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Review

Microalgae harvesting techniques: A review

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ABSTRACT

Microalgae with wide range of commercial applications have attracted a lot of attention of the researchers in the last few decades. However, microalgae utilization is not economically sustainable due to high cost of harvesting. A wide range of solid - liquid separation techniques are available for microalgae harvesting. The techniques include coagulation and flocculation, flotation, centrifugation and filtration or a combination of various techniques. Despite the importance of harvesting to the economics and energy balance, there is no universal harvesting technique for microalgae. Therefore, this review focuses on assessing technical, economical and application potential of various harvesting techniques so as to allow selection of an appropriate technology for cost effectively harvesting of microalgae from their culture medium. Various harvesting and concentrating techniques of microalgae were reviewed to suggest order of suitability of the techniques for four main microalgae applications i.e biofuel, human and animal food, high valued products, and water quality restoration. For deciding the order of suitability, a comparative analysis of various harvesting techniques based on the six common criterions (i.e biomass quality, cost, biomass quantity, processing time, species specific and toxicity) has been done. Based on the order of various techniques vis-a-vis various criteria and preferred order of criteria for various applications, order of suitability of harvesting techniques for various applications has been decided. Among various harvesting techniques, coagulation and flocculation, centrifugation and filtration were found to be most suitable for considered applications. These techniques may be used alone or in combination for increasing the harvesting efficiency.

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1. Introduction

Algae are eukaryotic photosynthetic microorganisms with variety of species living in an extensive variety of ecological circumstances such as freshwater, sea water, snow, soil, hot spring, etc. About 50,000 species of algae are present, but only around 30,000 of them have been analyzed (Richmond, 2004). These photosynthetic microorganisms can grow rapidly in large quantity requiring inorganic compounds such as CO_2, light energy source and nutrients like nitrogen and phosphorous for their growth (Grima et al., 2003). Algae are broadly divided into filamentous (frequently detach from the lake bottom to form raft-like masses over the water surface), macroalgae (algae that can be seen without the help of a microscope) and planktonic forms (free floating microscopic plants that are identified under microscope and usually measured in micrometers). The presence of these algae in water generally gives indication of higher concentration of nutrients in given water body. Depending upon the nutrient concentration lakes are classified as oligotrophic, mesotrophic, eutrophic and hypereutrophic. An overabundance of nutrient cause algal blooms which may cause clogging of screens, taste and odour problems in drinking water supply, poor aesthetic appearance of water body and loss in economy through decline in recreational uses the water bodies. Some of the harmful algal blooms produce toxins that can pose serious threats to animals and humans. However, when such conditions arises that hinders the use of the lake, control measures such as controlling agricultural, urban or storm water runoff, proper septic system management and controlled use of fertilizers should be taken to prevent human-induced fresh-water algal blooms. These measures are not too effective for immediate change so for many lakes limited algae control is opted in lake management programs.

The use of algae is very old and the phycologists are continuously working to get hold of their economic importance and many beneficial as well as harmful economic aspects of algae. Some of the beneficial roles exhibited by the algae are as primary producers, source of food for animals and humans, antibiotics and medicines, purifier of wastewater, biofuel, fertilizer and pollution controller by fixing CO_2. Microalgae biomass can be converted to biofuels such as biohydrogen, biodiesel, methane, etc (Demirbas, 2010). Nutraceuticals, fatty acids, stable isotopic biochemical, phycobiliproteins, carotenoids were also reviewed as commercial application of microalgae (Milledge, 2011). Beside extensive research on microalgae their commercial application is still uneconomical. Commercial development of microalgal biotechnology is well documented by Olaizola (2003). Cost for microalgae harvesting generally found to be 20–30% of the microalgal biomass cost (Mata et al., 2010; Grima et al., 2003; Verma et al., 2010). The most challenging task for algal biofuel production is harvesting with nearly half of the microalgal biomass cost (Greenwell et al., 2009). Their frequent harvesting from dilute suspension in comparison to conventional crops makes it more expensive. Also, harvesting of algae require high energy input. Amer et al. (2011) estimated that harvesting and dewatering equipment may cost 90% of total cost for producing algal biomass from open ponds.

In the present paper current techniques used for harvesting and concentrating microalgae are reviewed to assess their technical, economical and application potential and to suggest order of suitability of the techniques for four main microalgae applications i.e. biofuel, human and animal food, high valued products, and water quality restoration.

2. Algae harvesting techniques

Algae harvesting refers to the separation or detachment of algae from its growth medium. The harvesting method intensely depends on the physiognomies of the micro algae chosen, density and size of the microalgal cell, specifications of the final product and on allowability for reuse of the culture medium (Uduman et al., 2010; Amaro et al., 2011; Rawat et al., 2011). Regardless of the objective of harvesting process, growth in dilute suspension (0.02–0.05% dry solids), small cell size (<30 μm), negligible density difference of microalgal cells to their culture medium, negatively charged surface (zeta potential) and their high growth rates which needs frequent harvesting compared to land crops make harvesting process a challenging task (Edzwald, 1993; Amer et al., 2011; Zamalloa et al., 2011).

Currently algae harvesting involves mechanical, chemical, biological and electrical based methods. The macroalgae harvesting is simple and laborious work, whereas for microalgae harvesting relatively elaborated chemical or mechanical means are used. Grima et al. (2003) mentioned mechanical methods as the most reliable and commonly used methods for harvesting microalgal biomass. However, when mechanical methods are headed by coagulation and flocculation step, they improve harvesting efficiencies and reduce operation and maintenance costs. Several techniques such as flocculation, flotation, filtration and centrifugation, or a combination of any of these techniques are usually used to harvest and concentrate the algal biomass (Demirbas, 2010; Ho et al., 2011). The advantages and disadvantages of various harvesting techniques are presented in Table 1. A review on dewatering microalgae biomass concluded that “currently there is no superior method of harvesting and dewatering” (Uduman et al., 2010). So combination of several separation techniques has been proposed. Most of the times centrifugation and filtration are preceded by coagulation and flocculation to improve the harvesting efficiency and to reduce costs (Grima et al., 2003). Two methodologies that generally applied for microalgae harvesting are two step process and one step process (Uduman et al., 2010; Brennan and Owende, 2010). In two step process, the dilute algal suspension is concentrated to algal slurry of 2–7% TSS and then in second step the algal slurry is dewatered to 15–25% TSS, requiring more energy (Chen et al., 2011). The effectiveness of the harvesting process can be described in terms of recovery efficiency (RE) and the concentration factor (CF) (Pahl et al., 2013). The RE and CF together indicate separation efficiency of the process in terms of mass and volume of recovered microalgae biomass. The RE and CF are defined as following:

RE = *mass of the cell removed/mass of the cells in initial culture*
CF = *concentration of algae in final product/initial concentration of algae in culture*

The mass and concentration of microalgae cells can be estimated by measuring Chlorophyll-content, microalgae cell count, absorbance (optical density), dry weight and ashfree dry weight of the culture.

Table 1
Advantages and Disadvantages of Various Algae Harvesting Techniques (Abdelaziz et al., 2013; Barros et al., 2015).

Harvesting Technique	Advantages	Disadvantages
Coagulation/flocculation	• Fast and easy technique • Used for large scale • Less cell damage • Applied to vast range of species • Less energy requirements • Auto and bioflocculation may be inexpensive methods	• Chemicals may be expensive • Highly pH dependent • Difficult to separate the coagulant from harvested biomass • Efficiency depends upon the coagulant used • Culture medium recycling is limited • Possibility of mineral or microbial contamination
Flotation	• Suitable for large scale • Low cost and low space requirement • Short operation time	• Needs surfactants • Ozoflotation is expensive
Electrical based processes	• Applicable to all microalgal species • No chemicals required	• Metal electrodes required • High energy and equipment costs • Metal contamination
Filtration	• High recovery efficiency • Cost effective • No chemical required • Low energy consumption (natural and pressure filter) • Low shear stress • Water recycles	• Slow, requires pressure or vacuum • Not suitable for small algae • Membrane fouling/clogging and replacement increases operational and maintenance costs • High energy consumption (vacuum filter)
Centrifugation	• Fast and effective technique • High recovery efficiency (>90) • Preferred for small scale and laboratory • Applicable to all microalgae	• Expensive technique with high energy requirement • High operation and maintenance costs • Appropriate for recovery of high-valued products • Time consuming and too expensive for large scale • Risk of cell destruction

2.1. Centrifugation

Centrifugation is an expansion of gravity sedimentation where centrifugal force replaces gravity for separating microalgae from their growth medium. Centrifugal separation of the microalgae depends on the cell settling characteristics (cell size and negligible density difference of microalgal cells to their culture medium), cell slurry retention time in the centrifuge. Heasman et al. (2000) obtained harvesting efficiencies of >95, 60, and 40% at 13000×*g*, 6000×*g* and 1300×*g*, respectively and concluded that cell harvesting feasibility considerably depend on the microalgal species and on type of centrifuges. Several centrifuges were examined for microalgae separation. These include disk stack centrifuges, perforated basket centrifuges, imperforated basket centrifuges, decanters and hydrocyclones (Pahl et al., 2013). Centrifuges operated in continuous mode were more demanding as in the batch mode operation they had to be stopped for removing the solids. Centrifugation harvesting is generally characterized by high separation efficiency (>90%) under low flow rates and high energy utilization. However, Dassey and Theegala (2013) demonstrated that high biomass separation efficiency can be sacrificed for processing large volume of culture, resulting in net lower energy intake. They harvested 28.5% of algal biomass at a rate of 18 L/min, resulting 82% decrease in energy consumption.

Disc stack centrifuges are the common industrial centrifuges with force applied equal to 4000–14,000 times gravitational force (Milledge and Heaven, 2011). They are extensively used for high value algal products (Grima et al., 2003). Disc stack centrifuges concentrate microalgae with sizes between 3 and 30 μm to 0.02–0.05% (Milledge and Heaven, 2011) but they generally require high energy consumption (Uduman et al., 2010). Shen et al. (2009) estimated harvesting cost as 14% of total production cost and Verma et al. (2010) estimated harvesting cost as 20% of the algal biomass cost. Milledge and Heaven (2011) reported poor energy return of the biodiesel produced by centrifugation as more energy is consumed than that produced. Instead that using pre-concentrated algal biomass can improve this energy return.

Decanter centrifuges are as effective as solid bowl centrifuges but require high energy consumption (Milledge and Heaven, 2013). For large volumes hydrocyclone type centrifuge can be operated continuously with low maintenance requirements but its high energy demand and high capital cost restricted its use to high valued biomass. Application of hydrocyclone type centrifuge for algae harvesting was studied by Mohn (1980) for *Coelastrum algae* and reported that its reliability is poor with 0.4% solids and concentration factor 4. The solid concentration of harvested algae slurry was low, with incomplete solid-liquid separation. Milledge and Heaven (2013) suggested that hydrocyclone type centrifuge could be used to pre-concentrate microalgae prior to another harvesting technique. Compared to other microalgae harvesting methods offers many advantages such as can be applied to all microalgal species, have high recovery rate and chemicals free biomass. But for large scale application the energy consumption, treatment time, maintenance and capital cost, all are generally very high (Mata et al., 2010).

2.2. Membrane process

In membrane process algae culture is allowed to pass through filters operating under gravity, pressure or vacuum force to hold back algae in a thick paste form. The system can either be continuous or discontinuous. The quality of the harvested biomass is good as compared to other harvesting techniques as the cells are less disrupted and no chemicals are required in membrane harvesting (Wicaksana et al., 2012). Several filter assembles have been used for harvesting algae but they hindered by low throughput and rapid fouling (Milledge and Heaven, 2013). Depending on solvent/solute properties, hydrodynamic conditions and membrane characteristics there are wide variety of filter designs (Mo et al., 2015). Microfiltration (MF) (0.1–10 μm), macrofiltration (10 μm), dead end filtration, ultrafiltration (UF) (0.02–0.2 μm), tangential flow filtration (TFF), vacuum filtration and pressure filtration are few filtration forms (Milledge and Heaven, 2013; Pragya et al., 2013). Typical membrane materials include polyvinylidene fluoride (PVDF), polyacrylonitrile (PAN), polyether sulfone (PES) and polytetrafluoroethylene (PTFE), polyethersulfone polyvinyl-pyrollidone (PES-PVP), polyvinyl choloride (PVC) (De Baerdemaeker et al., 2013; Drexler and Yeh, 2014), an active layer of cellulose triacetate on a robust nonwoven polyester polyethylene backing (CA), ceramic filtering layers (alumina, zirconia or silica layers) on porous

metal supports (C+M) and ceramic filtering layers (TiO_2, Zr-O_2-TiO_2 or others) on porous ceramic supports (Al_2O_3 or ZrO_2) (C+C) (Mo et al., 2015; Mancinelli and Halle, 2015). Mo et al. (2015) suggested that membranes with ceramic filtering layers over ceramic supports (C+C) appear to be the best choice for inorganic materials. Rossi et al. (2004) and Rossignol et al. (1999) evaluated different organic membranes and among them neutral hydrophilic polyacrylonitrile (PAN) performed best in terms of permeation flux and cleanability for microalgae dewatering.

Ultrafiltration is a potential substitute for recovery but because of its high flux requirement and high operating and maintenance costs, they are generally not used for microalgae harvesting (Rawat et al., 2011; Grima et al., 2003). Mo et al. (2015) recommended UF membranes for long duration use for microalgae harvesting. De Baerdemaeker et al. (2013) and Drexler and Yeh (2014) found similar results, with UF membranes signifying better flux over a long period and fouling resistance than MF membranes but Sun et al. (2013) compared their performance and showed that they have very similar performance in terms of permeate flux under the same operation conditions at low transmembrane pressure (TMP). Membrane performance also depends on material and surface properties (charge, hydrophobicity). Rossi et al. (2004) studied surface properties of different membranes. They observed that while keeping other parameters constant, positive charge membranes perform worse than their neutral counterparts. Similarly, under same operating conditions PAN (neutral and hydrophilic) performs better than PES (neutral and hydrophobic) in terms of permeate flux (Rossignol et al., 1999). Sun et al. (2014) evaluate polysulfone membrane (PS), fluoro polymer membrane (PVDF), regenerated cellulose acetate membrane (RCA) and observed that the permeate flux increased with increasing TMP or increased in the cross-flow velocity for all membranes. Combinations of several harvesting techniques have been assumed to be economical by many researchers so it was tested with filtration also. Liang et al. (2008a) selected coagulation - settling as the best pretreatment method prior to UF. De Baerdemaeker et al. (2013) used submerged flat panel membranes prior to centrifugation and Bilad et al. (2012, 2013) used submerged microfiltration and magnetically induced membrane vibrating (MMV) system with centrifugation. All three pre-concentration steps resulted in low energy consumption of 0.169 kWh/kg, 0.91 and 0.77 kWh/m^3, respectively.

Algal secretions of organic matter during growth period or upon cell lysis mostly contribute to fouling and along with them iron and manganese may also contribute to the fouling (Drexler and Yeh, 2014; Kimura et al., 2004). These organic matters are also known as extracellular polymeric substances (EPS), algogenic organic matter (AOM), and extracellular organic matter (EOM). The main mechanisms for UF membrane fouling by EOM may be the cake layer formation, hydrophobic adhesion or pore plugging (Qu et al., 2012). Hydrophilic regenerated cellulose acetate (RCA) membrane had a much lower fouling tendency than hydrophobic PS and PVDF membranes (Sun et al., 2014). Liang et al. (2008a) investigated hydraulic cleaning methods for UF membrane fouling and concluded that backwashing followed by forward flushing with 20 min duration was most effective. For maximizing flux recovery they used combination of sodium hydroxide (0.02 N) and sodium hypochlorite (100 mg/L) for 4 has cleaning agents. Kimura et al. (2004) also used NaOH and NaOCl for renewal of membrane permeability. Fouling formation can be reduced by coating the membrane surface with hydrophilic polyvinyl alcohol polymer resulting in more hydrophilic surface. Hwang et al. (2013) obtained 36% increase in maximum flux by surface-coated membrane. Climatic conditions such as temperature and radiation also affect rate of membrane fouling and was found to be minimal at 28 to 35 °C (Babel et al., 2002). Pretreatment with Ozone shows reduction of cake resistance of 70–93% (Babel and Takizawa, 2011). Liang et al. (2008b) reported that repeated chemical cleaning may reduce the membrane unit's service life and suggested to inactivate algae before feeding into UF membrane. They used combination of permanganate and chlorine as pretreatment for inactivating algae. Preozonation improved microfiltration performance by reducing cake compressibility and the biomass loading for both hydrophobic and hydrophilic membranes. However, dissolved polysaccharide released during preozonation is adsorbed onto the hydrophobic membrane increasing its fouling resistance (Hung and Liu, 2006).

According to the flow of feed water filtration can be Tangential flow filtration (TFF) or Dead end filtration (DEF) using either UF or MF membranes. TFF also known as Cross Flow Filtration is a high rate method with about 70–89% removal efficiency (Rawat et al., 2011). During TFF diluted algal biomass solution is allowed to pass parallel to the membrane while filtrate passes through perpendicularly. TFF is an energy efficient harvesting method (Danquah et al., 2009) with preserving the integrity (motility features and reproductive capabilities) of the algal cells during harvest (Petrusevski et al., 1995). Danquah et al. (2009) concentrated *T. suecica* 151 times while requiring energy 2.15 kWh/m^3. Similarly Bhave et al. (2012) concentrated the *N. oculata* biomass 75 times (150 g/L) with hollow fiber and tubular membranes while requiring energy 0.3–0.7 kWh/m^3. The DEF (also known as Direct Flow Filtration) is not economical to use due to quick fouling of membranes as the diluted algal biomass solution is allowed to pass perpendicular to the membrane. Due to quick fouling researchers preferred dead end filtration for carrying out studies on membrane fouling and on cake behaviour under filtration (Babel et al., 2002; Babel and Takizawa, 2010, 2011).

2.3. Coagulation and flocculation

Microalgae cell have negative surface charge, density near to the growth medium and found in dispersed state. This stable system results in slow natural sedimentation. These microalgae can be successfully harvested using pre flocculation or coagulation (Chen et al., 2011). Chemicals called flocculants which neutralize the negative charge and allow agglomeration of microalgae are used. Flocculation has been proposed to be a superior technique for harvesting microalgae as it can be used for large scale with a wide range of microalgae species (Grima et al., 2003). Flocculation can be induced by electrostatic patch (or patching), bridging or sweep flocculation (Vandamme et al., 2013). Chen et al. (2011) mentions surface charge neutralization to be the major mechanism involve in microalgae flocculation. Ideally, chemical coagulant should be sustainable and renewable resulting in no biomass contamination, allow reuse of culture medium, cheap, nontoxic, effective in low doses and rather be extracted from renewable resources (Grima et al., 2003). Earlier many researchers have used a widespread selection of salts as coagulants for microalgal harvesting. Inspite of being economical, the chemicals used for flocculation can be hazardous and contaminate the algal biomass (Lee et al., 2009). Depending upon chemical composition flocculants can be inorganic, organic/polyelectrolyte flocculants (Chen et al., 2011).

Inorganic chemical flocculants are multivalent cations such as aluminium sulfate, ferric chloride and ferric sulphate which form polyhydroxy complexes at optimal pH resulting in neutralization and reduction of negative surface charges on micro algal cells are used for flocculating microalgal cells (Chen et al., 2011). Effectiveness of these multivalent salts depends on their electronegativity and solubility. More electronegative ion has faster coagulation. Similarly salts with lower solubility are more effective (Barros et al., 2015). Although flocculation is easily achieved by metal coagulants, they are not eco-friendly and resultant increase in dissolved solids,

contaminated biomass, alteration in growth medium and colour hinders their application for algae harvesting as biofuel or animal feed stock. Aluminum salts observed to be more efficient then ferric salts (Shelef et al., 1984). Organic flocculants or polyelectrolytes (polyacrylamide or polyethyleneimine) can be cationic, anionic, or non-ionic. Cationic polymers flocculate because they physically link cells together whereas the anionic or non-ionic fail to make microalgae flocs due to electro-repulsion. The flocculating power of the polyelectrolyte hangs on the properties such as charge and functional groups on the surface of microalgae, growth medium pH and density of the algal culture (Chen et al., 2011). Granados et al. (2012) reported that cationic polyelectrolytes were more effective than the metal salts and achieved upto 35 times concentrated biomass. Cationic polyelectrolytes with high charge density are more effective flocculants to harvest microalgae and the effective dose decreases with an increase in molecular weight of coagulant whereas anionic polyelectrolyte fail to flocculate (Uduman et al., 2010; Granados et al., 2012).

Flocculation can be carried out by chemicals or by microorganisms but the excess flocculant requirement often makes this method too costly for large scale operations. Other disadvantages such as high sensitivity to pH, contamination of harvested biomass and recycling of flocculants, limit its applicability for food or feed purposes downstream and probably making it uneconomical for commercial use (Pushparaj et al., 1993; Grima et al., 2003). This method is more productive at high biomass concentrations and low stirring speeds. Ionic strength, pH, molecular weight and the charge density of the flocculant can also affect flocculation efficiency (Grima et al., 2003). Similarly, presence of nutrients i.e phosphorous and nitrogen, alkalinity, ammonia, dissolved organic matter, algal type and temperature of the algal culture can also influence the optimal coagulant dose (Show et al., 2015). Flocculation efficiency is significantly affected by the polymer dosing and it is difficult to achieve optimum polymer dosing because of the fluctuation of many variables during the algal growth cycle. Low dosing results in weak bridging and loose flocs whereas high dosing results in reduction of bridging potential due to electrostatic hindering (Gerardo et al., 2015). During stationary growth phase low zeta potential and low metabolic activity with high intercellular interactions can be considered advantageous to harvest microalgal biomass (Danquah et al., 2009). Flocculation is generally used in combination with other harvesting techniques for economical harvesting.

2.3.1. Autoflocculation

Flocculation of certain microalgae can occur naturally without addition of supplementary chemicals in response to environmental stresses such as changes in nitrogen, pH and dissolved oxygen and it is known as auto-flocculation (Schenk et al., 2008; Uduman et al., 2010). Natural gravity settling proves to be rapid, inexpensive and relatively simple, and less disruptive to cells than centrifugation (Knuckey et al., 2006). It is induced at elevated pH. Knuckey et al. (2006) observed precipitate formation of Ca and Mg hydroxides in seawater as the pH was increased above 10. Similarly, Christenson and Sims (2011) reported that calcium phosphate precipitate which results due to an increase in pH neutralize the negatively charged microalgal cells. Wu et al. (2012) stated > 90% biomass recovery for both freshwater and marine microalgae by increasing the pH upto 10.6. Similarly, Vandamme et al. (2012) increased pH upto 10.8 using NaOH, KOH and $Ca(OH)_2$ and achieved 98% biomass recovery whereas $Mg(OH)_2$ contributed 98% recovery at pH 9.7 within 30 min. Perez et al. (2014) harvested *Chlorella vulgaris* using $Mg(OH)_2$ at pH 10.5 with recovery efficiency > 95% within 30 min. Pezzolesi et al. (2015) observed that at high pH flocculated cells were less compact and dense than those at lower pH values and they were easily re-suspended in the algal medium. They also observed that decreasing the pH at 4 results in 95% biomass harvesting within 60 min (Pezzolesi et al., 2015). Similarly, *Chlorococcum nivale, Chlorococcum ellipsoideum, Scenedesmus* sp. were harvested with >90% efficiency at pH 4 in 15 min. Many researchers stressed for need of more research to explain exact mechanisms behind environmental modification for inducing autoflocculation (Park et al., 2011; Pragya et al., 2013). Autoflocculation could be non-toxic but it is slow and unreliable also environmental modification may be uneconomical for commercial use (Schenk et al., 2008; Lee et al., 2009).

2.3.2. Electrolytic process

Electro-flocculation technology is used for algae harvesting with advantages such as non-species specific, easy to control, low chemical usage, more expected results, no left over anions (e.g., chloride and sulfate) and low power consumption compared to centrifugation (Vandamme et al., 2011; Lee et al., 2013; Chen et al., 2015). The coagulants are generated by the electrolytic oxidation of sacrificial electrode which destabilized the microalgal suspension. This destabilized phase again aggregates to form flocs and moves towards anode with a removal efficiency of 80–95% (Chen et al., 2011). The efficiency of the process depends on electrode material, electrolysis time, current density, pH and composition of the microalgae suspension. Among tested electrode materials, aluminum was better than iron (Lee et al., 2013; Xu et al., 2010; Dassey and Theegala, 2014). This process is generally used in conjunction with flotation (Chen et al., 2015). The disadvantages of this process include need of electrode replacement and maintenance, increase in temperature of algal suspension, changes in pH and left over metals in the algal concentrate (Vandamme et al., 2011).

2.3.3. Bio-flocculation

Bioflocculation is the process where other micro-organisms produce some bioflocculants which flocculates algae in suspension. Bio-flocculants can be the EPS produced by bacteria, microalgae, and fungi (Shelef et al., 1984; Grima et al., 2003). EPS addition either crude or purified form is uneconomical (Pahl et al., 2013). Bacteria are provided to the algal growth medium with a suitable organic carbon source so that they do not affect microalgal biomass productivity. Bacterias have been widely applied in treatment of water and wastewater including soil remediation (Chen et al., 2015). *Nannochloropsis oceanica* was harvested by bacterial strain (*Solibacillus silvestris*) with 88% efficiency (Wan et al., 2013), whereas Lee et al. (2009) harvested *Pleurochrysis carterae* with 90–94% efficiency using microbes. Similarly, Oh et al. (2001) harvested green algae (*B. braunii, S. quadricauda* and *S. capricornutum*) using bacteria (*Paenibacillus* sp.) within efficiency range of 91–95% but the cyanobacteria (*A. flosaquae* and *M. aeruginosa*) was harvested within a lower efficiency range of 38–49% only. Zhou et al. (2013) and Xie et al. (2013) floccuated *Chlorella vulgaris* using pallet forming fungi (*Aspergillus oryzae*) and filamentous fungi (*C. echinulata*), respectively with removal efficiency of <97%. The addition of flocculating species to harvest non-flocculating microalgae is promising method as it does not require chemicals or specific cultivating conditions. However, they are not recommended for biofuel production because of low lipid contents (Salim et al., 2011). Salim et al. (2012) reported that the application of bioflocculation followed by centrifugation reduces the energy demand for harvesting. This process is highly species dependent. Salim et al. (2012) harvested *Chlorella vulgaris* upto 55% using microalgae (*Ettlia texensis*) but with *Scenedesmus obliquus* only 34% of biomass was harvested. However, using bacteria (*Paenibacillus* sp.*) Chlorella vulgaris* removal efficiency was increased to 93% (Oh et al., 2001).

2.4. Flotation process

Flotation is a gravity separation process in which air or gas bubbles are used to carry the suspended matter to the top of a liquid surface where they can be collected by skimming process (Singh et al., 2011). Due to low density and self-float characteristics of some micro-algal species this method can be comparatively fast and more effective compared to sedimentation (Edzwald, 1993; Singh et al., 2011; Hanotu et al., 2012). Flotation separation has shown efficient harvesting of both fresh water as well as marine microalgae (Liu et al., 1999; Garg et al., 2015). The attachment of suspended particles to the air or gas bubbles depends on many factors including size of suspended particle, likelihood of collision and adhesion. The main advantages are short operation time, low space requirement, large scale harvesting and high flexibility with low initial cost (Rubio et al., 2002; Hanotu et al., 2012). This process generally requires flocculants and often proceeded by coagulation and flocculation (Rubio et al., 2002). The surfactants increase the probability for the air bubble and suspended particle to adhere (Gerardo et al., 2015). The factors that influence the flotation efficiency include the type of collector (surfactant or flocculants), pH and ionic strength in the medium, type of bubble formation, recycling rate, air tank pressure, hydraulic retention time and particle floating rate (Phoochinda and White, 2003). Hanotu et al. (2012) showed that use of micro sized bubbles were effective for separation of algal biomass from growth medium. High surface area and low rise velocity of micro-sized bubbles leads to faster attachment of the algal cells. For flotation to be successful the cell must be hydrophobic with high molecular weight (Hanotu et al., 2012; Henderson et al., 2009) and this can be achieved through the addition of surfactants or coagulants (Garg et al., 2014). Flotation processes are classified according to the method of bubble size production as dissolved air flotation (DAF), dispersed air flotation (DiAF), electrolytic flotation and ozonation-dispersed flotation (ODF) (Chen et al., 2011; Pragya et al., 2013).

2.4.1. Dissolved air flotation

Dissolved air flotation (DAF) uses small bubbles of sizes ranging from 10 to 100 μm, generated when air is dissolved in water under very high pressure (Edzwald, 1993; Chen et al., 2011). The bubbles forces the suspended algae cells to float to the surface which later can be skimmed off, making it an energy intensive process. However, an oversized bubbles break up the floc (Park et al., 2011). Henderson et al. (2009) used surface modified bubbles for the treatment of algae using DAF with 60, 63 and 95% removal efficiency for metal coagulant (aluminium sulphate), cationic surfactant (CTAB) and cationic polymer (PolyDADMAC) respectively. Ometto et al. (2014) used Ballasted Dissolved Air Flotation (BDAF) technique and reported it to be a more consistent and sustainable harvesting system than DAF with 99% cells recovery and reduction of 80 and 95% in energy inputs and coagulant demand, respectively. Wiley et al. (2009) reported high energy requirement of 7.6 kWh/m^3 for DAF.

2.4.2. Dispersed air flotation

In dispersed air flotation (DiAF) bubbles of 700–1500 μm size are produced by continuously passing air through a porous material (diffusers or spargers) or through a high speed mechanical agitator. This system consumes less energy, but requires costly equipment and high pressure drop for generating bubbles (Chen et al., 2011). Some synthetic as well as natural collectors, such as sodium dodecylsulfate (SDS), N-cetyl-N-N-N-trimethylammonium bromide (CTAB), saponin, chitosan, etc. have been used to support the flotation process (Nguyen et al., 2013; Kurniawati et al., 2014). Phoochinda and White (2003) studied DiAF as a function of the collector type, aeration rates and the pH of algal suspension with three surfactants, such as CTAB, SDS and the non-ionic Triton X-100, for flotation of *Scenedesmus quadricauda*. CTAB and SDS increased the aeration rates and reduced the size of air bubbles with algal removal efficiency of 90 and 16%, respectively. However, decreasing pH values of the algal suspension increased the removal efficiency to 80% for SDS but for CTAB no improvement in removal efficiency was seen. Similarly, Liu et al. (1999) reported increase in flotation efficiency of anionic SDS (40 mg/L) from 20 to 90% by using chitosan (10 mg/L). However, chitosan (10 mg/L) use with cationic CTAB reduced flotation efficiency to 10%. Kurniawati et al. (2014) observed that when saponin was used alone it was not too effective for flotation separation of algae but with the pre-flocculation using 5 mg/L of chitosan, separation efficiency of >93% microalgae cells was observed at 20 mg/L of saponin. Similarly, Nguyen et al. (2013) studied DiAF by CTAB and observed that removal efficiency increases from 65.1 to 83.1% with increase in CTAB dose from 20 to 60 mg/L. The turbidity removal reached >90% by using pre-ozonation for 30 min. Similarly, Coward et al. (2013) combines dispersed air flotation with foam fractionation and there model revealed that highest concentration factors were gained using the following variables and variable interactions: cationic CTAB, lower surfactant concentrations, and CTAB combined with high column heights consuming only 0.015 kWh/m^3 energy. Microalgae harvested by foam flotation using the surfactant CTAB have high lipid content than from cells harvested by centrifugation (Coward et al., 2014). This system can be used for harvesting microalgae for low-value products for example, biofuels. Recently Garg et al. (2015) efficiently harvested marine microalgae with 23-fold increase in algal concentration and over 99% algal recovery using advanced flotation machine with an appropriate collecting agent dodecyl pyridinium chloride (DPC).

2.4.3. Electrophoresis technique

Electroflotation is a process of floating of microalgae cells to water surface with the formation of fine hydrogen bubbles by electrolysis. Hydrogen evoluted at cathode separates microalgal biomass from the growth medium. Hydrogen bubbles sticks to the microalgal flocs and force them to float to the surface. No chemicals required and non-species specific are few benefits of this method while cathodes fouling and high power requirements, are the main disadvantages of this method (Chen et al., 2011).

2.4.4. Ozonation-dispersed flotation (ODF)

Nowadays, flotation with ozone (Dissolved Ozone Flotation, DOF) is getting more attention among the researchers even though this method is considered as costly process for water and wastewater treatment. In this technique instead of atmospheric air ozone gas is used to produce charged bubbles. Ozone being a strong oxidizing agent oxidise the soluble organic compounds and the charged bubbles separates the microalgae, thus interaction of these two processes can lead to better treatment effects. The biopolymers which are released during cell lysis acts as coagulant which is beneficial for effective separation. Ozoflotation was successfully applied to remove *Chlorella vulgaris* and *Scenedesmus obliquus* with turbidity removal 98 and 95%, respectively (Cheng et al., 2010, 2011). Similarly, Betzer et al. (1980) reported 98% algae or suspended solid removal with ozone dose ranging from 15 to 50 mg/L. Orta et al. (2014) achieved 79.6% TSS and 97.8% turbidity removal with 0.23 $mgO_3/mg_{\text{dried biomass}}$ within 5 min. Furthermore an increase in fatty acid methyl esters (FAME) when using ozone compared to using centrifugation was also observed. Similarly, harvesting of *C. vulgaris* by ozoflotation increase its lipid content by 24% (Cheng et al., 2011). However, contamination problems may occur when used at large scale.

3. Order of suitability of harvesting techniques

To decide order of suitability of the harvesting technique for four main microalgae applications (i.e. biofuel, human and animal food, high valued products, and water quality restoration), six criterions as described in Table 2 play main role. The selection of these criterions is based on literature related to microalgae biomass applications and on the fact that the selected criterions should be important and common for all considered microalgae applications. Similar selection of criterions was done by Al Hattab et al. (2015) for selecting appropriate method for harvesting microalgae biomass. A comparative analysis of various harvesting techniques based on the six important criterions is presented in Table 3 and it was used for deciding order of suitability of harvesting techniques for each criteria. The order of suitability of harvesting techniques for six criterions is given in Table 4.

Table 5 shows the order of criterion taken into consideration for deciding suitability of harvesting techniques for various applications.

Based on the order of various techniques vis-a-vis various criteria (Table 4) and preferred order of criteria for various applications (Table 5), order of suitability of harvesting techniques for various applications is summarised in Table 6.

Coagulation and flocculation, filtration and centrifugation are among top preferences of harvesting techniques depending on microalgae applications. Based on the six important criterions, coagulation and flocculation is best option for production of biofuels and water quality restoration. Similarly for human and animal food and high valued products, the best options for harvesting biomass are filtration and centrifugation, respectively. For biofuel, filtration is preferable over centrifugation to maintain low harvesting cost but for human and animal feed, centrifugation is

Table 2
Criterion Used for Deciding Order of Suitability of Harvesting Technique.

S.No.	Criterion	Description
1.	Biomass Quantity (BQn)	The method should produce large biomass quantity and can be applied at large scale.
2.	Biomass Quality (BQl)	The quality of the harvested cells should be good i.e. AOM or EOM are not released from the cell.
3.	Cost (C)	Low operational cost.
4.	Processing Time (PT)	The rate of harvesting should be fast.
5.	Species Specific (SS)	Method should be species dependent with high dewatering efficiency.
6.	Toxicity (T)	Biomass produced should not be toxic.

Table 3
Comparative Analysis of Various Harvesting Techniques.

S.No.	Criteria	Coagulation and Flocculation	Flotation	Filtration	Electrical Based Processes	Centrifugation
1.	BQn	Suitable for large scale application	Suitable for large scale application	Unsuitable for high concentration and large scale application	Unsuitable for large scale application	Unsuitable for large scale application
2.	BQl	Less cell damage	No cell damage	No cell damage	Damaged cells	Damaged cells
3.	C	Low cost method, some coagulants may be expensive	Low cost method but requires surfactants	Membrane replacement and pumping are expensive	High energy and equipment cost	Expensive method
4.	PT	Easy and fast method	Short operation time	Short operation time	High rate of harvesting	High rate of harvesting
5.	SS	Applicable to all microalgal species	Applicable to all microalgal species	Unfeasible for very small sized microalgae	Applicable to all microalgal species	Applicable to all microalgal species
6.	T	Metal contamination	Surfactants may be toxic	No toxicity	Metal contamination	AOM and EOM released cause toxicity

Table 4
Order of Suitability of Harvesting Techniques for Various Criterions.

Order	BQn	BQl	C	PT	SS	T
I.	Coagulation and Flocculation	Filtration	Coagulation and Flocculation	Centrifugation	Coagulation and Flocculation	Filtration
II.	Flotation	Flotation	Filtration	Electrolytic Process	Electrolytic Process	Centrifugation
III.	Filtration	Coagulation and Flocculation	Flotation	Coagulation and Flocculation	Centrifugation	Coagulation and Flocculation
IV.	Electrolytic Process	Electrolytic Process	Electrolytic Process	Flotation	Flotation	Flotation
V.	Centrifugation	Centrifugation	Centrifugation	Filtration	Filtration	Electrolytic Process

Table 5
Order of Criterion for Deciding Suitability of Harvesting Techniques for Various Applications.

Order	Biofuel	Human and Animal Food	High Valued Products	Water Quality Restoration
I.	BQn	BQl	T	C
II.	C	T	SS	PT
III.	PT	SS	BQl	BQn
IV.	BQl	BQn	BQn	BQl
V.	SS	PT	C	T
VI.	T	C	PT	SS

Table 6
Order for Suitability of Harvesting Techniques for Various Applications.

Order	Biofuel	Human and Animal Food	High Valued Products	Water Quality Restoration
I.	Coagulation and Flocculation	Filtration	Centrifugation	Coagulation and Flocculation
II.	Filtration	Centrifugation	Coagulation and Flocculation	Filtration
III.	Centrifugation	Coagulation and Flocculation	Filtration	Flotation
IV.	Flotation	Flotation	Flotation	Centrifugation
V.	Electrolytic Process	Electrolytic Process	Electrolytic Process	Electrolytic Process

preferable over coagulation and flocculation because of toxicity in later case. However, for high valued products, coagulation and flocculation is preferable over filtration as low dose of coagulants are required for specific species of microalgae and for water quality restoration, filtration is preferable over flotation because large quantity of water is involved in water quality restoration. Electrolytic process is not efficient for any of the considered application because of their non-feasibility for large scale application, high energy and equipment cost, damaged harvested cells, and metal contamination. Overall coagulation and flocculation, filtration and centrifugation may be used alone or in combination for harvesting microalgae for various uses of microalgae and water quality restoration.

4. Conclusion

Based on the six important criterions i.e. biomass quantity, biomass quality, cost, processing time, species specific and toxicity, coagulation/flocculation, centrifugation and filtration are the top three preferred harvesting techniques for applications such as biofuel, human and animal food, high valued products, and water quality restoration. These techniques can be used alone or in combinations for increasing the harvesting efficiency. The combinations of several harvesting techniques depending on the species under consideration, desired final product concentration and its quality may be used for algae harvesting. The combinations using flocculation as primary harvesting step to concentrate the algal biomass from the growth medium lead to the effective harvesting. Filtration and coagulation both techniques yield high quality as well as high concentrations of solids in harvesting. Nonetheless they are time consuming with high operating and capital cost. Flotation and flocculation concentrates microalgae upto 6% solids algal biomass at low cost compared to filtration and coagulation. Flotation and flocculation are easy, fast and used at large scale with low space requirement but required chemicals may be expensive and contaminate the harvested algal biomass. Centrifugation harvesting is fast, effective and suitable for all microalgal species but high operating and maintenance cost makes them too expensive for large scale application. Centrifugation or filtration can be used after flocculation as the less volume of biomass is to be processed which proves to be economical. Among various combination, flocculation and sedimentation is better and low cost option for harvesting microalgae. Replacing chemical coagulants with natural organic ones can solve the problem of high cost and contamination of harvested algal biomass.

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