Chapter 8
Improvement of Harvesting Technology for Algal Biomass Production

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8.1 Introduction

Global demands for biomass utilization as food, feed, biofuels and chemical production have been increased to a great extent. For a sustainable future it is necessary to minimise the environmental impact of our activities keeping in mind the socio-economic parameters along with operational efficiency. Our continuous dependence on fossil fuels is unsustainable because of its dwindling world reserves and global warming due to its use. Recent research has focussed on the development of renewable and potentially carbon neutral biofuels. First generation biofuels derived from terrestrial crops has impacted the environment in a big way by hastening deforestation and environmental pollution. The food vs. fuel debate has also come into force. Replacing them with second generation biofuels which is derived from lignocellulosic feedstock has addressed majority of the problems. But a concern over land usage and competition still remains. Third generation biofuels derived from microalgae seem to be the solution to the demand for alternative energy sources which is devoid of the major drawbacks associated with first and second generation of biofuels.

Microalgae are photosynthetic microorganisms with simple growing requirements (light, sugars, CO_2, N, P, and K) that can produce lipids, proteins and carbohydrates in large amounts over a short period of time. These products can be processed into both biofuels and valuable co-products. Research on microalgal cultivation has focussed on high-value products. High rate algal biomass production for low-value applications can be realized only when the technologies are cost-efficient and environment friendly. This led to the scientists focussing on the increase of algal biomass production by utilising different methods. The research concentrated on improvement of photobioreactors for biomass production (Morweiser et al., 2010),

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selection of suitable strains for different products (Larkum et al., 2012) and genetic engineering of metabolic pathways (Georgianna and Mayfield, 2012). To reduce the cost of microalgal biomass production, research on downstream processing of biomass is essential (Greenwell et al., 2010).

Today, microalgal production is rapidly moving from laboratory to pilot scale and commercial scale demo installations (Georgianna and Mayfield, 2012), prompting the need for cost and energy efficient downstream processing technologies. A major hurdle to cost effective production of algal biomass is the harvesting of microalgae. The microalgal cells are very small in size. So, it is required to separate a very small amount of biomass from a large volume of culture medium. A high biomass concentration leads to mutual shading of the microalgal cells and thus a reduction in productivity; therefore, biomass concentrations in microalgal cultures are usually low: 0.5 g L^{-1} in open pond reactors and 5 g L^{-1} in photobioreactors. This indicates that a large volume of water has to be removed to harvest the biomass.

8.2 Surface Characteristics of Microalgae

Algae are unique eukaryotic microorganisms, which convert sunlight, water and CO_2 to biomass resource with the process of photosynthesis. The factors which determine the stability of algal cells in the medium are surface charge, size and density. These factors influence the separation characteristics of the algal cells from the aqueous medium. The interactions between the algal cells as well as interactions with the surrounding medium govern the stability of the cells (Tenney et al., 1969). The settling rate of biomass is governed by the size and cell density which plays an important role in separation through sedimentation and centrifugation.

Suspended particles usually carry a positive or negative surface charge in water. They attract ions with an opposite charge from the solution to maintain their electrical neutrality. This is known as the counter ion effect. Together they form an electrical double layer consisting of the particle surface charge and the counter ions. A dense layer of ions is formed by the counter ions called the Stern layer. It is inaccessible to other counter ions. A balance is created further away from the particle surface between electrostatic attraction and thermal diffusion. This leads to the formation of a diffuse layer further away from the particle surface which results in an exponential decrease of the potential difference between the particle surface and the bulk solution with distance from the particle surface. An electrical repulsion between the particles is a result of the cloud of counter ions surrounding the particle. The ζ potential is the potential difference between the bulk fluid and the layer of counter ions that remains associated with the charged particle when the particle is moving through the solution (the slipping plane). The ζ potential can be estimated from the mobility of the charged particles in an electric field; therefore, it is a useful indicator of the degree of repulsion between charged particles in a suspension. A high ζ potential (>25 mV, positive or negative) leads to strong electrical repulsion between particles and the suspension is said to be stable. When the ζ potential is close to zero, particles can approach each other to a point where they will be

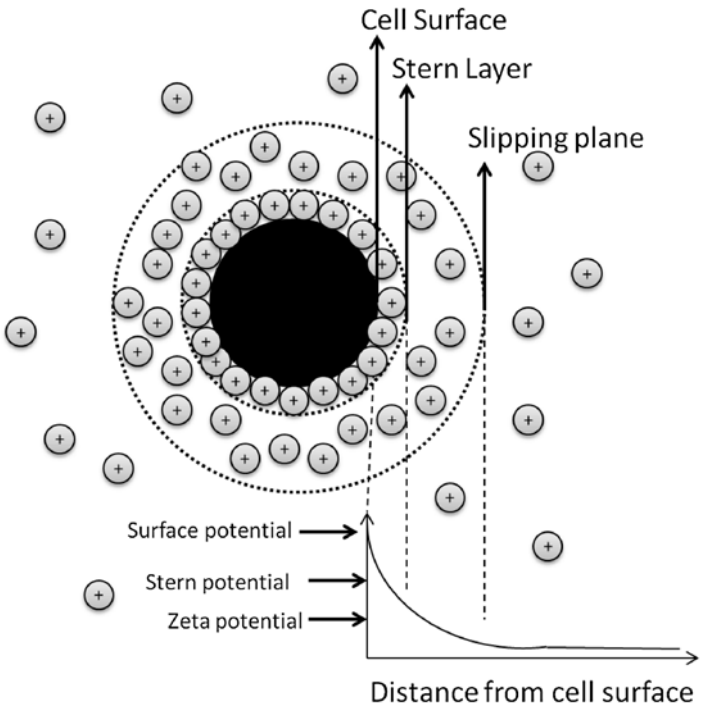

Fig. 8.1 Negatively charged microalgal cells surrounded by an electric double layer of charged ions (modified from Vandamme et al., Trends in Biotechnology, 2013).

attracted by Van der Waals forces. This leads to aggregation of particles. A similar observation is for microalgal cells where they are stabilized in media by the surface charge of the cells. The cell surface predominantly has carboxylic (-COOH) and amine ($-NH_2$) groups which leads to a surface charge. The carboxylic groups dissociate and are negatively charged above pH 4–5, whereas the amine groups are uncharged at this pH. This results in a net negative surface charge above pH 4–5 (Fig. 8.1).

8.3 Methods of Algae Harvesting

The further processing of algal biomass to value added products requires the algal culture to be devoid of water. This is an important step in the life cycle assessment (LCA) of the process as harvesting of biomass incurs the highest of the total production costs (20 %–30 %) (Rawat et al., 2011). An efficient harvesting method is required for large scale extraction of products from microalgae (Amaro et al., 2011; Uduman et al., 2010b). Selection of the appropriate harvesting method is of great importance to the economics of biofuels production. The different factors which

determine the appropriate method of harvesting depends on the microalgal characteristics, i.e., the density and size of algal cells as well as the product derived from the algal biomass (Amaro et al., 2011). The ideal harvesting method should not be dependent on the algal species, should be energy efficient and environment friendly and may also release the desired product from the algal biomass (Chen et al., 2011). In order to emerge as a sustainable process, microalgae have to be primarily dewatered and concentrated from dilute cultures. This is one of the major challenges which one has to overcome (Uduman et al., 2010a). Secondly the algal cells are highly stable in the medium owing to their negative charge and excess algogenic organic materials they produce during their metabolism (Amaro et al., 2011). Many harvesting strategies, like centrifugation, sedimentation, flocculation, flotation and micro-filtration, can be used to harvest microalgae (Amaro et al., 2011), electrophoresis (Amaro et al., 2011; Uduman et al., 2010a) and any combination of these (Rawat et al., 2011).

Microalgae harvesting can generally be divided into a two-step process (Fig. 8.2), including:

- Bulk harvesting: The purpose of this is to separate microalgal biomass from the bulk suspension. By this method, the total solid matter can reach 2–7 % w/v using flocculation, flotation, or gravity sedimentation (Brennan and Owende, 2010).
- Thickening: The purpose of this harvesting is to concentrate the slurry with filtration and centrifugation. This step needs more energy than bulk harvesting (Brennan and Owende, 2010).

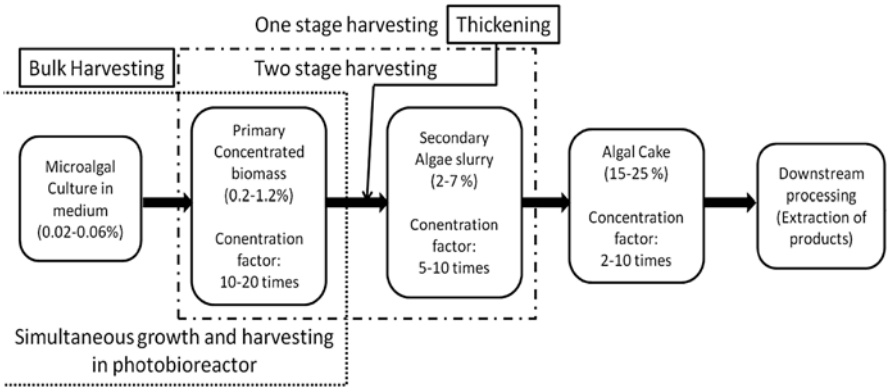

Fig. 8.2 Schematic representation of the essential steps of harvesting of microalgal biomass.

8.3.1 Screening

Two primary screening devices used for microalgal harvesting are microstrainers and vibrating screen filters because of their mechanical simplicity and availability in large unit sizes. A microstrainer is an array of rotating filters with very fine mesh screens which are frequently backwashed. They have several advantages, such as simplicity in function and construction, easy operation, low investment, negligible abrasion as a result of absence of quickly moving parts, being energy intensive and having high ion ratios. The concentration of microalgae plays an important part in determining the filtration efficiency of the device. A high microalgal concentration leads to clogging of the filtration screens whereas a low microalgal concentration may lead to inefficient separation (Wilde et al., 1991). Studies on microstraining by Molina Grima et al. (2003) confirmed this result and concluded that it would be necessary to flocculate the cells before microstraining. A highly efficient method for microalgal harvesting may be tangential flow filtration where 70–89 % recovery of microalgal biomass is possible (Petrusevski et al., 1995). In addition, tangential flow filtration retains the structure, properties and motility of the collected microalgae. Although the successful laboratory studies for concentrating microalgae, used in downstream fractionation (Rossignol et al., 1999; Rossi et al., 2004), a definitive study on large-scale algal harvesting is yet to be studied. Lazarova et al. (2006) showed that the cost of microfiltering river water can be as low as 0.2 kW h^{-1} m^{-3} of water processed. Decreasing the process volume by at least a factor of 100 significantly lowers the costs of disruption and fractionation stages downstream. Several variables associated with the choice of membranes and type of organisms could increase this cost, and there is a considerable scope for optimization of this process. As a guide to potential improvement, the costs of desalination by reverse osmosis, where a far higher pressure process is used, have fallen dramatically (85 %) over the past decade to give a total production cost of about \$1 m^{-3} and with desalination energy costs being as low as 3 kW h^{-1} m^{-3}. This is largely down to a better membrane technology; greater membrane longevity, increased scale of operation and better system management and such advances might also be expected in membrane separation processes for harvesting of microalgae (Greenwell et al., 2010).

8.3.1.1 Microstraining

The rotary drum of microstrainers is covered by a straining fabric, stainless steel or polyester. Concentrations of algae through microstrainers was very low which required further dewatering. Continuous backwashing was required in order to increase the concentration of the harvested microalgal biomass from high rate clarification ponds. Small microalgae could not pass through the microstrainers leading to very dilute slurry which required further concentration (Koopman et al., 1978; Shelef et al., 1980). Successful studies were conducted on *Microactinium* and *Scenedesmus* species with the smallest mesh size of 23 μm. Greater success in

reducing suspended solids of a stabilization lagoon effluent from about 80 to 20 mg l^{-1} or less by rotary microstrainers mounted with 1 μm screen has been achieved (Wettman and Cravens, 1980). Thickening of *Coelastrum proboscideum* to about 1.5 % suspended solids by microstrainers was reported when operating at a cost of about DM 0.02 m^{-3} and an energy consumption of 0.2 kW h^{-1} m^{-3} (Mohn, 1980). Problems encountered with microstrainers included low harvesting efficiency and difficulty in handling particle fluctuations. These problems may be overcome in part by varying the drum rotation speed (Reynolds et al., 1975). Another problem associated with microstraining was the build up of bacterial and algae biofilm slime on the fabric or mesh. Periodic fabric or mesh cleaning may help inhibit this biomass growth.

8.3.1.2 Vibrating Screen

Earlier studies by Mohn (1980) reported the harvesting of *Coelastrum* algae by vibrating screen. Higher algae solids concentration of 7–8 % has been harvested under batch operation in comparison with lower algal solids contents of 5–6 % when operated in continuous mode. In a commercial production, vibrating screens were used for harvesting *Spirulina* as a food source which are multicellular and filamentous blue-green microalgae belonging to two separate genera *Spirulina* and *Arthrospira* (Habib et al., 2008). The vibrating screen filtration used for harvesting achieved very high algae removal efficiency of up to 95 % for harvesting up to 20 m^3 h^{-1} from which algal slurry of 8–10 % solid contents was produced. Compared to inclining screens counterpart with a filtration area of 2–4 m^2 per unit, the vibrating screens required only one-third of the area.

8.3.2 Coagulation–Flocculation

Coagulation–flocculation is a process in which algal cells aggregate together to form larger clumps. These larger clumps can be easily filtered or rapidly settle down to be harvested easily. Chemicals used as algal coagulants could be broadly grouped into two categories – inorganic and long-chain organic coagulants. Flocculation can be achieved in several ways and a wide range of approaches for flocculating microalgae have been explored in recent years. These approaches range from traditional flocculation methods that are widely used in other fields of industry (e.g., chemical flocculation) to novel ideas based on the biology of microalgae (e.g. bioflocculation) and the use of emerging technologies (e.g. use of magnetic nanoparticles).

8.3.2.1 Chemical Flocculation

Water treatment and mining industries widely use chemical flocculants like ferric chloride and alum. Although metal salts are being applied for harvesting microalgae (e.g., *Dunaliella*; Ben-Amotz and Avron, 1990), their use results in high

concentrations of metals in the harvested biomass. These metals remain in the biomass residue after extraction of lipids or carotenoids (Rwehumbiza et al., 2012). Further use of the protein residue of algal biomass as animal feed is restricted as the accumulated metal ions may interfere in the metabolism of the animals. The valorization of the protein fraction as animal feed is said to be important for making microalgal biofuels economically viable (Wijffels et al., 2010). Despite this shortcoming, metal coagulants provide a good model system to study the interaction between flocculants and microalgal cells because their properties are well understood (Wyatt et al., 2012; Zhang et al., 2012). Other commonly used chemical flocculants in other industries are synthetic polyacrylamide polymers. These may however contain traces of toxic acrylamide and thus also contaminate the microalgal biomass (Bratby, 2008). Flocculants based on natural biopolymers are therefore a safer alternative. As microalgal cell surfaces are negatively charged, the biopolymers used for flocculation should be positively charged, which is rare in nature. A well-known positively charged biopolymer is chitosan, which is derived from chitin, a waste product from shellfish production. Chitosan is a very efficient flocculant but it works only at low pH, but pH in microalgal cultures is relatively high (Chang and Lee, 2012). An alternative to chitosan is cationic starch, which is prepared from starch by addition of quaternary ammonium groups. The charge of those quaternary ammonium groups is independent of pH and therefore cationic starch works over a broader pH range than chitosan (Vandamme et al., 2010). Other examples of biopolymers that can be used to flocculate microalgae are poly-γ glutamic acid (an extracellular polymer produced by *Bacillus subtilis*) (Zheng et al., 2012) or polymers present in flour from *Moringa oleifera* seeds (Teixeira et al., 2012). Coiling of polymer flocculants at high ionic strengths is a general problem faced which makes it ineffective (Uduman et al., 2010a). Therefore, they are less suitable for harvesting microalgae cultivated in seawater.

8.3.2.2 Autoflocculation

An increase in pH above 9 leads to the spontaneous flocculation of microalgae (Spilling et al., 2011). The property of CO_2 sequestration by microalgae leads to the decrease in pH of the medium during photosynthesis. This causes the spontaneous aggregation of the cells which leads to flocculation. Autoflocculation is associated with the formation of calcium or magnesium precipitates. Depending on the conditions, these precipitates carry positive surface charges and can induce flocculation through charge neutralization and/or sweeping flocculation. When calcium ions are in excess in the medium, they interact with the negatively charged surface of the cells resulting in positively charged aggregates of calcium phosphate (Christenson and Sims, 2011; Schlesinger et al., 2012). This type of flocculation requires a high concentration of phosphates in the medium. So as to make the process sustainable, microalgae growing in wastewater containing high amounts of phosphate may be harvested by this method (Lundquist et al., 2010). Magnesium hydroxide or brucite also precipitates at high pH. These precipitates are positively charged up to pH 12

and can therefore also interact with the microalgal cell surface to cause flocculation (Vandamme et al., 2012; Wu et al., 2012). Most waters contain sufficiently high background concentrations of magnesium for this process to occur. Calcium carbonate or calcite also precipitates at high pH but whether it can induce flocculation of microalgae remains to be demonstrated. Flocculation at high pH is caused by formation of inorganic precipitates and not by pH as such; therefore, the harvested biomass contains high concentrations of minerals (Show et al., 2013). Although these have a low toxicity, it is nevertheless preferable to remove them from the biomass.

8.3.2.3 Physical Flocculation Methods

The above mentioned methods highly contaminate the algal biomass by introducing different chemical compounds. This can be minimized by using only physical forces to harvest the biomass. For instance, flocculation of microalgae can be accomplished by applying a field of standing ultrasound waves. On a laboratory scale ultrasonic methods may be feasible but large scale applications are still to be tested (Bosma et al., 2003). In electro coagulation flocculation, flocculation is induced through electrolytic release of metal ions from a sacrificial anode (Vandamme et al., 2011). The efficiency of this method might be improved by changing the polarity of the electrodes (Kim et al., 2012). It is similar to flocculation by metal salts but the contamination of algal biomass by metals is to a minimum in case of electroflocculation. OriginOil claims to have developed a solution for this problem. Its method uses only electromagnetic pulses to neutralize the surface charge of microalgal cells and induce flocculation (Gouveia, 2011). Recently, several studies have explored the use of magnetic nanoparticles to harvest microalgae. Magnetite (Fe_2O_3) nanoparticles may adsorb directly on the microalgal cells, upon which the cells can be separated from the medium by applying a magnetic field. This method thus combines flocculation and separation in a single process step (Cerff et al., 2012). Magnetite nanoparticles seem to adsorb more easily on some microalgal species than on others (Xu et al., 2011). Adsorption can be improved by coating the nanoparticles with cationic polymers (Lim et al., 2012; Liu et al., 2009). An advantage of using magnetite nanoparticles for harvesting microalgae is that the nanoparticles can be recovered after harvesting and subsequently reused (Cerff et al., 2012).

8.3.2.4 Bioflocculation

Spontaneous flocculation of microalgae is a common phenomenon observed in microalgal blooms in lakes, rivers or ponds. This spontaneous flocculation is assumed to be caused by extracellular polymer substances in the medium and is called bioflocculation. Bioflocculation is often successfully used for harvesting microalgae in facilities where micro-algae are used in wastewater treatment (Craggs et al., 2012). Further research is required to understand the underlying mechanism

associated with bioflocculation. This may lead to a sustainable and cost effective process of harvesting of algal biomass. Some microalgal species flocculate more readily than others and such naturally bioflocculating microalgae can be mixed with other species to induce flocculation (Schenk et al., 2008; Taylor et al., 2012). There are indications that bioflocculation may be initiated by info chemicals (Eldridge et al., 2012). Recently, an info chemical isolated from a senescent and flocculating culture of a *Skeletonema* species was found to be capable of inducing flocculation in a culture of another species of microalgae (Salim et al., 2012). Bacteria or fungi can also induce bioflocculation of microalgae. Some fungi, for instance, have positively charged hyphae that can interact with the negatively charged microalgal cell surface and cause flocculation (Zhou et al., 2012; Zhang and Hu, 2012). Specific consortia of bacteria can also induce flocculation of microalgae (Gutzeit et al., 2005; Lee et al., 2008). Co-flocculation using bacteria or fungi require an organic carbon source in the medium. This can be provided by using wastewater for growth of microalgae. The presence of an organic carbon source in wastewater allows both the organisms to thrive together. This results in a culture of mixed algal–bacterial flocs that can be easily harvested (Van Den Hende et al., 2011; Su et al., 2011). The use of bacteria or fungi as a flocculating agent avoids chemical contamination of the biomass but results in microbiological contamination, which may also interfere with food or feed applications of the microalgal biomass. But if the biomass is being used for biofuels, specific species of fungi high in intracellular contents may be grown with microalgae which do not need separation later on.

8.3.3 Filtration

Filtration is a method in which the algal culture is run through filters which hold them and let the water pass through them. This is a continuous process which results into a thick paste of algae. Microfiltration, dead end filtration, vacuum filtration, pressure filtration, ultra filtration, and tangential flow filtration (TFF) are a few different filtration forms (Harun et al., 2010). The application of filtration is generally limited to the laboratory scale. Large scale applications often lead to membrane clogging, formation of compressible filter cakes and high maintenance costs limiting its acceptability. Separation of small microalgae through filtration is costly which limits its usage to filamentous or large microalgae. Generally tangential flow filtration is used for separation of microalgae. Another advantage of TFF is that it maintains the structure, properties and motility of the filtered microalgae. But the costs incurred during pumping and replacement of filtration membranes limits its utility in the large scale. Application of pressure or vacuum is used for recovering large microalgae but a higher concentration of biomass is required for this. A higher power consumption for these operations is required (in the range of 0.3–2 kW h^{-1} m^{-3}) which is almost similar to the power consumed during centrifugation (Molina Grima et al., 2003). Larger algae can be effectively recovered by vacuum filtration in combination with filter aid, while micro-filtration or ultra filtrations are effective

in recovering smaller algae. Another filtration method called tangential flow filtration is a high rate method. About 70 %–89 % algae was recovered using this method (Rawat et al., 2011). Considering the output and initial feedstock concentration, according to recent studies, TFF and pressure filtration are energy efficient harvesting methods. Issues like back mixing make simple filtration methods, for example dead end filtration, inadequate for dewatering microalgae culture. However, when used along with centrifugation, give better separation. Filtration methods, in spite of being an attractive dewatering option, have extensive running costs and hidden pre-concentration requirements (Harun et al., 2010).

8.3.4 Gravity Sedimentation

Common applications of sedimentation include separation of microalgae in water and wastewater treatments. Density and radius of algae cells and the induced sedimentation velocity influence the settling characteristic of suspended solids (Brennan and Owende, 2010). Sedimentation is a very simple process but the rate of sedimentation is very slow (0.1–2.6 m h^{-1}) (Choi et al., 2006). High temperature environments such as temperate climates may lead to the degradation of algal biomass. Enhanced microalgal harvesting by sedimentation can be achieved through lamella separators and sedimentation tanks (Uduman et al., 2010a). The success of solids removal by gravity settling depends highly on the density of microalgal particles. Studies by Edzwald (1993) showed that low-density microalgal particles are unsuccessfully separated by settling. Flocculation is frequently used to increase the efficiency of gravity sedimentation.

8.3.4.1 Clarification in Simple Sedimentation Tank or Pond

Reports on algal sedimentation in ponds were always accompanied by flocculation methods. Operation involving fill-and-draw cycle for secondary pond gave rise to significant removal of algae from facultative oxidation pond effluent (Benemann et al., 1980). Similar secondary ponds were used for algae settling from high rate oxidation pond effluent (Adan and Lee, 1980; Benemann et al., 1980). Well clarified effluent and algae slurry up to 3 % solids contents were achieved attributable to algae autoflocculation which enhanced the settling. Coagulant dosing in a settling tube to promote algae sedimentation was investigated by Mohn (1980). The batched operation achieved an algal concentration of 1.5 % solids content. Separation of algae in sedimentation ponds is a simple and inexpensive process. However the rate of sedimentation was influenced by the use of flocculants. So an in-depth study of the flocculating nature of microalgae is required in order to use sedimentation tanks or ponds for harvesting of microalgae. Different flocculants can be screened for this purpose in order to determine the best one for the process.

8.3.4.2 Lamella Type Sedimentation Tank

Modifications to the simple sedimentation tanks were applied in which flat inclined plates were introduced in the tanks. The slopes of the plates were designed such that the down gliding of the settled algal particles were collected in a sump from which they were removed by pumping (Mohn, 1980; Shelef et al., 1984). Algae were concentrated to 1.6 % solids content, and coagulant dosing was suggested if suspension of tiny algae such as *Scenedesmus* was fed into the system (Mohn, 1980). Operational reliability of this method was fair and further thickening of algae slurry was required.

8.3.4.3 Flocculation–Sedimentation

A flocculation followed by gravity sedimentation process for algae separation has been studied (Golueke and Oswald 1965). Treating high rate oxidation pond effluent, the process achieved up to 85 % of the algal biomass using alum as a coagulant. This was found to be an effective process where algae slurry with 1.5 % w/v of solid content was achieved. A comparison of the flocculation–sedimentation process with flocculation-flotation method indicated that the latter exhibited very clear optimal operating conditions for algae separation (Friedman et al., 1977; Moraine et al., 1980).

8.3.5 Floatation

Some algae may not have a settling velocity which may not be significant for gravitational separation. In turn they float on the water surface and are hard to settle down. This problem may be solved by use of floatation techniques for harvesting. Flotation was simply gravity thickening upside down. Instead of waiting for the particles to settle to the bottom, liquid–solid separation was brought about by introducing air bubbles at the bottom of a flotation tank. The combined buoyancy of the particulate matter and the bubbles encouraged them to rise to the surface. Once the particles had floated to the surface, a layer of thickened slurry will be formed which could be collected by a skimming operation (Chen et al., 1998). A coagulant in an optimal dose was required for efficient harvesting of algal biomass by the floatation method (Bare et al., 1975). Different coagulants had been used in flotation systems. Chemicals such as aluminium and ferric salts, and polymers were used to facilitate the flotation with the overall objective to increase allowable solids loadings, percentage of floated solids, and clarity of the effluent. The time factor which was limiting in the case of sedimentation was overcome using the floatation technique. Flotation systems also offered higher solids concentrations and lower initial equipment cost. There are three basic variations of the flotation thickening systems: dissolved-air flotation, electro-flotation (or called electrolytic flotation), and dispersed-air flotation.

8.3.5.1 Dissolved-Air Flotation (DAF)

In the dissolved-air flotation (DAF) system, a liquid stream saturated with pressurized air is added to the DAF unit where it is mixed with the incoming feed. As the pressure returns to atmosphere, the dissolved air comes out of solution forming fine bubbles bringing fine particles with them, which rise to the surface where they are removed by a skimmer. Use of DAF process for algae separation in conjunction with chemical flocculation has been reported (McGarry and Durrani, 1970; Bare et al., 1975; Sandbank, 1979). The parameters responsible for determining the quality of the clarified effluent were recycling rate, air tank pressure, hydraulic retention time and particle floating rate (Bare et al., 1975; Sandbank, 1979). The concentration of the slurry depended on the skimmer speed and its overboard above water surface (Moraine et al., 1980). DAF was used for cleaning algae pond effluents with a high efficiency of thickening (up to 6 %). DAF along with floatation could further increase the concentration of the harvested algae (Bare et al., 1975; Friedman et al., 1977; Moraine et al., 1980). Optimization of parameters for DAF yielded higher separation efficiency of the biomass.

8.3.5.2 Electro-Flotation

During electrolysis the water splits into hydrogen. Hydrogen in the form of gas bubbles attaches to algal particles. This helps the algae particles to float on the water where they can be removed by skimming the surface. Further discussion of research on electro-flotation will be presented in Section 8.3.7.

8.3.5.3 Dispersed Air Flotation

Introduction of non-pressurized air into the floatation tank could provide us with an alternative to DAF. DAF could be modified by combining agitation with air injection to form foam. The algal cells could then be separated by froth floatation or foam floatation. Different factors such as aeration rate, pH of the algae suspension and temperature of operation were significant in determining the separation efficiency by foam floatation (Phoochinda and White, 2003; Phoochinda et al., 2004). *Scenedesmus quadricauda* was used to study dispersed air floatation. Surfactants such as cetyl trimethyl ammonium bromide (CTAB) and sodium dodecylsulfate (SDS) were also added to increase the separation efficiency. A very high algal removal was observed using CTAB (90 %) as compared to SDS (16 %). Algal removal efficiency could be increased to 80 % by adjusting the pH of the algal suspension. Reports by Golueke and Oswald (1965) suggested that very few reagents (2 out of 18) gave high removal efficiency. Another study suggested that dispersed air floatation was governed by the controlled pH of the medium (Levin et al., 1962). Critical pH level was recorded at 4.0, being attributed to the changes in the algae surface characteristics.

8.3.5.4 Ozone Flotation

Few reports studied the effect of ozone floatation for algae recovery (Betzer et al., 1980; Benoufella et al., 1994; Jin et al., 2006; Cheng et al., 2010, 2011). Ozone gas modified the cell surface of the algae which promoted the floatation of the cells and release of some agents which aided in floatation. A pilot plant study was conducted using ozone floatation on *Microcystis* (Benoufella et al., 1994). Different aspects of ozone floatation such as oxidising properties of ozone and floatation properties of the cells were studied. They indicated that ozone was responsible for inactivation of the cells. Ozone floatation in association with coagulation flocculation was found to be an efficient process for removal of cyanobacteria. Ferric chloride was found out to be the most potent coagulant. Preozonation also influenced the enhancement of the coagulation efficiency. *Scenedesmus obliquus* FSP-3, a species with excellent potential for CO_2 capture and lipid production, was harvested using dispersed ozone flotation. Ozone produces effective solid–liquid separation through flotation while air does not (Cheng et al., 2011). The ozone dose required for harvesting of algae was similar to that used for purification of water. During ozonation, the algae removal rate, surface charge, and hydrophobicity of algal cells and fluorescence characteristics and proteins and polysaccharides contents of algogenic organic matter (AOM) were determined. The proteins released from the AOMs altered the hydrophobicity of the bubble surfaces leading to formation of a froth layer which helped in harvesting of the microalgae. Humic substances in the suspension scavenge ozone adversely affected the ozone flotation efficiency of algal cells.

8.3.6 Centrifugation

Centrifugation can be described by Stokes' law, which predicts that its velocity is proportional to the difference in density between the cells and medium on the one hand and on the square of the radius of the cells (Stokes radius) on the other hand. Although for bacteria gravitational force-based methods are not easy to apply, for yeast and microalgae with diameters >5 μm and relatively thick cell walls they are feasible. High operating costs incurred during centrifugation negate the reliability and efficiency of the method. Laboratory centrifugation tests were conducted on pond effluent at 500–1,000 g and showed that about 80–90 % microalgae can be recovered within 2–5 min (Molina Grima et al., 2003). Centrifuges are analogous to sedimentation tanks except that the suspended particles were accelerated in their separation from the suspension by a centrifugal force that was higher than the gravity force. Several centrifugation devices were examined for application in algae separation (Mohn and Soeder, 1978, 1980; Moraine et al., 1980; Shelef et al., 1980, 1984). Some of them were very efficient as one-step separation process while others were found either inefficient or required thickened feed slurry. Centrifuges operated in batch mode were less attractive, as their operation had to be stopped for the solids to be removed. Knuckey et al. (2006) also states that the exposure of microalgal

cells to high gravitational and shear forces can damage cell structure. According to Molina Grima et al. (2003), centrifugation is a preferred method, especially for producing extended shelf-life concentrates for aquaculture; however, they agree that this method is time-consuming and costly. Energy costs of about 1 kW h^{-1} m^{-3} have been quoted for centrifugation.

8.3.6.1 Solid-Bowl Decanter Centrifuge

The solid-bowl decanter centrifuge is a horizontal conical bowl which contains a screw conveyor that rotates in the same direction. Feed slurry enters at the centre and is centrifuged against the bowl wall. Settled solids were moved by the screw conveyor to one end of the bowl before discharge, while separated water formed a concentric inner layer which flowed over an adjustable dam plate and was discharged out of the centrifuge. The helical screw conveyor that pushed the deposited slurry operated at a higher rotational speed than the bowl. A solid bowl screw centrifuge was used to separate various types of algae (Mohn, 1980). A 22 % solids concentration was obtained in the separated algae when the feed suspension contained 2 % solids. Although the reliability of this centrifuge seemed to be excellent, the energy consumption was far too high. An attempt to concentrate an algal feed of 5.5 % solids derived from a flotation process by a co-current solid-bowl decanter centrifuge was not successful (Shelef et al., 1980). Subsequently, algae slurry concentration was improved to 21 % w/v TSS by reducing the screw conveyor speed to 5 rpm (Shelef et al., 1984). The solid-bowl decanter centrifuge was recommended for use concurrently with polyelectrolyte coagulant to increase the efficiency (Shelef et al., 1984).

8.3.6.2 Nozzle-Type Centrifuge

Continuous discharge of solids as a slurry was possible with the nozzle-type disc centrifuge. The shape of the bowl was modified so that the slurry space had a conical section which provided sufficient storage volume and afforded a good flow profile for the ejected cake (Shelef et al., 1984). The bowl walls sloped toward a peripheral zone containing evenly spaced nozzles. The number and size of the nozzles were optimized to avoid cake build up and to obtain reasonable concentrate of algal biomass. The application of nozzle-type disc centrifuge for algae harvesting, suggested by Golueke and Oswald (1965), investigated the influence of nozzle diameter on flow rate, algae removal efficiency and resultant slurry concentration. By comparing with other algae harvesting methods, it was concluded that the nozzle-type centrifuge seemed to be promising albeit it was less attractive because of power requirements and capitalization costs. In other studies, the centrifuge appeared to be more effective to harvest *Scenedesmus* than *Coelastrum* (Mohn and Soeder, 1978, 1980). By returning the centrifuge underflow to the feed, the solids content of the algae suspension (0.1 %) could be concentrated by a factor of

15–150 %. The reliability of this device could be ensured as long as the clogging of the nozzles was avoided.

8.3.6.3 Solid-Ejecting Disc Centrifuge

Solid-ejecting disc centrifuge provided intermittent solids ejection by regulating its valve-controlled peripheral ports by timer or an automatic triggering device. The advantage of this centrifuge for algae harvesting was its ability to produce algal cake in a single step without dosing with chemicals (Mohn and Soeder, 1978, 1980; Shelef et al., 1984). This centrifuge concentrated various types of microalgae effectively, achieving algal cake of 12–25 % solids (Mohn, 1980; Moraine et al., 1980). The extent of the algae suspension separation increased with increasing residence time (decreasing feed rate), and the ejected cake concentration was affected by the intervals between successive desludging (Shelef et al., 1984). Solid-ejecting disc centrifuges were found to be very reliable as the only setback reported was that solids finer than algae may be retained in the overflow which reduced the separation efficiency (Moraine et al., 1980). High capital and energy costs rendered this separation method unappealing.

8.3.7 Electrophoresis, Electroflotation and Electroflocculation Techniques

The electrolytic method is another potential approach to separate algae without the need to add any chemicals. In this method, an electric field drives charged algae to move out of the solution (Mollah et al., 2004). Water electrolysis generates hydrogen that adheres to the microalgal flocs and carries them to the surface. Electrocoagulation mechanisms involve three consecutive stages:

- Generation of coagulants by electrolytic oxidation of the sacrificial electrode
- Destabilization of particulate suspension and breaking of emulsion
- Aggregation of the destabilized phases to form flocs.

Electrical approaches to algae thickening included exploiting electrophoresis, electro-flocculation and electro-flotation. In a water solution, however, both electrophoresis and electroflocculation could occur under the same set of circumstances. If a vessel of algae in its growth medium was exposed to an electric field by placing metallic electrodes on two sides of the vessel and energizing them with a dc voltage, algae concentrations would occur at both electrodes (electrophoresis) and at the bottom of the tray (electro-flocculation). Research focussed on assessment of the factors influencing electrophoresis and electro-flocculation of algae in its growth medium was conducted (Pearsall et al., 2011) and the results showed that electrophoresis does occur but was complicated by the effects of the fluid motion. It

appeared that the coupling of the algal cell and the fluid could be sufficiently strong such that fluid motion effects could influence or dominate behaviour. Electroflocculation appeared to be a robust process (Azarian et al., 2007). It does, however, inherently leave electrically induced trace metal flocculants in the dewatered algae. In electro-flotation or electrolytic flotation, fine hydrogen gas bubbles formed during the electrolysis which would cause the algal particles to float to the surface where they would be removed. Efficient bench scale electro-flotation system for algae flocculation by using the magnesium hydroxide formed in the electrolysis to enable precipitation and consequently flocculation was reported (Contreras et al., 1981). Laboratory and field scale electro-flotation units for algae removal from wastewater oxidation pond effluent was also reported (Sandbank et al., 1974; Kumar et al., 1981). A 2 m^2 pilot scale unit was tested for clarification of high-rate oxidation pond effluent (Shelef et al., 1984). For satisfactory algae separation, electro-flotation was followed or operated concurrently with alum flocculation (Sandbank et al., 1974).

Azarian et al. (2007) investigated the removal of microalgae from industrial wastewater using continuous flow electro-coagulation. Different from electrolytic coagulation, electrolytic flocculation does not require the use of sacrificial electrodes. Electrolytic flocculation works based on the movement of microalgae to the anode to neutralize the carried charge and then form aggregates. Poelman et al. (1997) showed that the efficiency of algal removal is 80–95 % when electrolytic flocculation is applied. There are several benefits to use electrochemical methods, including environmental compatibility, versatility, energy efficiency, safety, selectivity, and cost effectiveness (Mollah et al., 2004). An investigation into the removal of microalgae electrolytically in batch and continuous reactors by flotation was conducted by Alfafara et al. (2002). The results for a batch system showed that by increasing the electrical power input, the rate of chlorophyll removal increased and the electrolysis time decreased. Gao et al. (2010a, b) studied the algae removal by electro-coagulation–flotation (ECF) technology and indicated that aluminum was an excellent electrode material for algae removal when compared with iron. The optimal parameters determined were current density = 1 mA cm^{-2}, pH = 4–7, water temperature = 18–36 °C, algae density = 0.55×10^9–1.55×10^9 cells L^{-1}. Under the optimal conditions, 100 % of algae removal was achieved with the energy consumption as low as 0.4 kW m^{-3}. The ECF performed well in acid and neutral conditions. At low initial pH of 4–7, the cell density of algae was effectively removed in the ECF, mainly through the charge neutralization mechanism; while the algae removal worsened when the pH increased (7–10), and the main mechanism shifted to sweeping flocculation and enmeshment.

Furthermore, initial cell density and water temperature could also influence the algae removal. Overall, the results indicated that the ECF technology was effective for algae removal, from both the technical and economical points of view (Gao et al., 2010a, b). Recently, OriginOil company is employing several next-generation technologies to greatly enhance algae cultivation and oil extraction (OriginOil, 2010), by going on to control the harvesting and oil extraction cycles in a high-speed, round-the-clock, streamlined industrial production of algae oil. In the pro-

cess, mature algae culture is injected through the OriginOil device, where Quantum Fracturing™, pulsed electromagnetic fields and pH modification (using CO_2) combine to break the cell walls, thereby releasing the oil within the cells. The processed culture now travels into a settling tank, or gravity clarifier, to fully separate into oil, water and biomass. Algae oil increases to the top for skimming and refining, while the remaining biomass settles to the bottom for further processing as fuel and other valuable products (OriginOil, 2010).

8.3.8 Ultrasonic Methods

Application of ultrasound technique to harvest microalgae has been reported in a laboratory scale study (Bosma et al., 2003). Algae separation process based on acoustically induced aggregation followed by enhanced sedimentation was carried out. Efficiencies higher than 90 % were recorded at high biomass concentrations and flow rates between 4 and 6 l d^{-1}. As much as 92 % of the algae biomass could be harvested with a concentration factor of 11. Attempts to harvest at higher efficiency were unfruitful due to small size and low particle density of the microalgae. Feed flow rate, biomass concentration and ratio between harvest and feed flows had a significant effect on the concentration factor. Use of ultrasound to improve the removal by coagulation of *M. Aeruginosa*—a common species of toxic algae—was investigated (Zhang et al., 2009). The results showed that sonication significantly enhanced the reduction of algae cells, solution UV 254, and chlorophyll-*a* without increasing the concentration of aqueous microcystins. The main mechanism involved the destruction during ultrasonic irradiation of gas vacuoles inside algae cells that acted as 'nuclei' for acoustic cavitation and collapse during the "bubble crush" period, resulting in the settlement of cyanobacteria. The investigation revealed that coagulation efficiency depended strongly on the coagulant dose and sonication conditions. With a coagulant dose of 0.5 mg L^{-1} and ultrasonic irradiation for 5 s, algae removal efficiency increased from 35 % to 67 %. Optimal sonication time was determined at 5 s, and further sonication would only marginally enhance the coagulation efficiency. The most effective sonication intensity was found to be at 47.2 W cm^{-2}, and the highest removal of the algae was recorded at 93.5 %. The authors recommended that this method could be successfully applied to natural water containing multiple species of algae. Ultrasonic harvesting on lab- or pilot-scale experiments has shown the merits that in addition to small footprint, the process could be operated continuously without evoking hydrodynamic shear stress on algal cells thus maintaining integrity of the algae (Bosma et al., 2003). The authors pointed out, however, that for industrial scale harvesting, centrifuges could better be used over the ultrasound aggregation sedimentation process because of lower energy requirement, better algae separation efficiencies and higher concentration factors.

8.4 Comparative Studies Amongst the Algae Separation Processes

Harvesting of microalgal biomass is one of the bottlenecks for biofuel production from microalgae (Li et al., 2011). It can be inferred from the above different harvesting methods that each of them have their own advantages and disadvantages and it also shows that efficiency of one method can be increased if integrated with another method, for example, integrating sedimentation with flocculation. Another such efficient method, which integrated electro-flocculation with dispersed-air flotation, was used for harvesting *Botryococcus braunii* (Li et al., 2011). According to another author, flocculation in combination with flotation or sedimentation followed by centrifugation or filtration is the most energy and cost efficient choice (Salim et al., 2011). Thus, integration of different methods is an efficient technology for harvesting microalgae. While undertaking research on harvesting, oil extraction, and refining processes for biofuel production from microalgae, nature and type of microalgal strain should be considered. Shape of algal cells, cell wall structure and oil composition vary from one algal strain to another; even two different cultures of the same strain are not similar in nature (Li et al., 2011). Although there are several biomass harvesting methods, Richmond (2004) suggested one main criterion for selecting a proper harvesting procedure is the desired product quality. In one hand for low value products, gravity sedimentation may be used, possibly enhanced by flocculation. Sedimentation tanks or settling ponds are also possible, e.g. to recover biomass from sewage-based processes. On the other hand for high-value products, to recover high-quality algae, such as for food, feed and nutraceuticals, it is often recommended to use continuously operating centrifuges that can process large volumes of biomass (Table 8.1).

8.5 Future Scope of Studies

Many research efforts are currently directed towards genetic modification of microalgae. Most recently published studies and granted patents in this field are aimed at increasing biomass productivity or increasing production of specific metabolites, most often lipids (Larkum et al., 2012; Georgianna and Mayfield, 2012). However, genetic modification may also be a promising way to harvest microalgae (Christenson and Sims, 2011; Georgianna and Mayfield, 2012). Here, achievements in genetic modification of yeast may be used as an example. In yeast, genetically modified strains have been developed that express flocculin proteins in their cell walls, causing the cells to aggregate (Govender et al., 2008). The expression of these proteins can be induced by an environmental trigger or during a specific growth stage. Sapphire Energy has described a method for flocculating microalgae in which ligand–receptor pairs can be expressed in different strains that are mixed to induce flocculation, or that are expressed sequentially in the same strain (Mendez et al., 2010). Genetic modification or selection may also be aimed at facilitating flocculation by other methods. For instance, a cell wall-deficient mutant of *Chlamydomonas*

Table 8.1 Comparative analysis between different methods of harvesting

Method	Advantages	Disadvantages
Screening	• Methods of screening are very simple	• Clogging of screens due to microalgal slime
	• Easy to operate	• High operational costs
Coagulation-flocculation	• Simple and easy to establish	• Chemical flocculants cause accumulation of harmful compounds in biomass
	• High efficiency	
	• Sustainable and low in energy input	• Not suitable for high value added products
Filtration	• High recovery efficiency of biomass	• Fouling of membranes
	• Can be scaled up	• Energy intensive process
	• Easy for bulk harvesting	
Gravity sedimentation	• Cost effective process	• Slow rate of separation
	• Easy to operate	• Not suitable for very small microalgae
	• Bulk harvesting	
Floatation	• Simple and effective process	• Low harvesting efficiency
	• Can be made efficient in combination with other harvesting methods	• Some methods may damage the cells
Centrifugation	• Highly efficient process	• Energy intensive process
	• Up to 98 % recovery of biomass	• High maintenance costs
	• Can be scaled up	
Electrophoresis	• High efficiency	• Energy intensive process
	• Combines different methods	• Non-sustainable
		• Scale up not possible
Ultrasonication	• Simultaneous recovery of product possible	• Energy intensive process
	• Easy to operate	• May lead to damage of cells
	• Can be scaled up	

has been found to flocculate much more easily under alkaline conditions than the wild type strain (Scholz et al., 2011). This indicates that minor genetic modifications may greatly facilitate flocculation. Future studies should concentrate on the genetic modifications of microalgae. The flocculating substances produced by different organisms could be used for cost efficient harvesting of microalgae.

8.6 Conclusion

Microalgae are considered as efficient feedstock for production of third generation biofuels. The varied uses of microalgal biomass make it an important commodity of the future. Research on decreasing the cost of algal biomass production and its

subsequent processing is on the rise. In order to come to a sustainable and cost efficient solution the problem of harvesting needs to be addressed. The cost of downstream processing of algal biomass alone accounts for most of the price required for the production. Efficient harvesting methods need to be developed in order to achieve the goal. The different techniques used for harvesting of microalgal biomass provide a roadmap for efficient separation of biomass. Some methods are too energy intensive to be used in a large scale. Use of bioflocculants for efficient separation of microalgal biomass is on the rise. Different flocculants such as guar gum, cationic starch and extracts have been used for efficient harvesting of microalgae. The biomass obtained after bioflocculation is devoid of any chemicals and can be used for further processing of the biomass.

New efforts concentrating on nanotechnology are also coming into focus. Magnetic nanoparticles are being used for effective harvesting of microalgal biomass. However, the large scale application of these methods still needs to be studied in depth. Genetic engineering of algae to produce external flocculants may be the path to the future. The algal cells can be modified as such they can express some surface agents which may help them to coagulate. This may probably present a cost effective and sustainable solution to the problem of dewatering of microalgal biomass. Fundamental research into infochemicals that induce flocculation in microalgae is urgently needed, because this may lead to a highly controllable method for inducing flocculation that avoids contamination. The same holds true for approaches to induce flocculation through genetic modification. Future studies should not only look at the efficiency of harvesting under specific conditions, but should also investigate how harvesting is influenced by properties of the microalgal cells or by culture conditions, particularly interference by organic matter in the culture medium. Cost is an important factor to consider when evaluating new flocculation methods for microalgae. Cost evaluation should not only take the cost of flocculation step itself into account, but also the influence on the entire production process.

References

Adan, B. and Lee, E.W. (1980). High rate algae growth pond under tropical conditions. Presented at a workshop on waste treatment and nutrient recovery. Singapore, 27–29 February, 1980.

Alfafara, C.G., Nakano, K., Nomura, N., Igarashi, T. and Matsumura, M. (2002). Operating and scale-up factors for the electrolytic removal of algae from eutrophied lake water. *Journal of Chemical Technology and Biotechnology*, 77, 871–876.

Amaro, H.M., Guedes, A.C. and Malcata, F.X. (2011). Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy*, 88(10), 3402–3410.

Azarian, G.H., Mesdaghinia, A.R., Vaezi, F., Nabizadeh, R. and Nematollahi, D. (2007). Algae removal by electro-coagulation process, application for treatment of the effluent from an industrial wastewater treatment plant. *Iranian Journal of Public Health*, 36, 57–64.

Bare, W.F.R., Jones, N.B. and Middlebrook, E.J. (1975). Algae removal using dissolved air flotation. *Journal of Water Pollution Control Federation*, 47, 153–169.

Ben-Amotz, A. and Avron, M. (1990). The biotechnology of cultivating the halotolerant alga *Dunaliella. Trends in Biotechnology*, 8, 121–125.

Benemann, J.R., Kopman, B.L., Weismsman, D.E., Eisenverg, D.E. and Goebel, R.P. (1980). Development of microalgae harvesting and high rate ponds technologies in California. *In:* Shelef, B., Solder, C.J. (Eds), Algae Biomass. Elsevier, Amsterdam.

Benoufella, F., Laplanche, A., Boisdon, V. and Bourbigot, M.M. (1994). Elimination of microcystis cyanobacteria (blue-green algae) by an ozoflotation process—A pilot-plant study. *Water Science & Technology*, 30, 245–257.

Betzer, N., Argaman, Y. and Kott, Y. (1980). Effluent treatment and algae recovery by ozone-induced flotation. *Water Research*, 14, 1003–1009.

Bosma, R., van Spronsen, W.A., Tramper, J. and Wijffels, R.H. (2003). Ultrasound, a new separation technique to harvest microalgae. *Journal of Applied Phycology*, 15, 143–153.

Bratby, J. (2008). Coagulation and Flocculation in Water and Wastewater Treatment. IWA Publishing.

Brennan, L. and Owende, P. (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14, 557–577.

Cerff, M., Morweiser, M., Dillschneider, R., Michel, A., Menzel, K. and Posten, P. (2012). Harvesting fresh water and marine algae by magnetic separation: Screening of separation parameters and high gradient magnetic filtration. *Bioresource Technology*, 118, 289–295.

Chang, Y.R. and Lee, D.J. (2012). Coagulation–membrane filtration of *Chlorella vulgaris* at different growth phases. *Drying Technology*, 30, 1317–1322.

Chen, C. Y., Yeh, K. L., Aisyah, R., Lee, D. J., & Chang, J. S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource technology*, *102*(1), 71–81.

Chen, M.Y., Liu, J.C. and Ju, Y.H. (1998). Flotation removal of algae from water. *Colloids and Surfaces B*, 12, 49–55.

Cheng, Y.L., Juang, Y.C., Liao, G.Y., Ho, S.H., Yeh, K.L., Chen, C.Y., Chang, J.S., Liu, J.C. and Lee, D.J. (2010). Dispersed ozone flotation of *Chlorella vulgaris. Bioresource Technology*, 101, 9092–9096.

Cheng, Y.L., Juang, Y.C., Liao, G.Y., Tsai, P.W., Ho, S.H., Yeh, K.L., Chen, C.Y., Chang, J.S., Liu, J.C., Chen, W.M. and Lee, D.J. (2011). Harvesting of *Scenedesmus obliquus* FSP-3 using dispersed ozone flotation. *Bioresource Technnology*, 102, 82–87.

Choi, S.K., Lee, J.Y., Kwon, D.Y. and Cho, K.J. (2006). Settling characteristics of problem algae in the water treatment process. *Water Science & Technology*, 53, 113–119.

Christenson, L. and Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances*, 29, 686–702.

Contreras, S., Pieber, M., del Rio, A., Soto, M.A., Toha, J. and Veloz, A. (1981). A highly efficient electrolytic method for microalgae flocculation from aqueous cultures. *Biotechnology and Bioengineering*, 23, 1165–1168.

Craggs, R., Sutherland, D. and Campbell, H. (2012). Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology*, 24, 329–337.

Edzwald, J.K. (1993). Algae, bubbles, coagulants, and dissolved air flotation. *Water Science & Technology*, 27, 67–81.

Eldridge, R.J., Hill, D.R.A. and Gladman, B.R. (2012). A comparative study of the coagulation behaviour of marine microalgae. *Journal of Applied Phycology*, 24, 1667–1679.

Friedman, A.A., Peaks, D.A. and Nichols, R.L. (1977). Algae separation from oxidation pond effluents. *Journal of Water Pollution Control Federation*, 49, 111–119.

Gao, S., Du, M., Tian, J., Yang, J., Ma, F. and Nan, J. (2010b). Effects of chloride ions on electrocoagulation-flotation process with aluminum electrodes for algae removal. *Journal of Hazardous Materials*, 182, 827–834.

Gao, S., Yang, J., Tian, J., Ma, F., Tu, G. and Du, M. (2010a). Electro-coagulation-flotation process for algae removal. *J Hazard Mater*, 177, 336–343.

Georgianna, D.R. and Mayfield, S.P. (2012). Exploiting diversity and synthetic biology for the production of algal biofuels. *Nature*, 488, 329–335.

Gouveia, L. (2011). Microalgae as Feedstock for Biofuels. Springer. (http://dz.doi.org/10.1063/1.3294480)

Govender, P., Domingo, J.L., Bester, M.C., Pretorius, I.S. and Bauer, F.F. (2008). Controlled expression of the dominant flocculation genes *FLO1*, *FLO5*, and *FLO11* in *Saccharomyces cerevisiae. Applied Environmental Microbiology*, 74, 6041–6052.

Greenwell, H.C., Laurens, L.M.L. , Shields, R.J., Lovitt, R.W. and Flynn K.J. (2010). Placing microalgae on the biofuels priority list: A review of the technological challenges. *Journal of the Royal Society Interface*, 7, 703–726.

Gutzeit, G., Lorch, D., Weber, A., Engels, M. and Neis, U. (2005). Bioflocculent algal-bacterial biomass improves low-cost wastewater treatment. *Water Science & Technology*, 52, 9–18.

Habib, M.A., Parvin, M., Huntington, T.C. and Hasan, M.R. (2008). A review on culture, production and use of *Spirulina* as food for humans and needs for domestic animals and fish. FAO Fisheries and Aquaculture Circular No. 1034, Food and Agriculture Organization of the United Nations, FAO Fisheries and Aquaculture Department Rome, Italy.

Harun, R., Singh, M., Forde, G.M. and Danquah, M.K. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews*, 14, 1037–1047.

Jin, P.K., Wang, X.C. and Hu, G. (2006). A dispersed-ozone flotation (DOF) separator for tertiary wastewater treatment. *Water Science & Technology*, 53, 151–157.

Kim, J., Ryu, B.G., Kim, B.K., Han, J.I. and Yang, J.W. (2012). Continuous microalgae recovery using electrolysis with polarity exchange. *Bioresource Technology*, 124, 164–170.

Knuckey, R.M., Brown, M.R., Robert, R. and Frampton, D.M.F. (2006). Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquaculture Engineering*, 35, 300–313.

Koopman, B.L., Thomson, R., Yackzan, R., Benemann, J.R. and Oswald, W.J. (1978). Investigation of the pond isolation process for microalgae separation from woodlands waste pond effluents. Final Report, U.C. Berkeley.

Kumar, H.D., Yadava, P.K. and Gaur, J.P. (1981). Electrical flocculation of the unicellular green algae *Chlorella vulgaris. Aquacultural Botany*, 11, 187–195.

Larkum, A.W.D, Ross, I.L., Kruse, O. and Hankamer, B. (2012). Selection, breeding and engineering of microalgae for bioenergy and biofuel production. *Trends in Biotechnology*, 30, 198–205.

Lazarova, V., Phillippe, R., Sturny, V. and Arcangell, J.P. (2006). Evaluation of economic viability and benefits of urban water reuse and its contribution to sustainable development. *Water Practical Technology*, 1, 1–11.

Lee, *A.K., Lewis,* D.M. and Ashman, P.J. (2008). Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *Journal of Applied Phycology*, 21, 559–567.

Levin, G.V., Clendenning, J.R., Gibor, A. and Bogar, F.D. (1962). Harvesting of algae by froth flotation. *Applied Microbiology*, 10, 169–175.

Li, Y.G., Xu, L., Huang, Y.M., Wang, F., Guo, C. and Liu, C.Z. (2011). Microalgal biodiesel in China: Opportunities and challenges. *Applied Energy*, 88(10), 3432–3437.

Lim, J.K., Chieh, D.C.J., Jalak, S.A., Toh, P.Y., Yasin, N.H.M., Ng, B.W. and Ahmad, A.L. (2012). Rapid magnetophoretic separation of microalgae. *Small*, 8, 1683–1692.

Liu, *D*., Li, F. and Zhang, B. (2009). Removal of algal blooms in freshwater using magnetic polymer. *Water Science and Technology*, 59, 1085–1092.

Lundquist, T.J., Woertz, I.C., Quinn, N.W.T. and Benemann, J.R. (2010). A Realistic Technology and Engineering Assessment of Algae Biofuel Production. Energy Biosciences Institute, University of California, Berkeley.

McGarry, M.G. and Durrani, S.M.A. (1970). Flotation as a method of harvesting algae from ponds. Research program report No. 5. Asian Institute of Technology, Bangkok.

Mendez, M., Behnke, C., Poon, Y. and Lee, P. (2010). Induction of flocculation in photosynthetic organisms. WO 2009158658.

Mohn, F.H. (1980). Experiences and strategies in the recovery of biomass from mass cultures of microalgae. *In:* Shelef, B., Solder, C.J. (Eds), Algae Biomass. Elsevier, Amsterdam, pp. 547–571.

Mohn, F.H. and Soeder, C.J. (1978). Improved technologies for harvesting and processing of microalgae and their impact on production costs. *Archives in Hydrobiology Bech Ergebn Lemnology*, 11, 228–253.

Molina Grima, E., Belarbi, E.H., Acién Fernández, F.G., Robles Medina, A. and Chisti, Y. (2003). Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances*, 20, 491–515.

Mollah, M.Y.A., Morkovsky, P., Gomes, J.A.G., Kesmez, M., Parga, J. and Cocke, D.L. (2004). Fundamentals, present and future perspectives of electrocoagulation. *Journal of Hazardous Materials*, 114, 199–210.

Moraine, R., Shelef, G., Sandbank, E., Bar Moshe, Z. and Schwarbard, L. (1980). Recovery of sewage borne algae: Flocculation and centrifugation techniques. *In:* Shelef, G., Solder, C.J. (Eds), Algae Biomass. Elsevier, North Holland.

Morweiser, M., Kruse, O., Hankamer, B. and Posten, C. (2010). Developments and perspectives of photobioreactors for biofuel production. *Applied Microbiology and Biotechnology*, 87, 1291–1301.

OriginOil (2010). http://www.originoil.com November 2010.

Pearsall, R.V., Connelly, R.L., Fountain, M.E., Hearn, C.S., Werst, M.D., Hebner, R.E. and Kelley, E.F. (2011). Electrically dewatering microalgae. *IEEE Transactions on Dielectrics and Electrical Insulation*, 18, 1578–1583.

Petrusevski, B., Bolier, G., van Breemen, A.N. and Alaerts, G.J. (1995). Tangential flow filtration: A method to concentrate freshwater algae. *Water Research*, 29, 1419–1424.

Phoochinda, W. and White, D.A. (2003). Removal of algae using froth flotation. *Environmental Technology*, 24, 87–96.

Phoochinda, W., White, D.A. and Briscoe, B.J. (2004). An algal removal using a combination of flocculation and flotation process. *Environmental Technology*, 25, 1385–1395.

Poelman, E., DePauw, N. and Jeurissen, B. (1997). Potential of electrolytic flocculation for recovery of micro-algae. *Resources Conservation and Recycling*, 19, 1–10.

Rawat, I., Kumar, R.R., Mutanda, T. and Bux, F. (2011). Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy*, 88, 3411–3424.

Reynolds, J.H., Middlebrooks, E.J., Porcella, D.B. and Grenney, W.J. (1975). Effects of temperature on oil refinery waste toxicity. *Journal of Water Pollution Control Federation*, 46, 2674–2693.

Rossi, N., Jaouen, O., Legentilhomme, P. and Petit, I. (2004). Harvesting of cyanobacterium *Arthospira platensis* using organic filtration membranes. *Food and Bioproducts Processing*, 82, 244–250.

Rossignol, N., Vandanjon, L., Jaouen, O. and Quemeneur, F. (1999). Membrane technology for the continuous separation microalgae/culture medium: Compared performances of cross flow microfiltration and ultrafiltration. *Aquaculture Engineering*, 20, 191–208.

Rwehumbiza, V.M., Harrison, R. and Thomsen, L. (2012). Alum-induced flocculation of preconcentrated *Nannochloropsis salina*: Residual aluminium in the biomass, FAMEs and its effects on microalgae growth upon media recycling. *Chemical Engineering Journal*, 200–202, 168–175.

Salim, S., Bosma, R., Vermue, M.H. and Wijffels, R.H. (2011). Harvesting of microalgae by bio-flocculation. *Journal of Applied Phycology*, 23, 849–855.

Salim, S., Vermuë, M.H. and Wijffels, R.H. (2012). Ratio between autoflocculating and target microalgae affects the energy-efficient harvesting by bio-flocculation. *Bioresource Technology*, 118, 49–55.

Sandbank, E. (1979). Harvesting of microalgae from wastewater stabilization pond effluents and their utilization as a fish feed. D.Sc. thesis presented to the senate of the Technion – Israel Institute of Technology.

Sandbank, E., Shelef, G. and Wachs, A.M. (1974). Improved electroflotation for the removal of suspended solids from algae pond effluents. *Water Research*, 8, 587–592.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O. and Hankamer, B. (2008). Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenergy Research*, 1, 20–43.

Schlesinger, A., Eisenstadt, D., Bar-Gil, A., Carmely, H., Einbinder, S. and Gressel, J. (2012). Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. *Biotechnology Advances*, 30, 1023–1030.

Scholz, M., Hoshino, T., Johnson, D., Riley, M.R. and Cuello, J.L. (2011). Flocculation of wall-deficient cells of *Chlamydomonas reinhardtii* mutant cw15 by calcium and methanol. *Biomass and Bioenergy*, 35, 4835–4840.

Shelef, G., Azov, Y., Moreine, R. and Oron, G. (1980). Algae mass production as an integral part of a wastewater treatment and reclamation system. *In:* Shelef, B., Solder, C.J. (Eds.), Algae Biomass. Elsevier, North Holland.

Shelef, G., Sukenik, A. and Green, M. (1984). Microalgae harvesting and processing: A literature review. Report prepared for the US Department of Energy, Technion Research and Development Foundation Ltd., Haifa, Israel.

Show, K.Y., Lee, D.J. and Chang, J.S. (2013). Algal biomass dehydration. *Bioresource Technology*, 135, 720–729.

Spilling, K., Seppälä, J. and Tamminen, T. (2011). Inducing autoflocculation in the diatom *Phaeodactylum tricornutum* through CO_2 regulation. *Journal of Applied Phycology*, 23, 959–966.

Su, Y., Mennerich, A. and Urban, B. (2011). Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. *Water Research*, 45, 3351–3358.

Taylor, *R.L.*, Rand, J.D. and Caldwell, G.S. (2012). Treatment with algae extracts promotes flocculation, and enhances growth and neutral lipid content in *Nannochloropsis oculata* – A candidate for biofuel production. *Mar. Biotechnol*., 6, 774–781.

Teixeira, C.M.L.L., Kirsten, F.V. and Teixeira, P.C.N. (2012). Evaluation of *Moringa oleifera* seed flour as a flocculating agent for potential biodiesel producer microalgae. *Journal of Applied Phycology*, 24, 557–563.

Tenney, M.W., Echelberger, W.F., Schuessler, R.G. and Pavpni, J.L. (1969). Algal flocculation with synthetic organic polyelectrolytes. *Applied Bacteriology*, 18, 965–971.

Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M., & Hoadley, A. (2010a). Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of renewable and sustainable energy*, *2*(1), 012701.

Uduman, N., Qi, Y., Danquah, M.K. and Hoadley, A.F. (2010b). Marine microalgae flocculation and focussed beam reflectance measurement. *Chemical Engineering Journal*, 162, 935–940.

Van Den Hende, S., Vervaeren, H., Saveyn, H., Maes, G. and Boon, N. (2011). Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio. *Biotechnology and Bioengineering*, 108, 549–558.

Vandamme, D., Foubert, I., & Muylaert, K. (2013). Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends in biotechnology*, *31*(4), 233–239.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B. and Muylaert, K. (2012). Flocculation of *Chlorella vulgaris* induced by high pH: Role of magnesium and calcium and practical implications. *Bioresource Technology*, 105, 114–119.

Vandamme, D., Foubert, I., Meesschaert, B. and Muylaert, K. (2010). Flocculation of microalgae using cationic starch. *Journal of Applied Phycology*, 22, 525–530.

Vandamme, D., Pontes, S.C.V., Goiris, K., Foubert, I., Jan Pinoy, L.J. and Muylaert, K. (2011). Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. *Biotechnology and Bioengineering*, 108, 2320–2329.

Wettman, J.W. and Cravens, J.B. (1980). Cost effective lagoon upgrading with microscreens. Proceedings of the 3rd Annual Pollution Control Association, Oklahoma, June 5, 1980.

Wijffels, R.H., Barbosa, M.J. and Eppink, M.H.M. (2010). Microalgae for the production of bulk chemicals and biofuels. *Biofuels, Bioproducts and Biorefining*, 4, 287–295.

Wilde, E.W., Benemann, J.R., Weissman, J.C. and Tillett, D.M. (1991). Cultivation of algae and nutrient removal in a waste heat utilization process. *Journal of Applied Phycology*, 3, 159–167.

Wu, Z., Zhu, Y., Huang, W., Zhang, C., Li, T., Zhang, Y. and Li, A. (2012). Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresource Technology*, 110, 496–502.

Wyatt, N.B., Gloe, L.M., Brady, P.V., Hewson, J.C., Grillet, A.M., Hankins, M.G. and Pohl, P.I. (2012). Critical conditions for ferric chloride-induced flocculation of freshwater algae. *Biotechnology and Bioengineering*, **109,** 493–501.

Xu, L., Guo, C., Wang, F., Zheng, S. and Liu, C.Z. (2011). A simple and rapid harvesting method for microalgae by in situ magnetic separation. *Bioresource Technology*, 102, 10047–10051.

Zhang, J. and Hu, B. (2012). A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. *Bioresource Technology*, 114, 529–535.

Zhang, X. et al. (2012). Influence of growth phase on harvesting of *Chlorella zofingiensis* by dissolved air flotation. *Bioresource Technology*, 37, 166–176.

Zhang, G., Zhang, P., & Fan, M. (2009). Ultrasound-enhanced coagulation for Microcystis aeruginosa removal. *Ultrasonics sonochemistry*, *16*(3), 334–338.

Zheng, H., Gao, Z., Yin, J., Tang, X., Ji, X. and Huang, H. (2012). Harvesting of microalgae by flocculation with poly (γ-glutamic acid). *Bioresource Technology*, 112, 212–220.

Zhou, W., Cheng, Y., Li, Y., Wan, Y., Liu, Y., Lin, X. and Ruan, R. (2012). Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. *Applied Biochemistry and Biotechnology*, 167, 214–228.