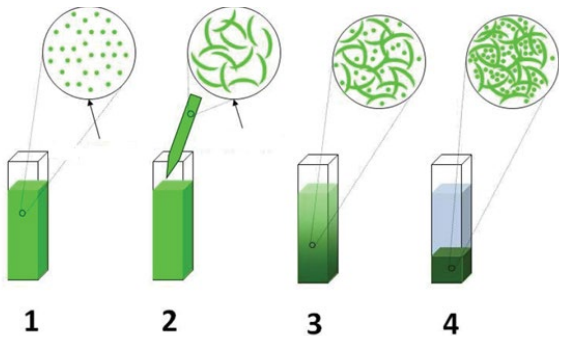


Figure 11: Flocculation harvesting process of microalgae [19].

Recycle rate: The recycle rate reported in the literature varied from 5 to 10%. Edzwald and Wingler [122] obtained a 97-99% removal efficiency of *Chlorella vulgaris* species with a recycle rate of 8%. Vlaski et al. [123] reported a removal efficiency of 94.5% for *Microcystics* with a recycle rate of 5-10%. Kempeneers et al. [124] achieved a removal efficiency of 80% for *Melosira cyclotella* with a recycle rate of 6%. Teixeira and Rosa [125] reported a 92-98% recovery efficiency of blue-green microalgae using a recycle rate of 8% and stated that the recycle system was vital for effective particle recovery but recycle rates past 8% illustrated little improvements. They also found that the addition of pressurized recycle system did not improve the recovery rate of the cells. This phenomena is attributed to the lack of particle destabilization since particle destabilization is vital to the effectiveness of dissolved air flotation as opposed to floc size. Gregory and Edwald [126] reported a recovery efficiency of 90-99% using dissolved air flotation with a recycle rate of 10%.

Hydraulic loading: The hydraulic loading rate for industrial air flotation applications ranges from 0.504 m/h to 40 m/h [127, 128]. Edzwald [129] reported that high rate dissolved air flotation techniques can be performed at hydraulic loadings of 20-40 m/h. Haarhoff and Rykaart [130] noted that increasing the hydraulic loading lowered bubbles formation. Dassey and Theegala [120] stated that increased hydraulic loadings decreased the time for air to dissolve which results in poor bubble productions.

Time: The time required for effective air floatation reported in the literature varied from 3 to 30 min. Edzwald and Wingler [122] noted that the time required to remove 96-99% of *Chlorella vulgaris* using air flotation was 5 min. Vlaski et al. [123] used an air flotation technique to concentrate the species *Microcystis aeruginosa* to 94.5% in 8 min. Kempeneers et al. [124] achieved an 80% removal efficiency of *Melosira cyclotella* using air flotation in 3 min. Xu et al. [103] noted a 93.6% recovery efficiency for *B. braunii* using air floatation in 14 min. Coward et al. [119] noted that the air flotation technique effectively harvested microalgae within 30 min.

Energy Consumption

The flotation technique is an energy intensive process as a result

Chemical Type	Flocculent	Reference
Inorganic	Ferric sulphate	Kown et al. [113]
	Ferric chloride	Papazi et al. [137]
	Aluminium chloride	Molina-Grima et al. [17]
	Aluminium sulfate	Oh et al. [136]
Organic	Magnafloc LT 25	Knuckey et al. [25]
	Zetag	t'Lam et al. [138]
	Praestol	Pushparaj et al. [139]
	Chitosan	Chen et al. [140]

Table 1: Chemical flocculent type and the effect on microalgae.

Flocculent Type	Effect on Microalgae	Reference
Inorganic	Toxic to the cells	Papazi et al. [137]
	Alter the color of the medium	Schenk et al. [11]
	Alter the pH of the media which may make it unsuitable for reuse	Molina Grima et al. [17]
Organic	High cell viability (>75%)	Harith et al. [24]
	No inhibitory effect the cells	Pushparaj et al. [139]
	Non-toxic	Vandamme et al. [141]

Table 2: Chemical flocculent type and the effect on microalgae.

of the high pressure required [108]. Algae harvest using dissolved air flotation is an efficient method but has high operational costs that are associated with the use of energy intensive compressor that function at pressures of 390 kPa [131-133]. However, the fluidic oscillator does not require much energy for operation and has been noted to consume 2-3 orders less energy than dissolved and dispersed air flotation methods [107,108].

Advantages and Disadvantages

Compared to sedimentation, flotation method is much faster and more effective for harvesting of microalgae [96]. Mohn [58] reported that dewatering microalgae using flotation methods (7% concentration) is much more rapid and efficient than the use of sedimentation (1.5% concentration). However, the flotation method has only been reported to be effective in microalgae harvesting on a bench scale and is not suitable for recovering microalgae cells on large scale [43]. In addition, this method is an energy intensive [16], has high operational costs and high energy is required for small bubble generation [58]. The cost of flotation has been reported to be as high as or even higher than the centrifugation method.

Chemical Harvesting Methods

On the basis of energy requirement, chemical flocculation as a dewatering method seems to be the most promising for large scale utilization [16,134, 135]. These flocculation methods work by concentrating the cells (coagulation) followed by settlement to the bottom of the cultivating apparatus due to the increased density of the concentrate as shown in Figure 11 [19].

Type of Chemical Flocculation

There are two types of chemicals (Table 1) that can induce flocculation: inorganic and organic polymers [136]. The effects of these polymers on microalgae are shown in Table 2 [11,17,24,137,139,141]. Typically, cationic, anionic and non-ionic polyelectrolytes are used to flocculate the microalgae cells [16].

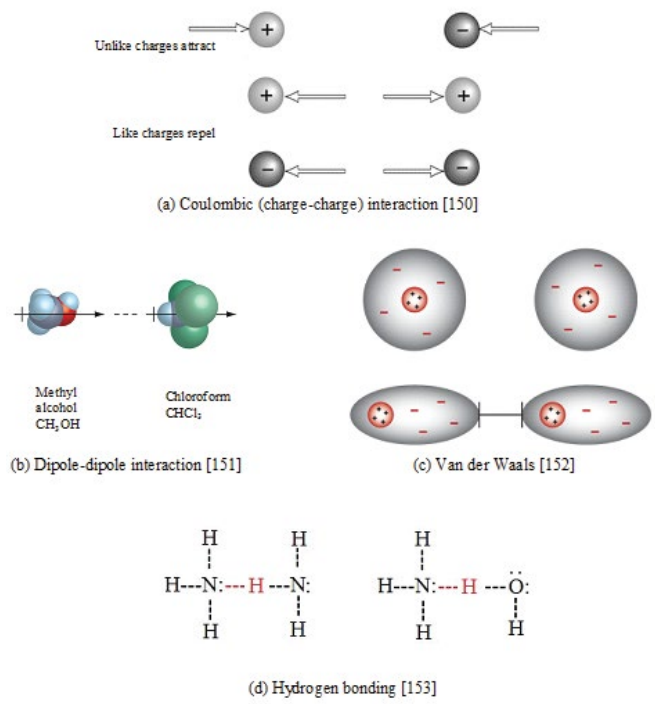


Figure 12: Methods of attachment of polymer to cell surface.

Inorganic Compounds: Microalgae flocculation using inorganic compounds works by charge neutralization [142]. The flocculation process using these compounds works in low pH environments in order to form cationic hydrolysis products [143]. Under optimal pH, these flocculants form polyhydroxy complexes. A large number of chemicals (ferric sulphate, ferric chloride, aluminum chloride, aluminum sulfate) have been tested with the microalgae inorganic flocculation process, the most effective of which was aluminum sulfate [136]. In wastewater treatment, multi-valent metal salts such as ferric sulphate, ferric chloride and aluminium chloride have been used to remove algae [16].

Papazi et al. [137] achieved a harvesting efficacy for *Chlorella minutissma* species greater than 85% using ferric salts. Kown et al. [113] reported a flocculation efficiency of 85.6% for *Tetraselmis sp*. using ferric sulfate (a dose of 0.7 g/L) at a pH of 4-8. Wyatt et al. [144] reported a harvesting efficacy greater than 90% for the *Chlorella zofingiensis* species using a ferric chloride concentration of 40% (w/v) at a pH of 4.0. Xuan [145] achieved a 90% removal efficacy for *Nannochloropsis sp*. using ferric chloride administered at 0.18 mg/l. Sukenik et al [146] achieved a flocculation efficiency greater than 80% for marine microalgae using ferric chloride. Bintisaarani [147] found the ferric chloride flocculation to be the most effective for harvesting *Nannochloropsis* species and reported a removal efficiency of 89% using a ferric chloride concentration of 0.9 M at a pH of 7.5.

Aluminium (alum) salts have been noted to effectively flocculate the microalgae species *Chlorella* and *Scenedesmus* [17]. Papazi et al. [137] found aluminum salts to be more effective in flocculating *Chlorella* species than ferric salts. Shelef et al. [43] noted that alum was a superior flocculating agent compared to ferric sulfate in terms of pH, amount of flocculent and the quality of the final water slurry. Kown et al. [113] reported a flocculation efficiency of 92.6% for *Tetraselmis sp*. using aluminium sulfate dose of 1.2 g/L at a pH of 5-6. Millamena et al. [148] stated that alum was effective in flocculating *Chaetoceros calcitrans, Tetraselmis chui, Skeletonema costarum* and *Isochrysis goibana* species at a pH of 6.5. Aragon et al. [149] used aluminium sulfate to harvest a culture made up of *Scenedesmus acutus* (80%) and *Chlorella vulgaris* (20%) using a dosage of 30-50 mg/L at a pH of 6-6.5.

Organic Compounds

Organic polymers (chitosan) or polyelectrolyte (polyelectolyte polyamine) flocculants are known as polymeric flocculants (synthetic and natural) that consist of both ionic and non-ionic species. The use of organic compounds for flocculation works by combining both particle bridging and charge neutralization. The charge density and polymer chain length determines the extent to which each is used. The process begins by the attachment of the polymer onto the microalgal surface through chemical or electrostatic forces.

The polymer is able to attach to the surface of the cells through Coulombic (charge-charge), dipole-dipole, van der Waals or hydrogen interactions as shown in Figure 12 [150-153]. Coulmbic force attraction works by having unlike charges on the surface of the polymer and the microalgae attach to one another, following the notion that like charges repel one another and unlike charges attract one another. Dipole-dipole interactions occur when two polar molecules approach one another and the partially negative portion bonds to the partially positive one. Van der Waals forces are the attraction of intermolecular forces between molecules. Hydrogen bonding is a type of dipole-dipole attraction in

which a hydrogen atom is bonded to a highly electronegative atom nearby [154]. In this manner, the polymer attaches to the surface leaving its tail out into the solvent forming loops. The loops and tails of the polymer allow it to attach to other cells to from bridges between them [155].

The efficiency of this flocculation process depends on the degree in which the microalgae cells cover the polymer. If the attachment of the polymer to the cell surface is less than the optimum amount, then it may not be able to withstand shear forces as a result of agitation. On the other hand, excess coverage of the polymer onto the cell surface can cause static hindering of the bridging process [156].

Recent studies have revealed that cationic polyelectrolytes flocculent agents are the most effective for microalgae recovery [16,30]. Granadoes et al. [30] noted that inorganic flocculants were less efficient in the flocculation of *Muriellopsis* sp. species than organic agents. Knuckey et al. [25] reported that adding 0.5 mg/L of non-ionic polymer Magnafloc LT25 (anionic polyacrylamide from BASF chemical company) to a medium with a pH adjusted to 10-10.6 effectively concentrated and settled a wide range of microalgae species at rates that are 200-800 times higher than the control. Harith et al. [24] maintained a high microalgae cell viability (75%) for the *Chaetoceros* species using Magnafloc LT25 flocculent at a dosage of 1 mg/L. They stated that increasing the Magnafloc LT 25 and Magnafloc LT 27 dosage did not increase the flocculation efficiency but increased the settling rates.

t'Lam et al. [138] reported a 98% flocculation efficiency for the species *P. tricorntum* using Zetag flocculent at 10 ppm, but only achieved a 52% recovery for the *N. oleoabundans* species. Udom et al. [157] found that using Zetag at a dosage of 34 mg/l flocculated microalgae and resulted in a 98% recovery efficiency. Buelna et al. [158] noted a 95-100% removal efficiency for *Chlorella* culture using 5 mg/L Zetag 63 at a pH of 6-9.

Pushparaj et al. [139] flocculated *Teraselmis* and *Spirulina* with a 70% biomass recovery efficiency using praestol (a cationic organic polyacrylamide based flocculent) with no inhibitory effects on recycled and reused media. However, inhabitation of flocculation has been noted for organic cationic polymers in environments with salinity above 5 g/L [17,25]. Sukenik et al. [146] found that the amount of flocculent required to remove 90% of microalgae from liquid suspension increased linearly with increased salinity. Danquah et al. [15] noted that the amount of energy required to achieve 15% (w/v) microalgae concentration using polymer flocculation was 14.81 kWh/m^3.

Chen et al. [140] reported that the general dosage range of chitosan required to effectively flocculate microalgae species was 5-200 mg/L. Xu et al. [159] noted a 99% clarification efficiency for *Chlorella sorokiniana* using chitosan at an optimal dosage of 10 mg/g dried microalgae and pH values below 7. Ahmed et al. [160] achieved a 99% flocculation efficiency in 20 minutes for *Chlorella* sp. with a chitosan dosage of 10 ppm. Chang and Lee [161] reported a flocculation efficiency of 99% for *Chlorella vulgaris* using chitosan at a dosage of 200 mg/L and a pH of 8.7. Sirin et al. [162] reported a flocculation efficiency of 92% in 10 min for the *Phaeodactylum tricornutum* species using chitosan (20 mg/L). Morales et al. [163] noted a 100 % flocculation efficiency for *Chlorella* sp. using chitosan at a concentration of 40 mg/L. Beach et al. [164] compared the chitosan flocculation, centrifugation and filtration methods for microalgae harvesting and noted that chitosan flocculation was the least energy consuming method of the three.

Factors Affecting Chemical Flocculation

Inorganic Flocculation

The factors affecting inorganics flocculation include: concentration of the flocculent, pH and the surface charge of the flocculent.

Flocculent concentration: The concentration at which the flocculent is administered into the system has been noted to affect the efficiency of the microalgae recovery. Rakesh et al. [165] used aluminium sulphate concentrations ranging from 50 to 300 mg/L for the recovery of *Chlorella sp*., *Chlorococcum sp*. and *Chlorella sorokiniana* and found 50 mg/L to be the most effective dose. Garzon-Sanabria et al. [166] evaluated the recovery efficiency of *N. oculata* using aluminum chloride at concentrations in the range of 50-100 mg/L and found 50 mg/L to be the most effective dose. Ferriols and Aguilar [167] reported on the use of calcium chloride and sodium hydroxide at concentrations of 100-200 mg/L for the recovery of *Tetraselmis terrahele* and achieved the highest recovery efficiency at 200 mg/L. Wyatt et al. [144] noted that in media with low algae concentrations, the concentration of ferric chloride required to flocculate *Chlorella zofingiensis* increases linearly with cell concentration.

pH: Varying the pH of the medium using inorganic flocculants can promote cell aggregation. Knuckey et al. [25] noted effective flocculation (>80%) of *Chaetoceros calcitrans*, *Chlorella muelleri*, *Thalassiosira pseudonana*, *Attheya septentrionalis*, *Nitzschia closterium*, *Skeletonema* sp., *Tetraselmis suecica* and *Rhodomonas salina* by altering the pH with the addition of sodium hydroxide. Garzon-Sanabria et al. [166] used aluminum chloride (50-100 mg/L) to modify the pH (4-7) and achieved the highest recovery efficiency of *N. oculata* using a dosage of 50 mg/L (pH =5.3). Lee et al. [168] noted that changing sodium hydroxide concentration affected the flocculation efficiency of *Botryococcus braunii* as a result of pH change in the medium. Sanyano et al. [169] successfully flocculated *Chlorella* sp. using ferric chloride at a pH of 8.1.

Surface charge: Microalgae surface cells are negatively charged, indicating that a positively charged flocculent would be required to bond the cells to one another [144,169]. Algal coagulation is induced by the attraction of the positively charged flocculent onto the negatively charged cell surface and the attachment of another algal cell onto the positively charged flocculent [161]. The efficiency of the flocculation is depended on the amount of flocculent available to bridge the algae to one another [144,170]. Wyatt et al. [144] noted that the positive nature of ferric chloride induced microalgae flocculation with a recovery efficiencies of 90% at a pH above 4.1 and below 8. Knuckey et al. [25] flocculated microalgae with an efficiency of 80% using Fe^{3+} ions. Garzon-Sanabria et al. [166] recovered *Nannocloris oculata* with a 90% efficiency using aluminium chloride to counteract the surface charge of the microalgae cells at a pH of 5.3. Sanyano et al. [169] successfully flocculated *Chlorella* sp. using ferric chloride. Lee et al. [171] achieved 100% flocculation efficiency in *Chlorella* sp. using synthesized cationic aluminum and magnesium organoclays.

Organic Flocculation

The factors affecting organic flocculation include: pH, charge on polymer, dosage and salinity.

pH: Some microalgae species can flocculate together by adjusting the pH [17]. Uduman et al. [16] stated that the pH and the chemical composition of the microalgal medium impact the amount of flocculent required. They noticed less electrostatic repulsion between colloids

at low pH levels resulting in increased amounts of bridging since the polymer chains are longer. They also found the dose of polymeric flocculent required to vary with microalgae concentration, because of the charge in surface area of algae. Knuckey et al. [25] reported that the non-ionic polymer Magnafloc LT25 settled a wide range of microalgae species effectively using a rate of 0.5 mg/L in a pH adjusted media to 10-10.6. Tenney et al. [172] noted the most effective flocculation resulted when using cationic polyelectrolytes at low pH levels. Ras et al. [47] noted that the *Chlorella* species flocculated when the pH was increased to 11-12. Lee et al. [173] stated that extreme pH levels can result in cell death or impairment.

Charge on polymer: The polyelectrolyte charge plays an important role in the flocculation process of microalgae. Anionic polyelectrolytes are not effective flocculent agents on their own due to the negatively charged microalgae cell surface because like charges repel one another and/or the length of the polymer is not sufficient enough to bridge the particles together [142,172]. It is for this reason that cationic polyelectrolytes are found to be much more effective in the flocculation of microalgae. Morrissey et al. [174] noted that the N,N-dimethylaminopropyl acrylamid polymer (positive character) resulted in recovery efficiencies of microalgae greater than 99% at a pH of 7 and increasing the pH to 13 (activating negative functionality) resulted in flocculation efficiencies of less than 12%. Chang et al. [161] noted that the positively charged surface of chitosan resulted in a 99% removal efficiency for *Chlorella vulgaris*.

Flocculent concentration: The amount of cationic flocculent required for effective bridging between the cells depend on the amount of negative charges present in the system, the surface charge density, the cell counts per volume, the total cell surface area and the charge density of the positively charged polyelectrolyte. The negative charge on the surface is induced by the functional groups (carboxyl groups) present on the microalga cells which have been noted to affect the isoelectric point of the cells [142]. Uduman et al. [16] reported that the growth phase and the metabolic conditions of the microalgal cells dictate the concentration and the reactivity of these functional groups. Granados et al. [30] showed that the species *Chlorella* and *Scenedesmus* were effectively flocculated to a concentration of 2 g/L after 15 min using polyelectrolyte dosages of 2-25 mg/g. Tenney et al. [172] stated that the cationic polyelectrolyte polyamine was effective in flocculating the microalgae cells at a dosage of 2.5 mg/l. Sukenik et al. [146] reported that marine microalgae *Isochrysis galbana* and *Chlorella stigmatophora* require 5-10 times more flocculent dosages than those required by freshwater microalgae.

Salinity: The salinity level affects the organic flocculation of microalgae. Bilanovic and Shelef [175] noted that the polyelectrolyte flocculent was inhibited in the marine medium due to its high salinity and observed effective flocculation at salinity levels below 5 g/l. This was attributed to the fact that high ionic strength causes the polymer to shrink in dimension, thus failing to form a bridge to link the microalgal cells. Schlesinger et al. [116] reported that the addition of alkali to *Chlamydomonas* did not result in rapid flocculation in the saline medium.

Advantages and disadvantages

In comparison with other methods, chemical flocculation is considered to be one of the best methods for cell harvesting because it can handle large amounts of microalgae, it can be used with a wide range of species, it is reliable and it is cost-effective [16,176].

The costs of inorganic flocculants are much less than those of organic ones [138,177,178]. However, the higher amounts required using inorganic flocculants can result in higher costs per unit of microalgae than the more expensive organic flocculants [58]. Sukenik et al. [146] reported that the optimal dosage of inorganic flocculent required to flocculate marine microalgae was 5-10 times higher than that required to flocculate freshwater microalgae. Shammas [177] also noted that the higher cost of organic coagulants can be offset by the low dosages required compared to those of inorganic flocculants.

Microalgae harvesting techniques using chemical flocculation are not environmentally friendly because they introduce chemicals into the system which increase the dissolved solids and change color [179]. Inorganic flocculants can be toxic and can also have negative effects on microalgae by modifying their growth media and changing their color which prevents the reuse and recycling of water [11,17,137]. Hee-Mock et al. [180]) and Vandamme et al. [181] stated that chemical flocculants that are toxic and carcinogenic and are not suitable for harvesting microalgae biomass that is being processed for food supplements and food additives. Therefore, selection of flocculent should be based on cost, toxicity and reusability of the media [17].

Auto-flocculation Harvesting Methods

Some microalgae species can flocculate spontaneously, a process known, in a response to certain environmental stresses. This phenomenon is known as autoflocculation.

Types of Environmental Stress

There are several factors that affect the efficiency of autoflocculation, which include: pH, dissolved oxygen content, nitrogen concentration and the amount of calcium and magnesium ions in solution.

pH

When the pH of the medium is increased the cells come together and settle by gravitational force. The addition of more bases into the medium increased the formation of dense flocs which result in less settling times. However, it is important to note that not all species flocculate with increased pH levels [17,182].

Harith et al. [24] noted that at pH values less than 10 only slight separations between the microalgae and liquid media occurred after 4 h and increasing the pH from 8 to 10 using NaOH and KOH increased the flocculation efficiency from 13 to 82% and from 35 to 78% in 4 h, respectively. Wu et al. [182] noted that a pH of 10.5 resulted in 90% flocculation efficiency for the freshwater species *Chlorella vulgaris*, *Scenedesmus* sp. and *Chlorococcum* sp. and a pH of 9.0-9.3 resulted in 90% flocculation efficiency for the marine species *Nannochloropsis* sp. and *Phaeodactylum tricornutum*. Horiuchi et al. [183] noted a 96% flocculation efficiency in the marine species *Dunaliella tertiolecta* when the pH was adjusted to 8.6. Millamena et al. [148] also noted effective flocculation of microalgae when the pH was maintained above 10 in salt water.

Dissolved Oxygen

Uduman et al. [16] noted that flocculation in some microalgae species can occur naturally with changes in dissolved oxygen concentration. Schenk et al. [11] reported that dissolved oxygen stress can result in microalgae flocculation. Liao et al. [184] reported that increased dissolved oxygen in solution triggers autoflocculation by increasing the binding sites available on the cell surface. Greater binding sites result in bulk formation of the cells which increases the weight of

Microorganism	Bio-Flocculated Microalgae	Reference
Bacteria		
Bacillus licheniformis	*Desmodesmus sp.*	Ndikubwimana et al. [197]
P. stutzeri & B.cereus	*Pleurochrysis carterae*	Lee et al. [173]
Paenibacillus sp.	*Chlorella vulgaris*	Oh et al. [136]
Paenibacillus polymixa	*Scenedesmus* sp.	Kim et al. [198]
Bacillus subtilis	*Chlorella vulgaris*	Zheng et al. [114]
Fungi		
Ankistrodesmus falcatus	*Chlorella vulgaris*	Salim et al. [19]
Scenedesmus obliquus	*Chlorella vulgaris*	Salim et al. [19]
Tetraselmis suecica	*Nannochloropsis oleabundans*	Salim et al. [19]
Skeletonema	*Nannochloropsis*	Schenk et al. [11]

Table 3: Bio-flocculation of microalgae by use of fungi and bacteria microorganisms.

the flocs and increases the settling rate). They also noted that increased photosynthetic activity by the microorganisms increases the dissolved oxygen content and the formation of dense flocs. Wilen and Balmer [185] noted that large flocs can be generated when the dissolved oxygen concentration is high in the media. Koopman et al. [186] noted that dissolved oxygen concentrations of 14-16 mg/l promoted flocculation in the system.

Nitrogen

Auto-flocculation in microalgae cells may be triggered naturally as a result of environmental stress caused by nitrogen concentration [11.16]. Sukenik and Shelef [187] noted that certain species of microalgae flocculate with one another as a result of nitrogen stress in the media. Becker [188] noted that microalgae cells can aggregate with one another as a result of nitrate assimilation. Assimilation of nitrate nitrogen increases the pH of the medium and promotes cell flocculation [182,189]. Nurdogen and Oswald [190] also noted that nitrate assimilation resulted in auto-flocculation in microalgae species. Nguyen et al. [191] noted a nitrate concentration of 840.4 mg/L was sufficient in flocculating *Chlorella vulgaris* in mBB medium.

Addition of Ca^{2+} and Mg^{2+}

Autoflocculation occurs in the culture media spontaneously as a result of coprecipitation of calcium and magnesium salts present in the media which results in change of the pH of the medium [43]. Smith and Davis [192] evaluated Mg^{2+}, Ca^{2+} and CO_3^{2-} ions for their effectiveness in flocculating and settling of microalgae cells and found that Mg^{2+} ion with high pH levels resulted in effective flocculation and rapid sedimentation. They achieved settling rates that were 100-fold higher than those achieved with sedimentation. The reason for this phenomenon is that magnesium hydroxide flocs are positively charged while calcium carbonate flocs are negatively charged [193]. Thus, destabilization of the negatively charged microalgae cells is greater using magnesium as opposed to calcium. The optimal pH for autoflocculation is strain specific [187]. Nguyen et al. [191] reported that the species *Chlorella vulgaris* autoflocculated with and efficiency of 90% by addition of Ca^{2+} and Mg^{2+} at concentrations of 120 mg/l and 1000 mg/l, respectively. Vandamme et al. [194] noted that addition of Mg^{2+} in *Chlorella vulgaris* culture induced autoflocculation.

Advantages and Disadvantages

The advantages of this harvesting technique are the simplicity and low costs. The process can be reverted by pH adjustment using HCl to decrease the pH back to 7.5-8 [25]. However, autoflocculation does not occur in all species making it an unreliable process [39].

Using pH flocculation is beneficial since pH induced flocculated cells were identical to non-flocculated microalgae cells. This means that this autoflocculation technique has low-shear force on the cells when compared to centrifugation [195]. Knuckey et al. [25] found that the chlorophyll a from *T. pseudonana* cultures were intact using pH-induced methods, but centrifuged microalgae cells only has slight chlorophyll a peaks. This is necessary when the microalgae biomass is required for

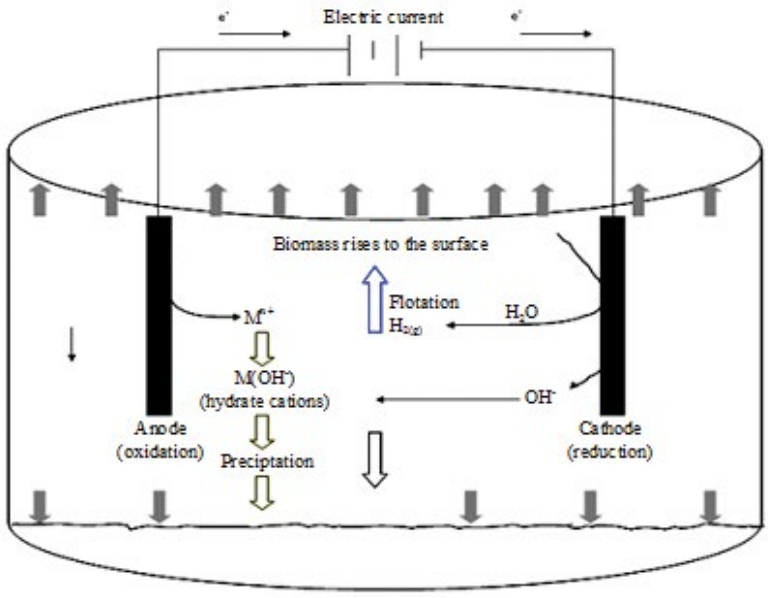


Figure 13: Dewatering microalgae through electrolytic coagulation [205].

use as feed diets or for use of extraction of certain compounds (such as chlorophyll a) from the cell. They also noted that the harvested microalgae species (used for oyster feed) using pH induced flocculation were a better diet choice compared to those harvested by centrifugation and much better than those harvested using ferric chloride flocculation processes. D'Souza et al. [196] also reported that pH induced harvesting of *C. muelleri* for feed to tiger prawn *P. mondon* were only slightly slower in developmental rate compared to those using fresh *C. muelleri*.

Bio-Flocculation Harvesting Methods

The use of microorganisms for the recovery of microalgae biomass has been investigated (Table 3) [11,19,114,136,173,197,198]. This method works by the addition of microorganisms to the culture which adhere to the microalgae cells causing the weight to increase and resulting in settlement of the cells to the bottom of the vessel. The supernatant containing the culture medium is decanted and washed with water in order to reduce the salinity [145,199].

Molina Grima et al. [17] noted the effective flocculation of *Chlorella* using bio-flocculent from bacteria species. Oh et al. [136] successfully harvested *Chlorella vulgaris* using the bio- flocculent bacterium *Paenibacillus* sp. Kim et al. [200] noted effective flocculation of the species *Scenedesmus* sp. using the bio-flocculent *Paenibacillus polymixa.* Ndikubwimana et al. [197] harvested the microalgae species *Desmodesmus sp.* using the bacterium *Bacillus licheniformis* with a 98% removal efficiency. Zhang and Hu [201] co-cultured the species *Chlorella vulgaris* with different filamentous fungi and extracted the microbial oil for transesterification into biodiesel.

Factors Affecting Bio-flocculation

The factors affecting bio-flocculation include: concentration of the bio-flocculent, pH and the selectivity of the microorganism.

Bio-flocculent Concentration

The rate at which bio-flocculation is achieved depends on the ratio of the bio-flocculent to the non-flocculating microalgae species. Bio-flocculent concentrations that are greater than the concentration of non-flocculating microalgae increase the rate of sedimentation [19]. Lee et al. [173] found that the addition of bacteria to the non-flocculating microalgae culture increased the rate of sedimentation. Salim et al. [19] successfully harvested non flocculating microalgae by addition of bioflocculating species and noted that the addition of bioflocculating microalgae induced the microalgae sedimentation and increased the efficiency of harvesting. Oh et al. [137] reported that the flocculation efficiency of *C. vulgaris* using the bacterium *Paenibasillus* sp. decreased with increasing dilutions of the bacterium. Zheng et al. [114] reported that the flocculation efficiency of *C. vulgaris* using the bio-flocculent *B. subtilis* increased with increasing concentrations of *C. vulgaris* biomass. Lee et al. [168] noted that *C. vulgaris* flocculation efficiency increased with increasing bacteria (*Flavobacterium*, *Terrimonas* and *Sphingobacterium*) concentration in the culture.

pH

The efficiency of bio-flocculation was noted to be affected by the pH of the medium. The pH alters the surface charge of the molecules in the medium which dictate the degree of attraction/repulsion. Oh et al. [136] reported that the flocculation efficiency of *C. vulgaris* using the bacterium *Paenibasillus* sp. increased with increasing pH from 5 to 11. Ndikubwimana et al. [197] noted that the flocculation efficiency of *Desmodesmus* sp. increased from 43 to 98% using the bacterium *Bacillus licheniformis* as the pH decreased from 7.2 to 3. Lee et al. [168] noted that pure *C. vulgaris* cultures showed no flocculation as the pH was increased from 3-11, but cultures with bacteria demonstrated increased flocculation efficiencies (from 43 to 94%) with increases in the pH over the range of 3-11. However, Zheng et al. [114] noted that the bio-flocculation efficiency using *B. subtilis* with microalgae species *Chlorella vulgaris* and *Chlorella protothecoides* were noted effected by pH.

Species Selectivity

Bio-flocculants are species specific which indicates that not all bio-flocculants will flocculate varying types of microalgae species. Oh et al. [136] reported that the bio-flocculent bacterium *Paenibasillus* sp. resulted in flocculation efficiencies in the range of 91-95% for *Botryococcus braunii*, *Scenedesmus quadricauda*, C. *vulgaris* and *Selenastrum capricornutum*, but efficiency in the range of 38 to 49% was noted for *Anabaena flos-aquae* and *Microcystis aeruginosa*. Grossart et al. [202] reported that bacteria were successful in aggregate formation of *Thalassiosira weissflogii* but has little effect on flocculation of *Navicula* sp. Oh et al. [136] and Kim et al. [198] noted that the flocculating with

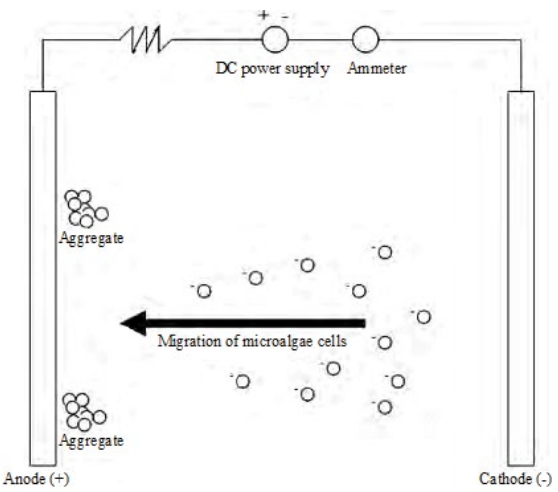


Figure 14: Dewatering microalgae through electrolytic flocculation [16].

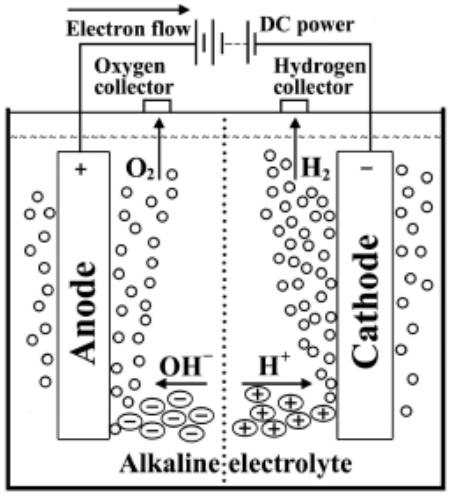


Figure 15: Dewatering microalgae through electrolytic flotation [212].

the bacterium *Paenibasillus* resulted in a flocculating efficiency of 83% and 95% with *Chlorella vulgaris* and *Scenedesmus* sp., respectively.

Advantages and Disadvantages

The advantages of using bio-flocculants include their biodegradability, non-toxic nature and the intermediates formed during degradation are not secondary pollutants [203]. Salim et al. [19] noted that a two step harvesting process using naturally flocculating microalgae to induce non-flocculating microalgae followed by centrifugation reduced the energy use from 13.8 MJ/kg (dry weight) to less than 2 MJ/kg (dry weight).

The disadvantages of this technique are that the microorganisms used to flocculate the algae are species-specific, and the recycling and recovery of these organisms can be difficult [17,204]. Oh et al. [136] stated that the bio-flocculants used to dewater the microalgae cells should be tested for acute oral toxicity in order for the retrieved biomass to be used in food additives and feed supplement. Vandamme et al. [181] indicated that the use of fungi or bacteria as flocculating agents results in microbiological contamination of the microalgae biomass, which needs to be assessed before use in feed or food products.

Electrophoresis Harvesting Methods

The electrolytic methods is used to eliminate the use of costly and toxic chemicals since microalgae behave much the same as colloid particles which allows for their separation from a water based medium by electric flied [16].

Types of Electrophoresis

The main electrophoresis methods that can be used for harvesting microalgae are: electrolytic coagulation, electrolytic flocculation and electrolytic flotation.

Electrolytic Coagulation

This type of electrophoresis method requires the use of both physical and chemical stimuli for the effective separation of microalgae biomass. The coagulation process is induced by the generation of current from an iron or aluminum electrode as shown in Figure 13 [205]. The amount of electrical current passing through the water medium dictates the amount of metal ions dissolved into the liquid suspension [206]. The metal ions released into the solution are metal hydroxides that contribute to the destabilization of colloid suspension and coagulate the biomass. Flocculation is achieved by the linking of the positively charged metal to the negatively charged microalgae cell and the movement toward the anode as a result of electrophoretic motion [179,206]. Rapid coagulation results from high current densities but the cost associated with the method is high.

Uduman et al. [207] used an aluminium electrode set at 5 V and achieved electrocoagulation efficiencies of 93.3% and 87.3% in 600 s for the species *Tetraselmis* sp. and *Chloroccum* sp., respectively. Azarian et al. [208] recovered 99.5% of total suspended solids in 15 minutes by electrocoagulation using a power source of 550 W with aluminium anode. They also noted that a power supply of 100 W required 30 minutes to achieve similar results. Ghernaout et al. [209] achieved a 99% removal of *Escherichia coli* in 20 min by electrocoagulation using aluminium electrode with a power supply of 12 W.

Electrolytic Flocculation

Electrolytic flocculation works by movement of negatively charged cells toward the anode as shown in Figure 14 [16]. At the anode, the cells lose their charge forming flocs that can be lifted to the surface by adhering to the bubbles formed by the electrolysis of the water [210].

Poelman et al. [210] tested the effectiveness of electrolytic flocculation in a 100 L vessel equipped with vertical electrodes and noted a removal efficiency of 80-95% in 35 min. They also noted that the rate of microalgae removal decreased with decreasing voltages and less energy was consumed when the total surface area of the electrodes was decreased and/or the distance between the electrodes was decreased.

Xu et al. [103] achieved a 93.6% recovery efficacy of *Botryococcus branii* after 30 minutes using electrolytic flocculation technique with a power supply of 6 W. Lee et al [168] noted a marine microalgae recoveries of 85% and 95% after 60 minutes using electrolytic flocculation with a power supply of 5 V and 5.2 V, respectively. Zenouzi et al. [211] reported a 97.4% removal efficiency of *Dunaliella salina* after 3 min using electrolytic flocculation.

Electrolytic Flotation

This technique is similar to electrolytic coagulation in that active metal anodes are used to flocculate the microalgae cells. The difference between the two techniques is that the cathode in electrolytic flotation is made from an inactive metal (steel) that is electrochemically nondepositing as shown in Figure 15 [212]. The inactive metal forms hydrogen bubbles from the electrolysis of the water. The particulates in the suspension attach to the gaseous bubbles and are lifted to the surface of the vessel [208,213].

Alfafara et al. [213] investigated the use of electrolytic floatation of microalgae cells using a polyvalent aluminium anode and titanium alloy cathode and found that increasing the electrical power decreased the electrolysis time and increased the rate of chlorophyll a removal. They also noted that the amount of chlorophyll measured was related to the concentration of microalgae removed by electrolysis. The usage of

Criteria	Importance	Description
Dewatering Efficiency	15	The system should be able to effectively concentrate and remove high percentage of the cells from their surrounding liquid media
Cost	15	The operational costs of the process should be low in order to reduce the total processing costs associated with microalgae recovery
Toxicity and health and environmental impact	15	The method should be non-toxic so that the retrieved algae biomass maybe processed for a number of value added products including ones for human consumption It should also be environmentally friendly in order to reduce the amount of toxic wastes produced
Suitability for Large Scale Use	15	The method should effective in handling large volumes for industrial production
Time	15	The rate of harvest should be quick to ensure the sustainability purposes
Species Specificity	10	The method should not be species or strain specific
Reusability of Media	8	The media should be recycled for reuse in order to minimize costs
Maintenance	7	Costs for maintaining the method should be low

Table 4: Criteria used for the comparative analysis of different harvesting techniques.

high electrical power is limited by the increase in heat and the increase in pH.

De Carvalho Neto et al. [214] reported a chlorophyll a removal efficiency of 99% using an electroflotation method running for 140 min with a power supply of 60 W. Ghernaout et al. [215] reported a microalgae removal efficiency of approximately 100% after 140 minutes in Ghrib Dam water using electroflotation method. Shelef et al. [43] noted that electroflotation technique resulted in total suspended solids in the range of 3-5%. Brennan and Owende [48] noted that there is little proof of the economic feasibility of this recovery method.

Energy Consumption

Dewatering microalgae by electrophoresis techniques requires less energy (0.2-2.1 Wh/g) compared to other harvesting methods (0.18-35.62 Wh/g). Vandamme et al. [141] reported that the amount of energy required to flocculate the freshwater microalgae *C. vulgaris* and the marine *P. tricornutum* species was 2.1 Wh/g and 0.2 Wh/g (dry weight), respectively. Gonzalez-Fernandez and Ballesteros [45]

Criteria	Description	Score
Dewatering Efficiency (15)	Settlement is based on density and since microalgae density is similar to that of water media, efficiency is low without the use of flocculants	5
Cost (15)	Minimum energy costs are required for this technique as gravitational forces are cost free	15
Toxicity and health and environmental impact (15)	This method is nontoxic to the cells, since it works by gravitational forces	15
Suitability for Large Scale (15)	Unsuitable for large scale use because of the long periods required for the process and it only works for microalgae cells with higher densities	5
Time (15)	Long periods of time are required to achieve settlement of microalgae cells through gravitational forces	2
Species Specificity (10)	Highly dependent on the type of species used. Species should have a higher density than that of water	4
Reusability of Media (8)	Method does not introduce any chemicals or alter the composition of the species/media	8
Maintenance (7)	No maintenance costs are required	7
Total (100)		**61**

Table 5: Evaluation of sedimentation.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective recovery of microalgae cells Depends on filter size and the size of microalgae cells	13
Cost (15)	Costs associated with pump and replacement of filters	9
Toxicity and health and environmental impact (15)	Cell composition remains intact and toxic chemicals are not required	15
Suitability for Large Scale (15)	Large pump and large filters are required for large scale	10
Time (15)	Rapid cell recovery	12
Species Specificity (10)	Dependent on the microalgae cell size	6
Reusability of Media (8)	Liquid media can be recycled	8
Maintenance (7)	Frequent filter replacement as a result of clogging	2
Total (100)		**75**

Table 6: Evaluation of vacuum filtration.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective in dewatering the microalgae. Suspended solids in the filtrate are low	13
Cost (15)	Costs associated with pump to create pressure and with filter replacements	9
Toxicity and health and environmental impact (15)	This method is non-toxic and cell composition is not altered	15
Suitability for Large Scale (15)	Suitable for large volumes but requires large filters and large pump	10
Time (15)	Relatively rapid cell recovery	12
Species Specificity (10)	Dependent on the size of the species	5
Reusability of Media (8)	Filtrate can be recycled and reused again for microalgae growth	8
Maintenance (7)	Costs associated with filter replacement	2
Total (100)		**74**

Table 7: Evaluation of pressure filtration.

Criteria	Description	Score
Dewatering Efficiency (15)	Complete removal of microalgae cells from the media	15
Cost (15)	Costs associated with pump and membrane	12
Toxicity and health and environmental impact (15)	This method is non-toxic	15
Suitability for Large Scale (15)	Suitable for large volumes of microalgae Smaller microalgae result in membrane clogging	10
Time (15)	Rapid cell recovery	12
Species Specificity (10)	Wide range of cell sizes can be used	8
Reusability of Media (8)	Filtrate can be recycled and reused again for microalgae growth	8
Maintenance (7)	Costs associated with filter replacement	4
Total (100)		**84**

Table 8: Evaluation of cross flow filtration.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective separation of solid particles from liquid suspensions	13
Cost (15)	Large amounts of energy are required for operation	8
Toxicity and health and environmental impact (15)	The use of toxic materials is not required Cell composition is not altered	15
Suitability for Large Scale (15)	Suitable for large volumes of microalgae	12
Time (15)	Rapid	15
Species Specificity (10)	No dependence on the type of species	10
Reusability of Media (8)	Supernatant can be easily recovered and recycled	8
Maintenance (7)	Not much maintenance is required	6
Total (100)		**87**

Table 9: Evaluation of disc stack centrifuge.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective separation of solid particles from liquid suspensions Solid concentrates are much more dense than those recovered using disc type	15
Cost (15)	More energy is required for operation compared to disc type	6
Toxicity and health and environmental impact (15)	The use of toxic materials is not required Cell composition is not altered	15
Suitability for Large Scale (15)	Suitable for large volumes of microalgae	12
Time (15)	Rapid	15
Species Specificity (10)	Suitable for larger species only	5
Reusability of Media (8)	Supernatant can be easily recovered and recycled	8
Maintenance (7)	Not much maintenance is required	6
Total (100)		**82**

Table 10: Evaluation of decanter centrifuge.

Criteria	Description	Score
Dewatering Efficiency (15)	Effectiveness depends on the likelihood of the cells coming into contact with the air bubbles in order to float to the surface Cell may rupture	12
Cost (15)	High costs are required for high speed agitation in order to produce the bubbles. Additional surfactants increase the costs	10
Toxicity and health and environmental impact (15)	The use of toxic materials is not required	12
Suitability for Large Scale (15)	Large volumes of microalgae can be used	13
Time (15)	The time required is dependent on the rate of agitation	10
Species Specificity (10)	Species should have high tolerance to avoid rupturing	5
Reusability of Media (8)	Media may be recycled for further use	8
Maintenance (7)	Not much maintenance is required	7
Total (100)		**77**

Table 11: Evaluation of dispersed air flotation.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective with the use of additional flocculants	10
Cost (15)	Operational costs are high, large amounts of energy would be required and the cost of flocculants for effective recovery is also high	9
Toxicity and health and environmental impact (15)	Inorganic flocculants are toxic	8
Suitability for Large Scale (15)	Large volumes of microalgae can be harvested	13
Time (15)	Dependent on the likelihood of the cells interacting with air bubble	10
Species Specificity (10)	Wide range of species can be used but species ability to adhere onto gas bubble is key	8
Reusability of Media (8)	Chemicals are not used and the medium can be recycled after it is saturated with air	5
Maintenance (7)	Not much maintenance is required	7
Total (100)		**70**

Table 12: Evaluation of dissolved air flotation.

noted that the marine microalgae required less energy for harvesting, because the marine medium allows for a higher conductivity that favors the electrocoagulation process. Kim et al. [200] reported that the electrical energy consumption of polarity exchange using two types of electrodes ranged from 1.19 to 1.23 Wh/g for 99% harvesting recovery of microalgae after 15 min. Bektas et al. [216] noted that dewatering of microalgae using electrocoagulation by 0.8-1.5 Wh/g of microalgae culture whereas cross flow filtration, pressure filters, vacuum filters, centrifugation and flocculation using polymers have consume 3.47, 0.18, 1.19, 1.67 and 35.62 Wh/g, respectively [15,17].

Advantages and Disadvantages

The advantages of the electrophoresis harvesting technique include: versatility, energy efficiency, safety, selectivity, environmental

Criteria	Description	Score
Dewatering Efficiency (15)	Effective with the use of additional flocculants	9
Cost (15)	Large amounts of energy would be required, but operational costs are much lower than those of dispersed and dissolved air techniques	10
Toxicity and health and environmental impact (15)	Coagulants are required for improving recovery effectiveness	10
Suitability for Large Scale (15)	Large volumes of microalgae can be harvested,	13
Time (15)	Dependent on the likelihood of the cells interacting the with air bubble	10
Species Specificity (10)	Can be used on a wide range of species but species ability to adhere onto gas bubble is key	8
Reusability of Media (8)	Recycled after it is saturated with air	6
Maintenance (7)	Not much maintenance is required	7
Total (100)		**73**

Table 13: Evaluation of fluidic oscillation.

Criteria	Description	Score
Dewatering Efficiency (15)	Cell concentration in liquid is low and depends on the position of the flocculent on the cell	10
Cost (15)	Large amounts of flocculants are required Does not require high amounts of energy	11
Toxicity and health and environmental impact (15)	Flocculating agents are toxic and not suitable for food additive and pharmaceutical products	5
Suitability for Large Scale (15)	Large volumes of microalgae suspensions can be used	15
Time (15)	Relatively fast	10
Species Specificity (10)	Wide range of species can be used but the process is dependent on the type of species used and how well the flocculent attaches to the cells	5
Reusability of Media (8)	The pH of the media left after harvest of microalgae is low which is not suitable for some microalgae species	2
Maintenance (7)	No maintenance required	7
Total (100)		**65**

Table 14: Evaluation of inorganic flocculation.

Criteria	Description	Score
Dewatering Efficiency (15)	Effectiveness is dependent on the position of the flocculent on the cell and the cell surface charge	11
Cost (15)	Expenses are associated with cost of flocculent Less amounts are required when compared to inorganic agents	11
Toxicity and health and environmental impact (15)	Organic compounds are non-toxic and can be used in the formation of addible and cosmetic by-products	15
Suitability for Large Scale (15)	Large volumes of microalgae suspensions can be used	15
Time (15)	Relatively fast	10
Species Specificity (10)	Process is dependent on the type of species used and how well the flocculent agent attaches to the cells	6
Reusability of Media (8)	Organic agents are non-toxic and the media can be recycled Changes in pH of media occur with flocculent addition and can affect the microalgae species	5
Maintenance (7)	No maintenance required	7
Total (100)		**80**

Table 15: Evaluation of organic flocculation.

Criteria	Description	Score
Dewatering Efficiency (15)	Flocculation is induced by pH change and separation depends on the response of the species to the environment	11
Cost (15)	Cost of chemicals purchased is relatively reasonable	12
Toxicity and health and environmental impact (15)	Chemicals used to induce flocculation can be toxic	8
Suitability for Large Scale (15)	Large volumes of microalgae suspension can be used Prolonged periods of time for sufficient settling is not suitable for large scale use	8
Time (15)	Long periods are required for the settling of the cells	8
Species Specificity (10)	Dependent on the response of the cell to the pH altered environment Is not suitable for all species	4
Reusability of Media (8)	The pH of the media is altered to induce the flocculation making the media unsuitable for reuse	2
Maintenance (7)	No maintenance required	7
Total (100)		**60**

Table 16: Evaluation of auto-flocculation.

compatibility and cost effectiveness [206]. Minimum energy is consumed when using optimum potential difference (0.331 kWh/m^3) by controlling the electrode surface area and distance between the electrodes. There are no added costs associated with flocculent products [16]. The costs associated with dewatering microalgae via electrolytic methods were significantly less than other harvesting methods such

Criteria	Description	Score
Dewatering Efficiency (15)	Effectiveness is dependent on the linkage of the bio-flocculent to the cells in order to increase their density	11
Cost (15)	Costs are associated with the purchasing the microorganisms and the maintenance of the culture	10
Toxicity and health and environmental impact (15)	Bio-flocculent species are non-toxic	15
Suitability for Large Scale (15)	Effective on large volumes of microalgae species	15
Time (15)	Based on the ability of the cells to link onto the microorganisms in order be more dense and improve settling time	10
Species Specificity (10)	Wide array of species can be used	8
Reusability of Media (8)	Microorganisms can be harvested and the medium can be recycled	3
Maintenance (7)	Maintenance of microorganism culture is required	4
Total (100)		**76**

Table 17: Evaluation of bio-flocculation.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective concentration of cells in liquid suspension	13
Cost (15)	High electrical power is required	11
Toxicity and health and environmental impact (15)	The addition of toxic chemicals is not required Cell composition can be altered	12
Suitability for Large Scale (15)	Large volumes require large power inputs which deem this method unsuitable for large scale	7
Time (15)	Rapid	15
Species Specificity (10)	Dependent on the charge of the species Conductivity of the water (marine water requires less energy)	3
Reusability of Media(8)	Microalgae can be harvested and the media can be recycled	5
Maintenance (7)	Effectiveness of electrode is reduced with continued use Frequent replacement of electrode maybe necessary	3
Total (100)		**69**

Table 18: Evaluation of electrolytic coagulation.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective in cumulating the cells together with one another	13
Cost (15)	Energy is required for rapid accumulation of the cells	11
Toxicity and health and environmental impact (15)	The addition of toxic chemicals is not required Cell composition can be altered	12
Suitability for Large Scale (15)	Unsuitable for large scale production because of energy requirement and the alteration of cell composition	8
Time (15)	Rapid	15
Species Specificity (10)	Dependent on the charge neutralization of the cell Conductivity of the water (marine water requires less energy)	5
Reusability of Media (8)	Media can be reused Cell composition can be altered	4
Maintenance (7)	Effectiveness of electrode is reduced with continued use Frequent replacement of electrode maybe necessary	3
Total (100)		**71**

Table 19: Evaluation of electrolytic flocculation.

Criteria	Description	Score
Dewatering Efficiency (15)	Effective in accumulating the cells together with one another Effectiveness is based on the likelihood that the cell comes in contact with the bubble in order to float to the surface	13
Cost (15)	High costs are associated with power supple	11
Toxicity and health and environmental impact (15)	The addition of toxic chemicals is not required Cell composition can be altered	12
Suitability for Large Scale (15)	Unsuitable for large scale production because of the energy required and the alteration of cell composition	8
Time (15)	Dependent on the likelihood that the cell adheres to a bubbles in order to float to the surface	10
Species Specificity (10)	Dependent on the charge neutralization of the cell Conductivity of the water (marine water requires less energy)	4
Reusability of Media (8)	Media can be reused	4
Maintenance (7)	Effectiveness of electrode is reduced with continued use Frequent replacement of electrode maybe necessary	3
Total (100)		**65**

Table 20: Evaluation of electrolytic flotation.

Criteria	Physical									Chemical		AF	BF	Electrophoresis		
	S	VF	PF	CFF	DSC	DC	DAF	DVF	FO	IF	OF			EC	EFC	EFT
Dewatering Efficiency (15)	5	13	13	15	13	15	12	10	9	10	11	11	11	13	13	13
Cost (15)	15	9	9	12	8	6	10	9	10	11	11	12	10	11	11	11
Toxicity and health and environmental impact (15)	15	15	15	15	15	15	12	8	10	5	15	8	15	12	12	12
Suitability for large scale (15)	5	10	10	10	12	12	13	13	13	15	15	8	15	7	8	8
Time (15)	2	12	12	12	15	15	10	10	10	10	10	8	10	15	15	10
Species specificity (10)	4	6	5	8	10	5	5	8	8	5	6	4	8	3	5	4
Reusability of Media (8)	8	8	8	8	8	8	8	5	6	2	5	2	3	5	4	4
Maintenance (7)	7	2	2	4	6	6	7	7	7	7	7	7	4	3	3	3
Total (100)	**61**	**75**	**74**	**84**	**87**	**82**	**77**	**70**	**73**	**65**	**80**	**60**	**76**	**69**	**71**	**65**

S: Sedimentation
VF: Vacuum filtration
PF: Pressure filtration
CFF: Cross flow filtration
DSC: Disc stack centrifugation
DC: Decanter centrifugation
DAF: Dispersed air flotation
DVF: Dissolved air flotation
FO: Fluidic oscillation
IF: Inorganic flocculation
OF: Organic flocculation
AF: Auto-flocculation
BF: Bio-flocculation
EC: Electrolytic coagulation
EFC: Electrolytic flocculation
EFT: Electrolytic flotation

Table 21: Comparative analysis.

as sedimentation with flocculants, centrifugation and flotation with flocculants [210]. This indicates that although higher electrical energy is consumed using electrolytic methods, the cost to harvest is much lower than other harvesting techniques.

Some of the drawbacks associated with this harvesting technique include: cathode fouling and change in cell composition [210]. The current intensity decreases by 5-10% upon reuse of the cathode due to internal resistance [141] and changes in cell composition can be induced using high current densities [45].

Combination of Methods

Cost effective methods for cell harvesting are vital for the economics of biodiesel production. Harvesting processes account for 20-30 % of the total biomass production costs [17]. Selection of harvesting technique is dependent on the size and density of the microalgae cells, conditions of the culture and concentration of biomass and target product value [13].

Schenk et al. [11] reviewed various harvesting methods and noted that the combination of flocculation with sedimentation or flotation with filtration or flotation with centrifugation to be the most economical alternatives. Brennan and Owende [48] and Uduman et al. [16] reported that energy can be conserved and cost can be reduced by harvesting the microalgae in a two-step process using two techniques. Initially, the microalgae are concentrated to 2-7% total suspended solids by the process of flocculation and the cells are further concentrated into a paste (suspended solid concentration of 15-25%) by a secondary harvesting step such as filtration or electrophoresis. Funk et al. [217] integrated dissolved air flotation with chemical flocculation (ferric sulfate) and noted increased recovery efficiency from 88% to 95% for *Chlorella vulgaris*.

Kim et al. [200] reported that a new and innovative technique for improving the economics of the electrophoresis processes is the combination of electrolytic coagulation and electrolytic flotation into continuous electrolytic microalgae. In this method, the current direction (polarity exchanges) is exchanged for the continuous harvest of microalgae and their cultivation. The current direction creates two phases by using a pair of electrodes. The first phase works to destabilize the negatively charged microalgae cells forming flocs. The formation of flocs is mediated by metal ions that are released from the electrode dissolving in solution. In the second phase, the metal ion generation is halted and the bubbles formed from both electrodes lift the flocs to the top of the solution causing them to float.

Xu et al. [131] used electroflocculation integrated with dispersed air flotation and noted a harvesting efficiency of 98.9% in 14 min. They noted that the cell aggregate increased with the integrated system as opposed to those observed with electroflocculation. The use of dispersed air flotation increased the rate of aggregate formation. However, the stress from continued air supplementation into the system disturbed the up-floated flocs into algal aggregates. Thus disturbance was avoided by halting the supplementation of air into the system once the aggregate size reached its peak value.

Comparative Analysis

Selection of Criteria

Eight criteria (Table 4) were used for the evaluation of microalgae harvesting techniques: (a) dewatering efficiency, (b) cost, (c) toxicity (d) suitability for large scale use, (e) time, (f) species specificity, (g) reusability of media and (h) maintenance. These criteria were selected based on the information reported in the literature about these microalgae harvesting methods. The comparative analysis was performed using these criteria to determine the most efficient, cost effective and environmentally friendly dewatering technique for a wide array of microalgae species that is suitable for large scale application.

Assigning Score to Each Criterion

Each of the selected criteria was assigned a score from 7 to 15 which was determined by the degree of importance of the criterion (Table 4). Higher values were given to the criteria that were deemed most important for development of an efficient and economic large scale dewatering method for microalgae. Lower values were given to criteria that were deemed necessary for determining a suitable method but were considered less important. These values were then used to determine the effectiveness of each harvesting method as shown in Tables 5-20.

Analysis

The sum of the scores obtained for each method are presented in Table 21. The results indicated that of the 16 methods used for microalgae harvesting evaluated in this study, 4 had scores of 80/100

or above and are, therefore, deemed suitable for harvesting a wide array of microalgae species at the industrial scale. These methods are disc stack centrifuge (87/100), cross flow filtration (84/100), decanter centrifugation (82/100) and organic flocculation (80/100).

Three of the nine physical harvesting methods (disk stack centrifugation, cross flow filtration and decanter centrifugation) were considered suitable for large scale harvesting of microalgae because they are highly effective in removing microalgae biomass from the liquid medium, non-toxic and rapid and the medium can be reused. The organic flocculation is suitable for microalgae harvesting on a large scale because it can be used with a wide range of microalgae, the organic chemicals are not toxic and the medium can be recycled.

The other 12 harvesting methods were deemed unsuitable for harvesting microalgae at the industrial scale because they did not meet the evaluation criteria (suitability for dewatering a wide array of microalgae species, suitability for large volumes, low operation costs and low maintenance). The other 6 physical methods were not as effective in removing the algae biomass, required long time, were not suitable for large scale, required high maintenance and were not effective for a wide array of microalgae. Autoflocculation techniques are unsuitable for large scale use because the chemicals used for pH change are toxic, are species specific, require long time and recycling of the medium requires additional costly treatment. The bio-flocculation technique depends on the desirable end product, since the microorganisms used for flocculation are harvested with the cells, the flocculating cultures must be adjusted for viable growth and the process is costly. Electrophoresis methods were deemed unsuitable for large scale microalgae harvesting because of difficulty in scaling up and the disruption of the cells can affect the quality and yield of the desired end product.

Conclusions

The major obstacle for using microalgae biomass on an industrial-scale for production of value added products is the dewatering step which accounts for 20-30% of the total costs associated with the process. A comparative analyses of 16 harvesting techniques that included 9 physical methods (sedimentation, vacuum filtration, pressure filtration, cross flow filtration, disc stack centrifugation, dispersed air floatation, dissolved air flotation, fluidic oscillation), 2 chemical methods (inorganic flocculation, organic flocculation), auto-flocculation method, bio-flocculation method and 3 electrophoresis methods (electrolytic coagulation, electrolytic flocculation, electrolytic floatation) were undertaken. Selection of the most suitable harvesting methods was based on the effectiveness, cost, toxicity, processing time, species specificity, maintenance and suitability for operating on large scale. Each of the selected criteria was assigned a score ranging from 7 to 15 depending on the degree of importance of the criterion.

The results indicated that of the 16 methods evaluated, 4 scored values of 80/100 or above and were deemed suitable for harvesting microalgae on an industrial scale. Three of which were physical techniques (disc stack centrifuge (87/100), cross flow filtration (84/100), decanter centrifugation (82/100)) and the forth was the organic flocculation (80/100) method. These techniques were deemed suitable for large scale use because of their effective dewatering ability, low operational costs, suitability for numerous species, rapidness, require minimal maintenance and being environmentally friendly. The other methods were deemed unsuitable because they are not suitable for dewatering a wide array of microalgae species, not suited for large volumes, costly and require high maintenance.

Although any of these four techniques is deemed suitable for harvesting of microalgae and can be used alone, a combination of methods can also be used to further enhance the recovery efficiency and improve the economics. The use of flocculation as an initial harvesting step to concentrate the algae suspension allows for effective removal of algae biomass from large liquid media. The costs associated with energy intensive centrifugation or filtration techniques can be reduced by using these methods as secondary techniques since less volume of microalgae suspension will be required to undergo the secondary treatment. It is, therefore, recommended that the use of centrifugation or filtration microalgae harvesting techniques be coupled (as secondary techniques) with organic flocculation (as an initial dewatering step) in order to improve the economics of the overall process.

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