International Journal of
Environmental Sciences & Natural Resources
ISSN: 2572-1119

Review Article
Volume 11 Issue 3 - May 2018
DOI: 10.19080/IJESNR.2018.11.555812

Int J Environ Sci Nat Res

Microalgae Biomass Harvesting Based on pH Induced, Chemical and Bioflocculants Mediated Flocculation-A Review

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Submission: May 04, 2018; **Published:** May 24, 2018

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Abstract

Harvesting of microalgae biomass using cost efficient method is a major challenge for obtaining value added products at larger scale. Although the use of centrifugation, filtration, and electrocoagulaton methods are feasible for high harvesting efficiencies, their energy requirements and cost remained worrisome for biomass harvesting. This review presents the flocculation methods involving pH induced, chemical flocculants and bioflocculants as promising approach for large-scale and low-cost harvesting of microalgal biomass. When using different flocculants for microalgae harvesting, it is necessary to consider flocculant type, dosage, cost and type of microalgae for efficient flocculation. Therefore, the selection of flocculation method should therefore be assessed based on multiple parameters on the level of each microalgae species.

Keywords: Microalgae; Flocculation; Biomass; Bioflocculation

Introduction

Microalgae slurry has a low amount of biomass due to suspension in large volume of water and must be dewatered. The common methods of dewatering include centrifugation Gerardo et al. [1]; Molina Grima et al. [2], various kinds of filtrations Gerardo et al. [1]; Danquah et al. [3]; Mo et al. [4], electrocoagulation Lee et al. [5] and forward osmosis (Mazzuca Sobczuk et al. [6]). Electrical based methods are based on electrophoresis of the algae cells Sridhar et al. [7]; Vandamme et al. [8]. Filtration and gravitational sedimentation are widely applied in wastewater treatment facilities to harvest relatively large (>70μm) microalgae but cannot be used to harvest smaller algae species Brennan and Owende [9]. Centrifugation is widely used to recover small sized microalgae biomass; however its application is restricted to algae cultures for high-value metabolites due to high energy requirements. It is essential to find a technology which is capable of processing large volumes of microalgae cultures to reduce the cost and increase the scale of microalgal biomass production. The surface of the cells is negatively charged and electrostatic repulsion between cells prevents them from coming together and spontaneously adhering to each other by van der Waals forces Ndikubwimana et al. [10]. Flocculation is widely considered as a promising approach for large-scale and low-cost harvesting of microalgal biomass Molina Grima et al. [2]; Coons et al. [11]; Vandamme et al. [12]. In a flocculation process, a cationic flocculant is used to neutralize the surface charge on the cells to facilitate spontaneous formation of cell aggregates, or flocs. Flocculants can induce flocculation through different mechanisms: by neutralizing the negative surface charge of the cells, by connecting individual cells, or by forming a precipitate that binds and enmeshes the cells.

pH based Microalgae Flocculation

Decreasing the pH of growth medium (pH 4.0) has resulted in higher flocculation efficiencies for *Chlorococcum nivale, Chlorococcum ellipsoideum* and *Scenedesmus* sp Liu et al. [13]. Reducing the culture pH below 6.5 using H_2SO_4 had greatly improved the harvesting efficiency for *Scenedesmus* sp however at pH below 3.5, harvesting efficiencies were reduced Das et al. [14]. Seo et al. [15] also found that harvesting efficiencies were higher at low pH adjusted cultures of *Chlorella sp*. Other studies reported that microalgae biomass formed spontaneous flocs when the culture pH was reduced to 4 by adding HNO_3 or HCl Liu et al. [13]; Pezzolesi et al. [16]. pH decrease-induced flocculation is not applicable for all microalgae and the flocculation efficiencies for the microalgal cells with small size (3-5 μm) are low. In a study by Liu et al. [17], *Chlorella zofingiensis* and *Chlorella vulgaris* were harvested using self-flocculating *Chlorococcum nivale, Chlorococcum ellipsoideum* and *Scenedesmus* sp. microalgae via pH decrease.

High pH has induced flocculation in *C. vulgaris* revealing the potential of pH change in the growth medium for microalgal biomass harvesting Vandamme et al. [8]. In *Chlorococcum sp*, maximum flocculation efficiency of 94% was obtained hen the pH of the growth medium was increased from 8.5 -12.0 through addition of NaOH Ummalyma et al. [18]. In a study by Yang et al. [19], pH induced flocculation using NaOH has resulted in 90% flocculation efficiency for *C. vulgaris*. Increasing pH to 11-12 has been shown to induce flocculation in *C. vulgaris* Ras et al. [20]. Alkaline flocculation using NaOH was reported as most efficient for harvesting of *Nannochloropsis, Chlamydomonas* and *Chlorella* sp by Lama et al. [21]. In some studies culture pH was raised above 11 to have higher harvesting efficiency Castrillo et al. [22]; Knuckey et al. [23]. The pH increase of culture medium could induce the flocculation efficiency of fresh water microalgae Halim et al. [24]. An optimum pH of 9.2 along with addition of alum resulted in 95% of biomass harvest for *Botryococcus sp* Gani et al. [25]. pH induced flocculation was found to be effective than flocculant based and elecro-flotation method for *Scenedesmus obliquus* and *C. vulgaris* Koley et al. [26]. Another study involving pH driven flocculation using potassium hydroxide at pH 10 produced efficient flocculation of *C. vulgaris* and *Scenedesmus* sp Vera Morales et al. [27]. The mechanism of pH induced flocculation for *Skeletonema costatum* and *Chaetoceros gracilis* were evaluated by Perez et al. [28]. Total biomass recovery was achieved at alkaline pH whereas maximum biomass recovery (60%) was reached for acid pH values. Flocculation activity of *Aspergillus niger* on *C. vulgaris* biomass was investigated by Li et al. [29]. The results indicated that flocculation mechanism is a pH and calcium dependent manner with hydrophobic interaction-involved process. Influence of pH, salinity and flocculant dosage on harvesting of *Chaetoceros gracilis* was studied by Perez et al. [30]. The results indicated that salinity influences the harvesting process and very low doses of flocculants were required when the salinity was reduced to 20 g/L between pH 5-9. The above results indicate that harvesting of microalgae biomass using pH adjustment is species specific.

Chemical Flocculants

Cationic starch nanoparticles have produced 90% flocculation efficiency for *C. vulgaris* which is 20% higher than the non-particulate starch Bayat Tork et al. [31]. Inorganic, organic and polyelectrolyte flocculants are frequently used for wastewater treatment to eliminate algae. Inorganic flocculants include salts of metals like aluminium and iron and organic flocculants include acrylamide, acrylic acid (synthetic), starch, chitosan and cellulose (natural). Higher dose of inorganic flocculants result in a higher cost per unit of microalgal cells flocculation. In comparison to inorganic flocculants, the organic flocculants are non-toxic, lesser sensitivity to pH and of lower dosages for flocculation process. However, presence of nutrients dissolved organic matter and algal type influences the optimal coagulant dose Bilanovic and Shelef [32]; Show et al. [33]. Polyelectrolytes flocculate cells based on the charge and functional groups on the surface of microalgae, growth medium pH and cell density Chen et al. [34] and cationic polyelectrolytes were more effective than the metal salts Granados et al. [35]. Combined use of Al^{3+} and cetyltrimethylammonium bromide exhibited larger algal floc size and maximum flotation recovery efficiency of 98.73% in *C. vulgaris* Xia et al. [36]. Flocculation is also achieved using solid acid carbons for *Desmodesmus communis* Pezzolesi et al. [16]. Ferric sulphate showed the highest flocculation efficiency at 150mg/L dosage for S. *obliquus* than other flocculants tested Abomohra et al. [37]. Cationic ions and cationic starch can enhance the flocculation of negatively charged particles through patch charge neutralization However, overdosing of flocculent leads to lower relative flocculation efficiency, due to electrostatic repulsion Liu et al. [38].

Chitosan is a low cost and nontoxic naturally occurring flocculant for microalgae harvesting for value added products Lersutthiwong et al. [39]. Chitosan contains amine functional groups which easily bind and bridges to produce larger, denser flocs, causing the faster settling of microalgae Lavoie and de la Noteb [40]; Ahmad et al. [41]. Harvesting of *C. vulgaris* using various concentrations of chitosan was tested by Rashid et al. [42] and it was found that 99% harvesting efficiency was achieved at 120 mg/L of chitosan at pH 6. More than 93% microalgae cells were separated when using saponin as bio-surfactant and chitosan as the flocculant under dispersed air flotation technique Kurniawati et al. [43]. In another study, over 99% of microalgae were harvested by using chitosan and iron oxide nanoparticle composites under a magnetic field Lee et al. [44]. Harvesting efficiency of inorganic and organic flocculants was studied by Kwon et al. [45] and the highest harvesting efficiency was 85.6 (aluminium sulfate), 92.6 (ferric sulfate) and 93 % (chitosan), and 91.3% for *Tetraselmis* sp. Tannin based cationic polymer was used for harvesting *Nannochloropsis oculata* which resulted in 99% flocculation efficiency under batch conditions Roselet et al. [46].

Bioflocculants

Several chemical flocculants including like ferric chloride, aluminium sulphate, ferric sulphate, chitosan and modified starch have been used to facilitate the aggregation of microalgae cells. However, high doses of chemical flocculants produce large amounts of sludge besides the high cost and possible pollution effects that may generate. Bioflocculation refers to naturally induced flocculation using secreted biolpolymers from microbial sources Christenson and Sims [47]. Bioflocculants address the cost and environmental concerns of chemical flocculants and decrease the harvest cost. High molecular weight extracellular polysaccharides and exudates of microbial cells could bridge and flocculate algae and microbial cells in suspension Pavoni et al. [48]; Salehizadeh et al. [49]. Polysaccharides from fungal hyphae and mycelia with active sites may enable their surface charge and capability of bio adsorption Tan et al. [50]. In a study by Zhang and Hu [51] involving co-cultivation of *Aspergillus niger* and *C. vulgaris*, 98.1 % microalgae cells were entrapped in the fungal clumps. Co-cultivation of *Cunninghamella echinulate* and *Chlorella vulgaris* resulted in complete harvesting of microlagal cells from the liquid medium at the fungi-to-algae ratios of 2:1 and 1:1

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Xie et al. [52]. Mixing of flocculating microalga (*Ankistrodesmus falcatus, S. obliquus* and *Tetraselmis suecica* with non-flocculating microalgae (*C. vulgaris* and *Neochloris oleoabundans*) has increased the initial sedimentation rate considerably by bridging and patching mechanism Salim et al. [53]. Similar positive effects on sedimentation rates are observed with bio-flocculation of *Pleurochrysis carterae* with bacteria Lee et al. [54]. Flocculation efficiency was improved by co-culturing of *C. vulgaris* and *Rhizobium radiobacter* in synthetic wastewater and the results showed that harvesting efficiency reached 45-50% with lipid content over 21% confirming the prospect of introducing bioflocculant producing bacteria for bioenergy production form microalgae Wang et al. [55]. *Nannochloropsis oceanica* was harvested by *Solibacillus silvestris* with 88% efficiency Wan et al. [56], whereas Lee et al. [54] harvested *Pleurochrysis carterae* with 90-94% efficiency using microbes. Flocculation of *C. pyrenoidosa* with *Aspergillus fumigatus* pellets was studied by Bhattacharya et al. [57]. The results revealed that 24h old fungal pellets flocculated at 38 °C and 1:5 fungal-algal ratio showed the best flocculation efficiency.

Bioflocculant (Poly γ-glutamic acid) produced by *Bacillus licheniformis* was reported to increase the flocculation efficiency of *Desmodesmus* sp from 43.8% to 98.2% at pH 3 Ndikubwimana et al. [58]. Similarly, poly γ-glutamic acid from by *B. subtilis* has induced 95% flocculation for *C. vulgaris* and *C. protothecoides* Zheng et al. [59]. In a study by Ma et al. [60], poly γ-glutamic acid was combined with calcium oxide for harvesting *C. vulgaris*. The results showed that combined flocculants significantly decrease the flocculants dosage and settling time of the microalgae. Bioflocculant from *Cobetia marina* has produced 92.7% flotation efficiency for *C. vulgaris* in the presence of $CaCl_2$ Lei et al. [61]. Oh et al. [62] reported that the harvesting efficiency of *C. vulgaris* was increased to 83% when using *Paenibacillus* sp as bioflocculant which is higher than aluminium sulphate and polyacrylamide produced flocculation. In some cases, actinomycetes and yeasts were also used as bioflocculants for microalgae harvesting. For example, Li et al. [63] had harvested *C. vulgaris* biomass by using *Streptomyces* sp with calcium through mycelial pellets. Bioflocculaltion of *Chlamydomonas reinhardtii* and *Picochlorum* sp by *Saccharomyces bayanus* var. *uvarum* were studied by Diaz Santos et al. [64-66].

Conclusion

Based on the above methods of flocculation, the flocculation methods can be used alone or in combination for increasing the harvesting efficiency. When using different flocculants for microalgae harvesting it is necessary to consider flocculant type, dosage, cost and type of microalgae for efficient flocculation. In addition, a flocculation method that is effective for one species may not necessarily be successful for other species of microalgae. Therefore, the selection of flocculation method should therefore be assessed based on multiple parameters on the level of each microalgae species.

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