BIOFLOCCULATION: A POTENTIAL MEANS OF HARVESTING MICROALGAE

Pratibha Kushwaha, Sushma Kumari, Kamleshwar Singh and K. Suresh Kumar*
Department of Botany, University of Allahabad, Prayagraj 211002, India.

Abstract
Global increase in demand for production of biofuels, coupled with potential efficacy of microalgae, has largely been the driving force for progression in microalgae-based research. Apart from their utilization in renewable energy, these photosynthetic organisms have several other high-value applications (e.g. in biopharmaceutical and nutraceutical industries). However, large-scale cultivation of microalgal biomass and manufacture of microalgal products, require cost-effective technologies. Microalgae have a low sedimentation velocity; moreover, their colloidal characteristic, together with the negative charge on the surface, makes harvesting difficult. Harvesting of microalgal biomass poses as a major bottleneck as it generally accounts for about 20 to 30% of the total cost of cultivation. Amidst a range of harvesting techniques adopted (coagulation, flocculation, and centrifugation), bioflocculation has emerged as a cost-effective, non-perilous, commercially suitable method for microalgal harvesting.

Keywords: algae, bacteria, bioflocculation, factors, flocculant, fungi, harvesting, microalgae, plants, self-flocculation.

Highlights
• This manuscript provides a brief on the various algal harvesting methods and focuses on the eco-friendly method of bioflocculation.
• Various bioflocculation strategies include: the use of animal, plant, bacteria, fungi and actinomycete-based flocculants, and, auto–flocculation.
• Production of bioflocculants is highly subjective to the culture medium composition and other physicochemical parameters.
• Influence of abiotic and biotic are also discussed here.

1. Introduction
The production of biomaterials, bioproducts, and bioenergy requires biomass; biological resources, such as energy crops, biomass residues from forest and crops, wastes and by-products from agro–industries and pulp and paper industries, wet organic wastes and the organic fraction of municipal solid wastes, have been used to produce bioenergy. More recently, the search for new sources of biomass, complementing traditional agricultural biomass for the production of food, feed, or bulk chemicals, have turned our attention towards algae (macro-and micro-algae) and aquatic plants, proposing them as attractive impending biological resources (Wijffels & Barbosa, 2010, Alam et al. 2016, Lago et al. 2019).

Microalgae (when cultivated under optimal culture conditions) constitute a significant fraction of that on trans-esterification yield third generation fuel (Barros et al., 2015). They are used as raw material for the production of biodiesel, biomethane, bioethanol, biobutanol and biohydrogen, and thus comprise a potential renewable fuel resource (alternative to fossil fuel). Oil accumulating microalgae are considered to be feed stock for the production of biodiesel (Umamalyma et al., 2017). The fact that microalgae have high growth rate, can be cultivated all year round using non–agricultural land, and, have good oil productivity (exceeding the best oilseed crops), establish their utilization in sustainable production for renewable energy (Uduman et al, 2010b, Haldar et al, 2018). However, their small size and low concentration in the medium pose a hitch in cultivation, harvesting and processing of these minifactories (Vandamme et al, 2012).

With technical advancements, nowadays several strategies (mechanical, chemical, electrical and biological) are available for separation and harvesting of microalgae. Based on the characteristics of the microalgae (size, density, etc.), the typical strategies employed in microalgal harvesting, include: gravity and centrifugation, sedimentation, ultrasonic aggregation, filtration, various forms of flocculation (e.g Chemical using inorganic and organic agents, alkaline flocculation, bio–flocculation using microorganisms, electrocoagulation, etc.), flotation, and, a combination of these techniques (Gultom & Hu, 2013). Table 1 elucidates several methods used for flocculation of algae. Nevertheless, each of these strategies is flanked with certain drawbacks; e.g using metal coagulants like aluminium sulfate or ferric chloride contaminates the recovered biomass with metals delimiting downstream applications. On the other hand, iron oxide based magnetic coagulants involve separation of microalgae using magnetic forces (Alam et al., 2016). Mechanical methods like centrifugation are energy intensive (Kandasamy & Shaleh, 2016). Flocculation is a phenomenon where solution comprising of solute particles forms an aggregate known as a floc; flocculation is a result of collision and adherence between solute particles in a suspension (Uduman et al, 2010a). This article providing an overview of the various methodologies used for microalgal harvesting discusses bioflocculation technology as a cost effective method for harvesting microalgae.
Table 1. advantages and limitations of different flocculation methods.

<table>
<thead>
<tr>
<th>Flocculation method</th>
<th>Advantages</th>
<th>Limitation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation</td>
<td>Fast method, High recovery efficiency</td>
<td>Expensive method, High energy requirements</td>
<td>Grima et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Suitable for almost all microalgal species</td>
<td>Suitable only for the recovery of high-valued</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>products, can not use at industrial scale.</td>
<td></td>
</tr>
<tr>
<td>Biopolymers</td>
<td>Biobased chemicals</td>
<td>Costly</td>
<td>Vandamme et al. (2011)</td>
</tr>
<tr>
<td>Flotation</td>
<td>Low cost method</td>
<td>Generally requires the use of chemical</td>
<td>Logan Christenson, Ronald Sims</td>
</tr>
<tr>
<td></td>
<td>Short operation times</td>
<td>flocculants, Unfeasible for marine</td>
<td>2011</td>
</tr>
<tr>
<td></td>
<td>Proven at larger scale</td>
<td>microalgae harvesting</td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td>High recovery efficiencies.</td>
<td>Membranes should be regularly cleaned</td>
<td>Barros et al. 2015</td>
</tr>
<tr>
<td>Bioflocculation</td>
<td>No addition of chemicals, Ecofriendly</td>
<td>To be confirmed at scale</td>
<td>Kandasamy and Shaleh 2016</td>
</tr>
<tr>
<td>Gravity sedimentation</td>
<td>Simple and the inexpensive method</td>
<td>Time-consuming</td>
<td>Alam et al., 2016</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>No contamination</td>
<td>High power consumption, heating, scalability</td>
<td>Alam et al., 2016</td>
</tr>
<tr>
<td>Magnetic coagulants</td>
<td>Separation is enhanced with magnetic force</td>
<td>Costly, established only on lab scale</td>
<td>Luo and Nguyen (2017)</td>
</tr>
<tr>
<td>Electrical method</td>
<td>Low energy requirement and reliable process</td>
<td>Contamination of biomass with metal</td>
<td>Ummalyma et al., 2017</td>
</tr>
<tr>
<td>Chemical flocculants</td>
<td>Well known technology, Reliable</td>
<td>Metal accumulation in biomass, toxic nature</td>
<td>Ummalyma et al., 2017</td>
</tr>
</tbody>
</table>

2. Microalgae harvesting: the technique

An ideal harvesting procedure is one that is generally effective for the most of microalgal strains, provides high biomass concentrations, and requires moderate finance, energy and maintenance; it could optionally facilitate recycling of the medium. According to Barros et al. (2015) harvesting of microalgae generally comprises a two-step separation process; it includes: thickening procedures (here, microalgal slurry is concentrated to about 2 to 7% of total suspended solids), and, dewatering procedures (that result in the concentration of microalgal slurry to 15 to 25% of total suspended solids). Selection of the appropriate harvesting method is especially of great economic interest, especially for biofuel production. Apart from species independency, and, sparse or no use chemical and energy (Chen et al., 2011), the characteristic of microalgae (e.g. density and size of microalgae), and, specification of desired product should be considered (Praga et al, 2013) while choosing an optimal harvesting method.

2.1. Screening:

Screening is the primary unit of operation in algae harvesting and wastewater treatment; it involves pre-processing of microalgal cultures. During the screening process, the algal biomass is introduced onto a screen of given aperture size; the efficiency of screening method depends on the spacing between the screen opening and cell size (Barros et al, 2015). Devices such as microstrainers and vibrating screens are primarily used for screening. In microstraining, a rotary drum is covered with a straining fabric, stainless steel or polyester, and suffers frequent backwash; here the flow-through rate essentially determined production costs. As larger microalgae can be effectively processed with larger openings, they result in faster flow rates and lower operational costs. Nevertheless, in this procedure a bacterial and microalgal biofilm formation occurs on the fabric or mesh, which demands constant maintenance. Mohn (1988) observed that while 1.5% total suspended solids (TSS) Coelastrum proboscideum is reported to be harvested by microstraining consuming 0.2 kWh/m3 the use of vibrating screens in continuous and batch mode allowed the recovery of 5 to 6% and 7 to 8% TSS, respectively. Vibrating screens are therefore considered area efficient. Commercial production of multicellular filamentous blue-green algae is generally carried out using this method.

2.2. Thickening

Overall the harvesting process can be divided into bulk harvesting (i.e. separating the microalgal biomass from the bulk culture by sedimentation, flotation and flocculation), and, thickening (concentrating the microalgal slurry after bulk harvesting, such as centrifugation and filtration) (Ferraro, 2017). In the process of thickening the harvesting slurry is concentrated with filtration and centrifugation; this step requires more energy than bulk harvesting (Chen et al, 2011). Thickening processes help increase the concentration of the microalgal suspension and reduces the volume to be processed, which contributes to considerable savings during downstream processes. Thickening increases the effective particle size prior to dewatering and significantly reduces its energy demand (Barros et al, 2015). Classically, thickening processes consists of gravity sedimentation, flotation, coagulation/flocculation (both chemical and biologically based), etc. (Barros et al, 2015).

2.2.1. Gravity sedimentation

In this process, the feed suspension is separated into concentrated slurry and clear liquid. Harvesting by sedimentation at natural gravity can be accomplished by lamella separators and sedimentation tanks; due to orientation of plates, lamella separators provide an increased settling area compare to conventional thickener (Salim et al, 2013). Although there is continuity in the pumping of microalgal suspension, there is discontinuity in the removal of slurry. At times, sedimentation flocculants are added to achieve microalgal separation. Although this is a rather inexpensive method of separating algae in sedimentation tanks, the reliability of gravity separation and settling is low.
2.2.2. Flocculation

Flocculation is an advance and effective technique as compared to sedimentation (Chen et al., 1998). It is a physicochemical type of gravity separation; in this method gas is bubbled through solid-liquid suspension and the gaseous molecules get attached to the solid particles carried to the surface of the liquid, thereby the particles get accumulated as “float” and could be removed. The size of the particle is an important parameter in flocculation; if the particle size is smaller, its more likely that the particles will be levitated by the bubbles. Generally if the particle diameter is <500μm, it can be used for flocculation (Matis et al., 1993). The second parameter is the particle being captured by bubble. There are two probabilities during the capturing process: (i) the probability of the collision between a bubble and a particle, and (ii) the probability of the adhesion of the bubble and the particle after collision has occurred. Flocculation is more advantageous as compared to centrifugation and filtration due to its low energy requirement, the low amount of floculants required, and its high scalability (Uduman et al., 2010).

2.2.3. Chemical coagulation/flocculation

Destabilization of colloidal suspensions by an electrolyte is referred as coagulation, while aggregation of the particles as a result of polymer addition is called flocculation (Branyikova et al., 2018). Coagulation/flocculation methods are generally performed after gravity sedimentation. In a coagulation process, pH adjustment or addition of electrolytes is carried out, while flocculation is the process wherein solute particles collide and adhere to each other. During flocculation, larger aggregates are formed from single cells; these aggregates are separated from the medium by simple gravity sedimentation (Ndikubwimana et al., 2014), leaving a clear supernatant. Therefore, flocculation refers to the aggregation of unstable and small particles through surface charge neutralization, electrostatic patching and/or bridging after addition of floculants (Matter et al., 2019). Flocculation can be induced by: 1) electrostatic patch (or patching) which occurs when a charged polymer binds to an opposite charged particle, locally reversing that charge and creating a patch that will connect with opposite charged patches, 2) bridging, which occurs when polymers or colloids bind to the surface of two different particles forming a bridge between them; and (iii) sweep flocculation, which occurs when particles are entrapped in a massive mineral precipitation (Barros et al., 2015, Singh & Patidar, 2018). Floccs formation facilitates separation (or recovery) by simple gravity-induced settling or any other conventional separation method. The flocculation process being simple and effective has been extensively scrutinized as a promising strategy for several algal species harvesting. Ideally chemical coagulation/flocculation leads to high efficiency biomass settling, allows reuse of culture medium, and is pretty inexpensive and safe when applied at a large-scale (Barros et al., 2015). Flocculation has been successfully utilized in various industries ranging from mining, brewing to water treatment. The flocculation chemicals that are added for better separation are known as floculants. Cell surface properties of microalgae (species, culture conditions and growth phase) play a vital role in any flocculation method. The presence of negative charge on microalgal surface does not facilitate self-aggregation of microalgal within the suspension; therefore, floculants are added to make the surface charge on the algae neutral. Flocculation increases the sedimentation rate by aggregating the microalgal cells, and therefore it eases the subsequent separation by sedimentation, centrifugal recovery, or filtration (Branyikova et al., 2018).

Microalg floculation methods include: Spontaneous and forced alkaline floculation, chemical flocculation with addition of floculants, physical flocculation induced by ultrasound or electric field, auto-flocculation provoked by extracellular polymeric substances (EPS), bio-flocculation involving other organisms or biological products, etc. According to Umamalyma et al. (2017) the factors to be considered while choosing a floculant include concentration of biomass, composition of the culture medium, pH and ionic strength and charge of the algal broth. Moreover, the smaller species have higher specific surface areas thus requiring a higher floculant dose per biomass weight (Branyikova et al., 2018). Microalgal growth phase also influences flocculation; moreover, the pH, dissolved CO₂, zeta potential and particle size suffer significant variations throughout the cultivation time. Generally, the stationary phase is best suited for harvesting microalgal biomass; in this phase, microalgae have lower metabolic activity and cell mobility, presenting higher intercellular interactions, as the zeta potential is lower (Barros et al., 2015).

2.2.3.1. Chemical flocculation

Several chemical floculants have been tested for microalgal harvesting. These include a variety of salts, inorganic chemicals, organic/polyelectrolyte floculant, etc. However, despite being economical, these chemicals could prove to be hazardous and often contaminate the algal biomass (Lee et al., 2009). Inorganic chemical floculants include multivalent cations (e.g. aluminium sulfate, ferric chloride and ferric sulphate), which form polyhydroxy complexes at optimal pH resulting in neutralization and reduction of negative surface charges on microalgal cells (Chen et al., 2011). Effectiveness of these multivalent salts depends on their electronegativity and solubility (e.g more electronegative ions have faster coagulation); further, salts with lower solubility are more effective (Barros et al., 2015).

Metal salts such as alum and ferric chloride are widely used for floculation in water treatment. Ferric chloride (FeCl₃), aluminium sulfate (Al₂(SO₄)₃) and ferric sulfate (Fe₂(SO₄)₃), have been effectively tested; Dissociation of these salts in the culture medium lowers electrostatic repulsion between the negatively charged cell surfaces, enabling cell aggregate formation (Barros et al., 2015). Although metal salts are used for harvesting microalgae (Dunaliella), their use could result in presence of high concentrations of metals in the harvested biomass; this metals often persist in the biomass residue post-extraction of lipids or carotenoids, which could interfere with its use in animal feed (Vandamme et al., 2013). Aluminum salts are more efficient than ferric salts. Metal coagulants offer good floculation, but they are not eco-friendly, contaminate biomass, alter the growth medium, and cause color change.

Organic floculants or polyelectrolytes (polyacrylamide or polyethyleneimine) can be cationic, anionic, or non-ionic. While cationic polymers flocculate because they physically link cells together, the anionic or non-ionic fail to make microalgal 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). Barros et al. (2015) reported that cationic polyelectrolytes with high charge density are more effective floculants to harvest microalgae and the effective dose decreases with an increase in molecular weight of coagulant whereas anionic polyelectrolyte fails to flocculate. Apart from the biomass, presence of nutrients (phosphorous and nitrogen), alkalinity, ammonia, dissolved organic matter, algal type, temperature of the algal culture, ionic strength, pH, molecular weight, coagulant dose and the charge density of the floculant influence flocculation efficiency. 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).

Magnetic particles hold tremendous potential as harvesting agents; the non-destructive nature of the magnetic field, flanked
with the particle biocompatibility, easy manipulation and regeneration, make utilization of magnetic particles as harvesting agents extremely competent (Branyikova et al, 2018). Magnetic harvesting of microalgae using an external magnetic field, after adsorption of a magnetic agent to microagal cells, has been considered as a single-step process, as flocculation and separation occur simultaneously here (Branyikova et al, 2018). The magnetic particles used for harvesting microalgae, could be in the form of uncoated magnetic iron oxide particles or as functional composites that can consist of a magnetic core coated with silica (this coating additionally carries specific functional groups such as polyethyleneimine or cationic polyelectrolytes such as chitosan, poly (diallyldimethylammonium chloride) and cationic polyacrylamide) (Branyikova et al, 2018) Fundamentally, it is important that a chemical coagulant should be sustainable and renewable resulting in no biomass contamination, lead to subsequent high efficiency biomass settling allow reuse of culture medium, be cheap and non–toxic when applied in large scale, be effective in low doses, allow the reuse of the culture medium, consider environmental impact and rather be extracted from renewable resources (Singh & Patidar 2018, Barros et al, 2015).

The use of naturally available coagulants/flocculants (phosphates, carbonates, calcium and magnesium ions, frequently found in wastewater, brackish water or seawater), have also been considered. However, phosphate–based coagulation is feasible only for phosphate–rich wastewater, and, microalgae stockpile it into their metabolic uptake (Barros et al, 2015); on the other hand, magnesium ions are easily obtained from wastewaters, and have similar efficiencies to those achieved with Al\(^{3+}\) and Fe\(^{3+}\). Limestone or dolomites could also be added, as they bring not only magnesium ions but also other carbonates, hydroxides and oxides, which present pH-related coagulation (Barros et al, 2015).

Polyelectrolyte flocculants are natural or synthetic polymers of ionic or non-ionic species; they facilitate the reduction required dose by increasing their molecular weight. These flocculants can be cationic, anionic or non–ionic; nevertheless, due to the net negative charge of microalgal cells, anionic or non–ionic polymers have no effect on their flocculation. Furthermore, some cationic polymers, such as chitosan, cationic polyacrylamides, cellulose, surfactants and other man–made fibers, have successful microalgae flocculating activity (Barros et al, 2015). Cationic polymers reduce microalgal cell surface electronegativity and bridge them to one another; further Barros et al. (2015) state that although polyelectrolyte flocculants effectively flocculate freshwater microalgae, their efficiency in case of marine microalgae is rather low. According to them, the ionic strength of sea and brackish waters (high salinity of the marine environment) proves to be inhibitory (as the polymers shrink to smaller dimensions, failing in bridging the cells). Evenmore, polyelectrolytes such as polyacrylamide would not be suitable for microagal harvesting due to their toxicity and inappropriateness for animal feeds. However, chitosan, a natural polymer derived from shrimp exoskeletons, is effectively used for harvesting both fresh and seawater microalgae as does not contaminate microagal biomass (Schlesinger et al, 2012), and adds bulk (Barros et al, 2015).

2.2.3.2. Physical flocculation methods

Flocculation of algal biomass could be induced using physical forces. Harvesting microalgae using physical flocculation is an appropriate scheme to avoid contamination induced by chemical flocculants addition. Physical flocculation methods include ultrasound, electro–flocculation, and magnetic separation (Wan et al, 2015, Vandamme et al, 2013). Flocculation of microalgae accomplished by applying a field of standing ultrasound waves is however more suitable at a laboratory scale rather than large scale. During the process of harvesting Monodus subterraneus, the microalgal cells aggregate into the knots of ultrasonic field with a high flocculation efficiency and estimated energy consumption of 345 kW/d (Bosma et al, 2003). Heng et al. (2009) used ultrasonic irradiation–coagulation and obtained satisfactory recovery of algae at optimal operating parameters (40 kHz, 60 W, and 15 s).

In the electrocoagulation method of flocculation, flocs are induced through electrolytic release of metal ions from a sacrificial anode. Here, the negatively charged microalgal cells move to anode and loose the charge; this enables the formation of microagal aggregates or flocs (Wan et al, 2015). In this process, the bubbles produced at the anode rise to the surface entrapping microagal aggregates or flocs, which can be swept off easily (Wan et al, 2015). The efficiency of this method could be improved by changing the polarity of the electrodes (Vandamme et al, 2013). However, electrocoagulation flocculation leads to minor contamination of the biomass with metals; in order to solve this issue, electromagnetic pulses could be given to neutralize the surface charge of microalgal cells and induce flocculation. Harvesting microalgae via electro–flocculation seems more cost–effective and feasible to scale up (Wan et al, 2015). Uduman et al. (2011) reported that Fe\(^{2+}\) from steel electrodes can benefit electro-coagulation of Chlorococcum sp. and Tetraselmis sp. However, aluminum electrodes prove to be more efficient and required lower energy input during cell harvest of Microcystis aeruginosa, P. tricornutum and N. oculata KMMCC-16 (Vandamme et al, 2013).

On the other hand, in a method that combines flocculation and separation in a single process, Cerff et al. (2012) used magnetic nanoparticles to harvest microalgae; Magnetite (Fe\(_3\)O\(_4\)) nanoparticles adsorb directly on the microagal cells, upon which the cells can be separated from the medium by applying a magnetic field. Nevertheless, this was species dependent. The adsorption properties of the nanoparticles could be improved by coating them with cationic polymers (Vandamme et al, 2013). The nanoparticles could also be recovered after harvesting and subsequently reused.

1.2.3.3. Bio-flocculation

In general, a flocculation process assisted with biological products/substances or organisms could be termed as bio-flocculation. Bioflocculation is a cheap, environmental friendly, sustainable approach, for harvesting algal biomass; it is generally applied in waste water treatment systems. Bioflocculation method has emerged as a cost effective and ecofriendly (safe to human health, biodegradable, and being free of secondary pollution) alternative for microagal harvesting (Kandasamy & Shaleh, 2016).
Charged cationic biopolymers can electrostatically interact with different cell surfaces resulting in flocculation through bridging by charge neutralization or electrostatic patch aggregation; use of polymers leads to more effective density (floc compaction), and, thereby improved sedimentation velocity (Branyikova et al., 2018). Figure shows the probable mechanisms of microalgae bioflocculation. Biopolymers like chitosan, cationic starch and tannins which are secreted from algal or bacterial source are also known as bioflocculants (Alam et al., 2016). At times, a bioflocculant combined with a chemical could also be used as a suitable alternative, e.g. bioflocculant from Paenibacillus polymyxa combined with cationic chemicals has been used for harvesting Scenedesmus sp. (Kim et al., 2011). Exopolysaccharide (EPS) synthesized by organisms such as bacteria, algae, fungi, and actinomycetes are reported to act as a bioflocculants (Kim et al., 2013). Poly (γ-glutamic acid) from Bacillus subtilis is also reported to be efficient in harvesting freshwater and marine microalgae (Branyikova et al., 2018). Furthermore, certain microalgal species that flocculate readily can be mixed with other species to induce mutual flocculation, e.g. Skeletonema species is reported to induce flocculation in other species of microalgae (Saltim et al., 2012). Guo et al. (2013) studied an extracellular biopolymer from Scenedesmus obliquus AS–6–1; this bioflocculant is a 127.9 kDa polysaccharide that flocculates freely-suspended microalgal cells. Nevertheless, Scenedesmus quadricauda, produces significant amounts of bioflocculant composed of sugar (56.7%) and protein (41%) (Rebah et al., 2018). Trending algal bioflocculation schemes have been deliberated below in brief.

**3. Autoflocculation/algae-algae flocculation**

In the process of auto–flocculation, suspended algal cells spontaneously aggregate, forming large flocs that induce their simple gravitational sedimentation (Matter et al., 2019). According to Ummalyma et al. (2017), auto-flocculation refers to the cell aggregation and adhesion of cells to each other in liquid culture, due to special cell surface properties or some other factors; this type of flocculation occurs naturally in certain microalgae. Autoflocculation occurs naturally in certain microalgal various factors are responsible for autoflocculation such as environmental stress, change in pH, dissolved oxygen, nitrogen and amount of calcium and magnesium in culture medium (Uduman et al., 2010 b), it could also occur due to some special cell surface properties. Some abiotic factors like pH affect autoflocculation of diatoms and green algae; however, the effect of pH varies with species of algae (Ummalyma et al., 2017). Nevertheless, all microalgal species do not autoflocculate and at times the process could be slow and unpredictable (Schenk et al., 2008). This process could occur naturally in microalgal cultures exposed to sunlight (in warm and sunny days) with limited CO₂ supply through photosynthesis, microalgae remove CO₂ dissolved in the culture medium, increasing its pH value. Autoflocculation is an actively investigated harvesting strategy of microalgae (Ummalyma et al., 2016), Table 2 demonstrates auto-flocculation of microalgal. According to Vandamme et al. (2012), pH range 10.5 to 11 is optimum for autoflocculation of Chlorella vulgaris. Increase in dissolved oxygen is favourable for autoflocculation of microalgae (Liao et al., 2011). Further, pH 9 to 9.3 results in 90% flocculation efficiency for the marine algae viz. Nanochloropsis and Phaeodactylum tricornutum (Wu et al., 2012). Dissolved oxygen concentration also influences autoflocculation of algae, for e.g. 2 mg/l produce larger flocs than dissolved oxygen concentration of 0.5 mg/l (Wilen & Balmer, 1998). Apart from magnesium and calcium hydroxide, this phenomenon can also be simulated by the addition of NaOH, which is a low cost product e.g. Horiuchi et al. (2003) flocculated Dunaliella tertiolecta by adding a NaOH solution; they observed a short settling time (few minutes) at a pH between 8.6 and 10.5, resulting in a 90% biomass recovery (Schlesinger et al., 2012). Autoflocculation is an effective and attractive alternative, as it is low cost, low energy, non-toxic to microalgae and does not require the use of flocculants, enabling simple medium reuse (Schlesinger et al., 2012), moreover, there is no extra expenditure in cultivation of microalgae.

Salim et al. (2014) proposed that glycoproteins are involved in cell flocculation of green microalgae E. texensis SAG79. However, Alam et al. (2014) reported that the cell wall polysaccharides of C. vulgaris JSC–7 are enriched with phosphodiester group; this is responsible for self–flocculating freely suspended microalgal C. vulgaris CNW11 and S. obliquus FSP with 80% flocculation efficiency. Nonetheless, the use of excessive volumes of the self–flocculating microalgal C. vulgaris JSC–7 has been reported to result in a higher harvesting efficiency of the freely suspended microalga C. vulgaris CNW–11 without addition of cations such as Al³⁺, Fe³⁺, or Cu²⁺ (Alam et al., 2014); a similar phenomenon was formerly reported for A. falcatus and S. obliquus AS–6–1. Biochemical investigation of the self–flocculating microalgal C. vulgaris JSC–7 and S. obliquus AS–6–1 (Alam et al., 2014)
2014, Guo et al. (2013) show that polysaccharides synthesized by these two strains are responsible for their self–flocculating properties. According to Salim et al. (2012), Harvesting microalgae using a disk stack bowl centrifuge consumed 13.5 MJ/kg of dry weight biomass, which is 50% of the total combustion energy of microalgae; the implementation of algal-algal bioflocculation, with a 1.3 ratio of flocculating and non–flocculating microalgae, could reduce energy consumption with approximately 12 MJ 215/kg of dry weight biomass. Thus, it is one of the most promising harvesting technologies for commercial algae production; nevertheless, it comprises the additional cultivation of self–flocculating microalgal species. Purified polysaccharides of C. vulgaris JSC–7 are also effective bioflocculants, inducing flocculation of the non–flocculating strains C. vulgaris CWN11 and S. obliquus FSP–3 (85% flocculation after 60 min of sedimentation, dose of 0.5 mg/L; Alam et al. 2014). Taylor et al. (2012) observed water–soluble extracts of the marine microalga Skeletonema marinoi induce flocculation of Nannochloropsis oculata.

Alam et al. (2016) report that bioflocculation caused increased biomass concentration. They suggest that both bridging and patching mechanisms underlie alga-algae bioflocculation process. Specifically, when a large network of microalgal cells are formed, the mechanism involved is bridging. On the other hand, in case the cells are more closely attached, the mechanism could be patching through the EPS excreted by flocculating microalgae (Alam et al. 2016).

**Autoflocculation induced by pH changes:**

Autoflocculation has been observed in several algal species, particularly under non-ideal culture conditions such as change in pH and cultural aging. Both alkaline and acidic conditions are reported to reduce the intensities of the negative surface charge of algal cells, thereby promoting their self–aggregation (Matter et al. 2019). Under alkaline conditions > pH 9, the changes in the surface charge of algal cells are mainly attributable to significant secretion of protective extracellular polymers; however, under acidic conditions the fluctuating dissociations of carboxyl and amine groups in the algal cell wall could cause changes in surface charge. Ummalyma et al. (2017) describe that when the pH of the medium is increased or decreased at certain point the cells come together and settle by gravitational force. The addition of more bases or acids into the medium could cause an increase in the formation of dense flocs which result in less settling times. Harith et al. (2009) observed that in case of microalgae Chaetoceros calcitrans, on increasing pH from 8.0 to 10.0 using NaOH and KOH, flocculation efficiency increased from 13 to 82% and from 35 to 78% in 4 h respectively. However, not all the microalgal species flocculate with increased or decreased pH level; auto–flocculation efficiency resulting from pH manipulation is largely species-dependent (Matter et al. 2019).

Further, at pH 10.5, 90% flocculation efficiency is reported in case of freshwater microalgae C. vulgaris, Scenedesmus sp. and Chlorococcum sp., while a pH 9.0 to 9.3 is optimum for 90% flocculation efficiency of marine algae Nannochloropsis sp. and Phaeodactylum tricornutum (Wu et al. 2012). Similarly pH 8.6 to 10.5 helps achieve 90% biomass harvesting in case of halo–tolerant microalgae Dunaliella tertiolecta, and pH 11.0 to 12.0 causes flocculation of fresh water microalgae Chlorococcum sp. RAP–13; a high harvesting efficiency of 94% was reported at pH 12. In fresh water microalgae Chlorococcum sp. RAP 13, flocculation occurs at pH between 11.0 to 12.0 (Ummalyma et al. 2017). In Chaetoceros calcitrans the harvesting efficiency is doubled with a slight increase in pH from 10 to 10.2 (Harith et al. 2009). Matter et al. (2019) cited several algae in this context, e.g. an increase in pH from 7 to 10 caused only slight improvement in auto–flocculation efficiency (10.4% to 33.2%) of S. obliquus NRCB1r1. High flocculation efficiency (90%) in case of three freshwater algal species, C. nivalis, C. ellipsoideum, and Scenedesmus sp. occur under acidic conditions (pH 4).

<table>
<thead>
<tr>
<th>Plant bioflouulant</th>
<th>Microalgae sp.</th>
<th>Types of microalgae</th>
<th>Harvesting efficiency (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>Thallessisira pseudomonas</td>
<td>Marine</td>
<td>&gt;90</td>
<td>Heasman et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Nonochloropsis oculata</td>
<td>Marine</td>
<td>&gt;90</td>
<td>Zhang et al., 2012</td>
</tr>
<tr>
<td>Poly–g-glutamic acid</td>
<td>Chlorella vulgaris, Nannochloropsis oculata, and Phaeodactylum Tricornutum</td>
<td>Marine</td>
<td>&gt;90</td>
<td>Stuart 2014</td>
</tr>
<tr>
<td>Cationic starch</td>
<td>Scenedesmus dimorphus</td>
<td>Fresh water</td>
<td>70–95</td>
<td>Banergee et al 2014</td>
</tr>
<tr>
<td>Guar gum</td>
<td>Chlamydomonas sp.</td>
<td>Fresh water</td>
<td>84</td>
<td>Rahul et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Chlorella sp.</td>
<td></td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>Botryococcus sp.</td>
<td>Marine</td>
<td>88.6</td>
<td>Kandasamy and Shaleh, 2016</td>
</tr>
<tr>
<td>Moong bean protein extract</td>
<td>Nanochloropsis sp.</td>
<td>Marine</td>
<td>&gt;90</td>
<td></td>
</tr>
</tbody>
</table>

**Age of Algae:**

Aging of algal cultures is often combined with the release of extracellular organic matter (EOM; mainly composed of proteins and polysaccharides) from algal cells into the aqueous environment. This EOM plays an important role in the self-flocculation of algal cells, specifically by forming biofilms and changing the surface charge of cells, presumably via neutralization (Matter et al. 2019). Nevertheless, the amount and composition of EOM largely depends on species, growth stages, and culture conditions (culture duration, pH, light, and temperature). Nonetheless, whole microbial cells without EPS can also be used to induce bioflocculation. The advantage of this type of flocculation is that there is no addition of chemical flocculant (Ummalyma et al. 2017). Contrariwise, Branyikova et al. (2018) mention that microalgae often excrete algogenic organic matter (AOM), consisting mainly of polysaccharides and proteins, into the growth medium; these AOM compete with flocculants for the algal cell surface and thus interfere in flocculation. Therefore, the quantity and composition of AOM along with the surface properties of the microalgal cells need to be considered while choosing a flocculant.

Yet another perspective of aging, auto-flocculation efficiency of S. obliquus does not exceed 5.5% at the early stationary growth-phase under neutral pH, but it is reported to increase to 24.4% (for the same culture and pH range) at the late stationary growth-phase (Matter et al. 2018). Similarly, in case of one-week-old culture and the three-week-old culture of Chlorococcum sp.
The composition and concentration of nutrients in the medium influence auto-flocculation efficiency and algae biomass production. Microalgae can also aggregate as a result of nitrate assimilation (where aggregation of cells occurs due to assimilation of nitrate as nitrogen source, which increases the pH of the medium and promotes auto-flocculation of cells). Nguyen et al. (2014) showed that nitrate concentration of 840.4 mg/L flocculated C. vulgaris in MBB medium. Nguyen et al. (2014) evidenced higher auto-flocculation efficiency for C. vulgaris occurs when nitrate (NO\textsubscript{3}\textsuperscript{-}) was used as the nitrogen substrate instead of ammonium (NH\textsubscript{4}\textsuperscript{+}); this is because of the fact that, under a high pH environment, there is co-precipitation of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions originally dissolved in the medium with algae cells, resulting from the nitrate assimilating metabolic activities of algal cells. Similarly, Tran et al. (2017) studied Nannochloropsis oculata at pH 10.4, reporting that the self-aggregation efficiency (~90%) of algal cells could be enhanced by the co-precipitation of Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. Negatively charged algal cells are destabilized due to the presence of oppositely charged metal ions. Under these circumstances, the absolute value of the zeta potential of algal cells decreases while increasing the Van der Waals forces, thereby promoting flocs formation between algal cells. In another study by Lv et al. (2018) auto-flocculation efficiency of Chlorococcum sp. was increased (63 to 84%) by increasing the ammonium concentration (10 to 50 mg/L); this was possibly due to the enhanced extracellular protein secretion at the higher ammonium concentration.

Wang et al. (2014) stated that addition of ions like Ca\textsuperscript{2+} and Mg\textsuperscript{2+} in the culture media spontaneously induces auto-flocculation of cells as a result of co-precipitation of calcium and magnesium that further causes a fluctuation in pH of the medium leading to effective flocculation of cells. Ca\textsuperscript{2+} and Mg\textsuperscript{2+} are used to autoflocculate microalga Chlorella (120 mg/L and 1000 mg/L respectively give 90% flocculation efficiency; Nguyen et al., 2014). Vandamme et al. (2012) also reported that addition of Mg\textsuperscript{2+} in C. vulgaris culture induced auto-flocculation. Among Mg\textsuperscript{2+}, Ca\textsuperscript{2+} and CO\textsubscript{3}\textsuperscript{2-} ions, at high pH levels the ion Mg\textsuperscript{2+} had effective flocculation and rapid sedimentation as compared to the other two ions (Ummalayma et al., 2017); this is probably because magnesium hydroxide flocs are positively charged whereas calcium carbonate flocs are negatively charged. Therefore, destabilization of the negatively charged microalgal cells is greater when magnesium ions are added into the medium than calcium ions.

Self-aggregation of microalgae can be triggered naturally with environmental stimulus such as nitrogen concentration stress in the suspended water or media, e.g. Scenedesmus dimorphus (Sukenik & Shelef 1984, Ummalayma et al., 2017).

**Role of Dissolved oxygen:**
Flocculation can also be induced in some microalgae species naturally in the medium because of the changes in concentration of dissolved oxygen content in the broth (Ummalayma et al., 2017, Schenk et al., 2008). Increased dissolved oxygen in a solution could trigger auto-flocculation of microalgae by creating more binding sites available on the cell surface; higher binding sites resulted in aggregate formation of the cells which increases the weight of the flocs and eventually leads to faster the settling rate (Ummalayma et al., 2017). Moreover, increased microalgal photosynthetic activity could also increase the dissolved oxygen content and the formation of dense flocs. Wilen and Balmer. (1999) emphasized high dissolved oxygen concentrations in the medium stimulate the auto-flocculation of microalgae; they state that dissolved oxygen concentrations of 14–16 mg/L promote flocculation.

**Algae-algae interactions and Spontaneous auto-flocculation:**
Some microalgae (described as self-flocculating microalgae) flocculate spontaneously; e.g. C. vulgaris JSC–7, S. obliquus AS–6–1, Ankistrodesmus falcatus and Etilia texensis SAG 79.80 (Alam et al., 2016). However, microalgae self-flocculation is different from flocculation stimulated by pH modulation. Ummalayma et al. (2017) acknowledged that cells flocculation generally exists in microorganisms, and, listed several self-flocculating microalgae such as C. vulgaris JSC–7, S. obliquus AS–6–1, Ankistrodesmus falcatus and Etilia texensis; however, they mention that the exact mechanism of this autoflocculation is still obscure. Biochemical analysis of two self-flocculating algae C. vulgaris JSC–7 and Scenedesmus obliquus as–6–1, have revealed that the polysaccharides synthesized by these are responsible for their self-flocculating properties; especially the purified polysaccharide obtained from C. vulgaris JSC–7 are effective bioflocculants which induce flocculation of non-flocculating strain (Alam et al., 2016). Marine microalgae Skeletonema marinoi also produce water soluble extracts which induce flocculation of Nonochloropsis oculata (Taylor et al., 2012). However, in the three self-flocculating microalgae C. nivalis, C. ellipsoideum and Scenedesmus sp., 90% flocculation efficiencies were observed at pH 4.5 (Ummalayma et al., 2017). Harvesting microalgae using self-flocculation which requires no extra expense in cultivation of microalgae or purification of bio-flocculant is a promising method for low cost harvesting. However, till date only few self-flocculation microalgae are reported; this does not meet the commercial demand for the application of this method as a harvesting technology for microalgae. Genetic modification of microalgae could be considered as an alternative in this perspective i.e. incorporating genes responsible for flocculation into microalgae without compromising their high biomass productivity of specific metabolites and high flocculation efficiency could be an option (Ummalayma et al., 2017) e.g a cell wall-deficient mutant of Chlamydomonas has been found to flocculate much more easily under alkaline conditions than the wild type strain (Scholz et al., 2011). However, there is an absolute need to pursue more research on self-flocculating microalgae to harvest non-flocculating oleaginous microalgae for industrial application. On the other hand, the addition of self–flocculating microalgae to non-flocculating microalgae has been known to promote faster flocculation and sedimentation, resulting in enhanced harvesting efficiency (Salim et al., 2012). In case of self–flocculating microalgae C. nivalis, C. ellipsoideum and Scenedesmus sp, Liu et al. (2013) reported maximum flocculation efficiencies (>90%) at pH 4.5; but these self-flocculating algae are used for the flocculation of the target microalgae (C. zofingiensis and C. vulgaris) with small size, flocculated by the pH decrease-induced flocculation method.

Overall, although auto–flocculation-based harvesting of algae is a potentially low-cost, eco-friendly biomass recovery strategy, that involves no chemical flocculant, it is generally slow and highly species-specific (Matter et al., 2019).

### 4. Animal based bio-flocculants

The natural polymer chitosan that is derived from shrimp exoskeletons have been effectively used in the harvesting of both fresh and seawater microalga and does not contaminate microalgal biomass (Schlesinger et al., 2012). Chitosan is cationic starch which is less pH-dependent, but is required at a higher dosage, is expensive and adds bulk (Barros et al., 2015). Chitosan has a net positive charge due to its high charge density, while overall charge of microalgae cells is negative; thus, the positively charged chitosan is strongly adsorbed on microalgae cells. Chen et al. (2014) explained that this results in most of the charged groups being close to the surface of the cells and effective destabilization of the microalgae. According to their report,
chitosan, first neutralizes charges on the microalgae cells, weakens the electrostatic repulsion between the microalgae cells, and thereafter, reduces the inter-particle repulsion; this is called charge neutralization. Chitosan has been effective in harvesting algae such as *C. vulgaris* and *N. oleoabundans*. Chitosan has unique properties like biodegradability, biocompatibility, renewability, bioactivity, and ecological acceptability, in addition to attractive physical and mechanical properties; it has been effectively used in wastewater treatment, biomedical engineering, and food processing (Chen et al., 2014). In fact, Matter et al. (2018) suggested harvesting of microalgae *S. obliquus* using a chitosan–alginate dual flocculation system.

### 5. Plant-based bioflocculant

Plant derivatives have recently emerged as attractive bioflocculants in the polymeric flocculants category (Table 3); their remarkable popularity in wastewater treatment is due to their biodegradability, stable hydrophilic nature, non-toxicity, wide availability from renewable resources and environmental friendliness (Ummalayma et al., 2017) e.g. protein from *Moringa oleifera* is an effective bioflocculant for *Chlorella* sp. and shows 90% flocculation efficiency (Ummalayma et al., 2017). Moong bean protein extract as an effective bioflocculant for *Nanochloropsis* having 81% flocculation efficiency at pH 2 has also been reported (Kandasamy & Shalea, 2016). Due to their biodegradability and high flocculation efficiency, Pal et al. (2008) recommend polysaccharide-based cationic flocculants as an alternative to the expensive, synthetic flocculants. The bridging mechanism is the main event occurring during flocculation by polymers (Pal et al., 2005); the extracellular matrix of green algae are supplemented with various sugars, polysaccharides and their derivatives like rhamnose, uronic acids, glucose, xylose, galactose, mannose, cellulose, pectin, pectic acids and ulvan along with other functional groups, these could be responsible for this mechanism. Furthermore, the presence of functional group like carboxyl, sulphate, amino and other negatively charged atoms in the above extracellular matrix impart an overall negative charge to the algal surface (Domozych et al., 2012). Further, Rahul et al. (2015) used cationic inulin for the harvesting *Botryococcus* sp. and achieved 88.6% efficiency (15 min at concentrations of 60 mg/L). Cationic inulin acts as bioflocculant, creating an electrostatic interaction between the opposite charges and cationic; the inulin neutralizes the negatively charged algal surface. This interface decreases the electrostatic repulsion between the cells, destabilizes the algal suspension and facilitates aggregation.

The positively charged polysaccharide framework concomitantly bridges several algal cells; thereby, this meshing–bridging action generates a structural complex in the form of bulky flocs. Once these flocs are created, they settle down faster and eventually get separated from culture broth (Rahul et al., 2015). On the other hand, cationic guar gum based flocculation of microalgae Chlamydomonas sp.and Chlorella sp. (flocculation efficiencies of 94 and 92% at concentrations of 100 ppm and 40 ppm respectively) have also been reported by Banerjee et al. (2013). Harvesting of *C. vulgaris* using seed powder of clearing nut (*Strychnos potatorum*) with 99.68% at a concentration of 100 mg/L for 150 agitation speed at 35 °C settled time of 30 min has been suggested by Razack et al. (2015) as an economically, effectively and an eco-friendly alternative. Banerjee et al. (2014) have ascertained the ability of cassia gum and guar gum (Chlamydomonas and Chlorella sp.) as a polymeric flocculants for algae recovery. Grafted agar and grafted guar gum have also been investigated as bioflocculants (Banerjee et al., 2013). With respect to waste water treatment, plants Plantago psyllium, Tamarindus indica, Moringa oleifera and Hibiscus esculentus have shown promising results; Anastasakis et al. (2009) showed that anionic polysaccharide of these plants are effective flocculants and remove 90% of total dissolve solid. Greenfloc 120 cationic starch is reported to be a good flocculant in case of *Parachlorella* and Scenedesmus (Vandamme et al., 2009).

### Table 3: plant-based bioflocculant for harvesting of microalgae.

<table>
<thead>
<tr>
<th>pH</th>
<th>Microalgae sp.</th>
<th>Flocculation efficiency (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 8</td>
<td><em>Chaetoceros calcitrants</em></td>
<td>85</td>
<td>Harith et al., 2009</td>
</tr>
<tr>
<td>pH 10.2</td>
<td><em>Chaetoceros calcitrants</em></td>
<td>90</td>
<td>Harith et al., 2009</td>
</tr>
<tr>
<td>pH 10.2</td>
<td><em>Chaetoceros</em></td>
<td>90</td>
<td>Harith et al., 2009</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td><em>Chlorella vulgaris</em></td>
<td>34</td>
<td>Salim et al., 2012</td>
</tr>
<tr>
<td><em>Skeletonema marinoi</em></td>
<td><em>Nonochloropsis oculata</em></td>
<td>90</td>
<td>Taylor, Rand and Caldwell 2012</td>
</tr>
<tr>
<td><em>Ettlia tenaxis</em></td>
<td><em>Chlorella vulgaris</em></td>
<td>55</td>
<td>Salim et al., 2011</td>
</tr>
<tr>
<td>pH 10.5</td>
<td><em>Scenedesmus sp.</em></td>
<td>90</td>
<td>Wu et al., 2012</td>
</tr>
<tr>
<td>pH 9</td>
<td><em>Ellipssoideum</em></td>
<td>90</td>
<td>Wu et al., 2012</td>
</tr>
<tr>
<td><em>Phaeodactylum Tricornutum</em></td>
<td><em>Nanochloropsis oculata</em></td>
<td>90</td>
<td>Wu et al., 2012</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td><em>Chlorella vulgaris</em></td>
<td>90</td>
<td>Wu et al., 2012</td>
</tr>
<tr>
<td>pH 9</td>
<td><em>C. vulgaris</em></td>
<td>85</td>
<td>Guo et al., 2013</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td><em>S. obliquus</em></td>
<td>80</td>
<td>Guo et al., 2013</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em> AS 6-1</td>
<td><em>Chlorococcum ellipsoideum</em></td>
<td>&gt;90</td>
<td>Liu et al., 2013</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em> AS 6-1</td>
<td><em>Scenedesmus</em></td>
<td>&gt;90</td>
<td>Liu et al., 2013</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em> JSC-7</td>
<td><em>Chlorella vulgaris CNW-11</em></td>
<td>80</td>
<td>Alam et al., 2014</td>
</tr>
<tr>
<td>pH 12</td>
<td><em>Chlorococcum sp.RAP13</em></td>
<td>94</td>
<td>Ummalayma et al. (2016)</td>
</tr>
<tr>
<td><em>Ankistrodesmus falcatus</em></td>
<td><em>Chlorella vulgaris</em></td>
<td>50</td>
<td>Ummalayma 2017</td>
</tr>
<tr>
<td><em>Tetraselmis suecica</em></td>
<td><em>Neochloris oleoabundans</em></td>
<td>72</td>
<td>Ummalayma 2017</td>
</tr>
</tbody>
</table>

Noor et al. (2016) reported flocculation efficiency of *Moringa oleifera* after oil extraction (MOAE) and non-extracted *Moringa oleifera* (MOWE) for algae *Nanochloropsis oculata* to be 93.77% (pH 7, 150 minutes, 5000 mg/L) and 70.56% (pH 7, 90 minutes, 4000 mg/L) respectively, which was less than aluminium sulfate (99.98% with short settling time, 30 minutes and 2000 mg/L of flocculant dosage at pH 6). However when the *Nanochloropsis oculata* was then fed to the *Brachionus plicatilis* (rotifers), the
concentrates of MOWE gave better than growth MOAE. Plant–based polymer mediated flocculation of microalgae is a less toxic, fast and low cost method for harvesting of algae biomass; however, there are certain concerns regarding the cost associated with the cationic quaternary amine group with regard to certain polymers.

6. Flocculation induced by fungus

Recently, a lot of attention has been focused on microbial flocculants produced by various microorganisms (actinomycetes, fungi, bacteria, and algae) widely distributed in soil and water. Microbial flocculants produced during the microorganism growth vary in composition (polysaccharides, proteins, DNA, cellulose, sugar, protein, polyamino acids, etc. Rebah et al, 2018); they are active biological compounds, biodegradable, without degraded intermediate pollutants, environment friendly, and have flocculation properties. Fungal hyphae and mycelia contain polysaccharides with active sites that are responsible for surface bioadsorption ability; these polysaccharides also enable the fungal cells to be charged. On the other hand, most microalgae cells have negative charges on their cellular surfaces and are capable of forming stable suspensions; nevertheless, the stability of these microalgal suspensions depends on the forces that: (i) interact between the cells themselves, and, (ii) between the cells and water. Hence they are considered as hydrophilic bio-colloids (Uduman et al, 2010b). The presence of functional groups like hydroxyl, carboxyl and amine on the surface give alga a negative charge, while the positively charged fungal mycelia reach inside the polysaccharide matrix and neutralize negative charge of algae (Grima et al, 2003). Aspergillus fumigatus efficiently flocculates a large number of microagal strains including marine as well as motile species (Wrede et al, 2014). While fungi can be easily cultivated, harvesting algae using fungal flocculation strategy does not involve toxic inorganic chemical compounds (Xie et al, 2013).

Table 4. Co-cultivation of fungi with microalgae

<table>
<thead>
<tr>
<th>Algae</th>
<th>Fungi</th>
<th>Flocculation efficiency (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. suecica</td>
<td>A. fumigatus</td>
<td>90</td>
<td>Muradov 2015</td>
</tr>
<tr>
<td>C. protothecoides</td>
<td>A. fumigatus</td>
<td>90</td>
<td>Muradov 2015</td>
</tr>
<tr>
<td>C. vulgaris</td>
<td>Aspergillus niger</td>
<td>80.3</td>
<td>Alam 2016</td>
</tr>
<tr>
<td>C. vulgaris UMN 235</td>
<td>A. oryzae</td>
<td>100</td>
<td>Alam 2016</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>S. bayanus var. uvarum</td>
<td>65</td>
<td>Alam 2016</td>
</tr>
<tr>
<td>Picoclorum sp. HMI</td>
<td>S. bayanus var. uvarum</td>
<td>50</td>
<td>Alam 2016</td>
</tr>
<tr>
<td>C. vulgaris</td>
<td>Saecharomyces postorianus</td>
<td>90</td>
<td>Ummalayma 2017</td>
</tr>
<tr>
<td>Nanochloropsis</td>
<td>Aspergillus nomius</td>
<td>94</td>
<td>Ummalayma 2017</td>
</tr>
<tr>
<td>C. vulgaris</td>
<td>Cunnighamella echinulata</td>
<td>99</td>
<td>Ummalayma 2017</td>
</tr>
<tr>
<td>C. vulgaris</td>
<td>A. nomius</td>
<td>97</td>
<td>Ummalayma 2017</td>
</tr>
<tr>
<td>Chlamydomonas sp.</td>
<td>S. bayanus var. uvarum</td>
<td>95</td>
<td>Ummalayma 2017</td>
</tr>
<tr>
<td>Picoclorum sp.</td>
<td>S. bayanus var. uvarum</td>
<td>75</td>
<td>Ummalayma 2017</td>
</tr>
</tbody>
</table>

According to Branyikova et al. (2018), specific consortia of positively charged fungi could induce bioflocculation of microalgal; they could be cultured separately or co-cultured with the microalgae. Co-culturing of fungi with algae for the purpose of harvesting of microalgal biomass has emerged as a new alternative in the recent years (Muradov et al, 2015). Co-cultivation could also be carried out in wastewater containing a carbon source. C. vulgaris co-cultivated with Aspergillus sp. is reported to demonstrate complete pellettization of spores; in addition, it also has the capability to remove nitrogen and phosphate from waste water treatment (Zhou et al, 2012). Table 4 demonstrates the co-cultivation of fungi with algae. Muradov et al. (2015) screened 33 fungal strains (from compost, straw and soil) for their flocculation efficiencies against microalgae used for biodiesel production (heterotrophic freshwater microalgae Chlorella protothecoides and the marine microalgae Tetraselmis suecica); they observed that co-cultivation of microalgae and filamentous fungus increased total biomass production, lipid yield and wastewater bioremediation efficiency. Thus fungi-aided microalgal flocculation is a significantly potent means of solving the major challenges faced by the commercialization of microalgal biotechnology; it is efficient and cost-effective for freshwater and seawater algal strains.

Gultom & Hu. (2013) reviewed utility of fungal bioflocculants, stating that filamentous fungi can have various morphologies in submerged cultures, depending on the organism and culture conditions. The morphologies that they described included: dispersed hyphae, microscopic aggregates, loose hyphal aggregates (clumps), and denser spherical aggregates (pellets). Fungal cell pellettization has been researched since decades (Zhang & Hu, 2012), it has been applied in the industrial processes to produce organic acid, pharmaceuticals, enzymes and other high-valued fermentation products, and wastewater treatment to remove pollutants. Many filamentous strains of fungi shows self-pellettization, that can be explained by either coagulative or non-coagulative mechanisms (Ummalayma et al, 2017).

The conditions for cell pellettization are strain-specific (Gultom and Hu, 2013); moreover, not all the filamentous fungal strains can form pellets during their growth. The proficiency of cell aggregation of filamentous fungal cells is mainly rooted to the production of hydrophobic proteins (i.e hydrophobins, a family of low molecular weight amphipathic proteins). Linder (2009) reported dimorphic fungal species where the hydrophobin was detected on the hyphal surface, while it was missing when the cells were grown in the yeast form. According to Feofilova (2010), these hydrophobic proteins coordinate the adherence of hyphae to solid substrates, which facilitates biofilm formation (attaching on a solid surface) and cell pellettization/granulation/aggregation (attaching on each other).

Overall the fungal pellettization can be categorized into two categories:

(i) Coagulative mechanism, where spores coagulate in the early stage of cultivation and develop into pellets through their intertwining hyphae; several fungi e.g., Aspergillus, basidiomycete, Phanerochaete chrysosporium, etc., follow the coagulative mechanism where fungal spores conglomerate at an early stage of development and then each pellet may arise from each spore aggregate. In coagulating pellet formation, the spores usually aggregate in the early stage of cultivation and pellets are formed out of these aggregates; this type of pellet formation can include Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, and the basidiomycete fungus Phanerochaete chrysosporium. On the other hand, Aspergillus, Basidiomycetes and Phanerochaeta produce dense spherical aggregates showing coagulative pellets.

(ii) Non-coagulative mechanism, where the spores germinate into hyphae, and then intertwine into pellets. Rhizopus sp, Mucor sp, Penicillium chrysogenum, follow the non-coagulative mechanism. For the non-coagulating type, pellets are formed out of one
spore; this type of pellet formation has been reported for some actinomycetes from the genus Streptomyces and for fungi belonging to Rhizopus sp. and Mucor sp. Gultom & Hu, (2013) described that the non-coagulative pellets are formed from one spore; moreover, they reported that some actinomycetes (i.e. genus Streptomyces) and fungi belonging to Rhizopus sp. and Mucor sp. show this type of pellet formation.

In a wastewater treatment context, Rebah et al. (2018) mention the utility of Aspergillus flavus (bioflocculant composed mainly of 69.7% polysaccharide and 28.5% protein; excellent FA <90% without cation), Aspergillus niger (bioflocculant composed of 66.8% polysaccharide and 31.4% protein), Phanerochaete chrysosporium (acidic polysaccharide; 93.5% FA of coal slurry), and Talaromyces sp. (proteoglycan with 84.6% polysaccharide and 15.2% proteins, 92.5% FA), Zang & Hu, (2012) reported A. flavus grown to form cell pellets and applied it for flocculation of C. vulgaris microalgae cultural broth; their zeta potential measurement revealed the average number for microalgae as 23.7 mV while A. flavus as +46.1 mV. There was charge difference between the microalgae and fungal cells; according to Zang & Hu, (2012), this surface charge might be the reason of co-pelletization of microalgae and fungal cell cultures. Zhou et al. (2013) suggested optimal parameters of bioflocculation of C. vulgaris UMN235 using fungus pelletization (Aspergillus oryzae) were 1.2x10⁶ spores/mL, 20 g/L glucose, and pH 4.0 to 5.0. Zhou et al. (2012) have also reported fungal bioflocculation of C. vulgaris. Talukder et al. (2014) reported that both marine (e.g. Nannochloropsis sp.) and freshwater (e.g. Chlorella vulgaris) microalgae were almost completely (94-97%) precipitated using the mycelium of Aspergillus nornias CCK–PDA7#. Al-Hothaly (2018) recognized that biofuel (biodiesel) produced from microalgae such as Botryococcus braunii is an alternative energy source, and optimized a method for the bio-harvesting of this microalgae using Aspergillus sp. in large–scale studies. Luo et al. (2019) cultured edible fungi Pleurotus ostreatus (100 rpm agitation) reporting lower pH of the Chlorella sp. suspension resulted in higher flocculation efficiency (maximum recovery efficiency reached 64.86% in 150 min). Interaction between the filamentous fungus Isaria fumosorosea and the microalgae C. sorokiniana was investigated by Mackay (2015), who observed that strict autotrophic conditions at pH 7-8, co-culture of microalgae (2-20 μm) with fungal blastospores resulted in the development of large pellets (1-2 mm) which may be easily harvested by sedimentation or filtration at 95% harvesting efficiency. Zhou et al. (2012) reported an alternative fungus pelletization assisted bioflocculation method for harvesting microalgae C. vulgaris UMN235 using pellet-forming fungal strain (Aspergillus oryzae).

7. Bacterial based bioflocculation

Several bacteria belonging to various classes (Actinobacteria, Alphaproteobacteria, Bacilli, Deltaproteobacteria, Gammaproteobacteria, Proteobacteria, etc.) are also reported to produce flocculants (Rebah et al., 2018; table 5). Bacterial communities play a significant role in microalgal aggregation in natural aquatic (Alam et al., 2016), thereby the idea of bacterial flocculants being used for microalgal harvesting seems convincing. Specific bacteria are also known to induce flocculation of microalgae (Gutziet et al., 2005, Lee et al., 2008). On culture, mixed algal bacterial flocs can be easily harvested by this technique. Extracellular bioflocculant from the bacterium Shimella albus xn–1 is reported to be useful for harvesting C. vulgaris (Li et al., 2018). The use of bacteria as a flocculating agent rules out the question of chemical contamination, but results in microbiological contamination, which needs to be considered in case of harvesting microalgal biomasses for food or feed applications (Vandamme et al., 2013).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Microalgae</th>
<th>Flocculation Efficiency (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumonia</td>
<td>Syneccovitis</td>
<td>95</td>
<td>Nie et al., 2011</td>
</tr>
<tr>
<td>Bacillus subtilis (y-PGA)</td>
<td>Chlorella vulgaris LICME 001</td>
<td>90</td>
<td>Zhang et al., 2012</td>
</tr>
<tr>
<td>Bacillus subtilis (y-PGA)</td>
<td>Chlorella vulgaris LICME 003</td>
<td>92</td>
<td>Zhang et al., 2012</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Chlorella zofingiensis</td>
<td>83</td>
<td>Agapke et al., 2014</td>
</tr>
<tr>
<td>Bacillus licheniformis CGMCC 2876 (y-PGA)</td>
<td>Desmodusmos sp. F51</td>
<td>92</td>
<td>Ndikubwimana et al., 2014</td>
</tr>
<tr>
<td>Paenibacillus sp.</td>
<td>Botryococcus braunii</td>
<td>91-95</td>
<td>Alam et al., 2016</td>
</tr>
<tr>
<td>Paenibacillus polymyxa AM49.</td>
<td>Scenedesmus sp.</td>
<td>95</td>
<td>Alam et al., 2016</td>
</tr>
<tr>
<td>Paenibacillus sp. AM49.</td>
<td>C. vulgaris</td>
<td>93</td>
<td>Alam et al., 2016</td>
</tr>
<tr>
<td>Solibacillus silvestris (proteoglycans)</td>
<td>Nannochloropsis oceanic</td>
<td>90</td>
<td>Ummalyma et al., 2017</td>
</tr>
<tr>
<td>B.subtilis - (y PGA)</td>
<td>Phaeodactylum Tricornutum</td>
<td>97</td>
<td>Ummalyma et al., 2017</td>
</tr>
</tbody>
</table>

Li et al. (2018) particularly report that the algal pH causes enormous effects on flocculation activity of bioflocculant, and basic pH values are helpful to promote flocculation activity of the bioflocculant. Unlike Li et al. (2018), Ndikubwimana et al. (2014) showed that the flocculation efficiency of bacterial bioflocculant on microalgae Desmodusmos sp. F51 was dependent on the initial culture pH, and the flocculation efficiency increased when the initial culture pH was changed from pH 7 to 3. Pérez et al. (2017) tested acidic pH (2 to 6) and basic pH (8 to12) for harvesting Skeletonema costatum and Chaetoceros gracilis microalgae, thereby confirming that the highest pH values (11, 11.5 and 12) caused higher flocculation activity than acid pH values; they suggest that the pH induced flocculation is an effective method for both species.

On the other hand, metal ions as coagulants are reported to improve the flocculation activity of bioflocculant; in fact, certain bioflocculants flocculate algal cells only when metal ions were added together as coagulants. Li et al. (2018) reported Ca²⁺ ion as one of the most suitable coagulant to improve flocculation activity of the bioflocculant FLC–hsn06. Similarly, Kim et al. (2011) used the bioflocculant produced by Paenibacillus polymyxa AM49 to harvest Scenedesmus sp., and confirmed that consecutive treatment with 8.5 mM CaCl₂ and 0.2 mM FeCl₃ as coagulants improved the flocculation activity up to 95%. According to Lei et al. (2015), a bioflocculant from Cobetia marina L03 effectively harvested microalgae C. vulgaris via flocculation–floation, wherein, the flocculation efficiency of 92.7% was observed using 20 mg/L bioflocculant in the presence of 5 mM CaCl₂. Their bioflocculant was stable at wide ranges of pH and temperature (Lei et al., 2015); this proves to be advantageous for its use in various applications involving a wide range of pH and temperatures. Paenibacillus sp. is also reported to show high flocculent activity for C. vulgaris.
Contrary, bioflocculant from Solibacillus silvestris could flocculate Nannochloropsis oceanica (90% flocculating efficiency) without adding any metal ions (Wan et al, 2013). Chemical analysis indicated that the purified bioflocculant was a proteoglycan composed of 75.1% carbohydrate and 24.9% protein (w/w); it exhibited no effect on the growth of microalgal cells and could be reused for economical harvesting of N. oceanica.

Alam et al. (2016) explained that there are two possible mechanisms involved in microalgal aggregation by bacteria: (i) aggregation triggered by compounds produced by bacteria, and (ii) direct interaction between the bacteria and microalgae, triggering aggregation. Such interactions could be facilitated by proteins on the bacterial cell wall or the flagellum (Alam et al, 2016). The bioflocculant could contain charged functional groups that could cause aggregation of microalgal cells via either charge neutralization and electrostatic patches, or via bridging (Ndiobikwimana et al, 2015). Charge neutralization in the presence of ions and attachment via cells or extracellular polymeric substances (EPS) are the main mechanisms underlying bacteria-associated bioflocculation (Alam et al, 2016). Ummalyma et al. (2017) reported that biopolymers EPS or glutamic acid secreted by microbes cause microbial flocculation of microalgae. According to Alam et al. (2016), if the major component of a bioflocculant is a glycoprotein, its stability depends on the relative protein and polysaccharide content. Aggregation could be facilitated by polysaccharides, proteins, or other bioflocculant agents that enhance microalgal sedimentation; here, polysaccharides have better bioflocculant properties than proteins. The molecular weight and the functional groups in the molecular chains is the most essential factor needed to be considered while studying flocculation activity of bioflocculants. While protein bioflocculants have a low molecular weight, and have few functional groups, polysaccharide bioflocculants have a high molecular weight and many functional groups. The functional groups determine the type charge, its distribution, and consequently the type of interaction. Pugazhendhi et al. (2019) and Sutherland et al. (2001) have described the mechanism and importance of functional groups and exopolymers in bioflocculation. During algal-bacterial flocculation, microalgal cells are not damaged and they maintain their integrity, which is an advantage. Moreover, in a large scale process, the media could be reused to minimize cost of nutrients and the water demand (Alam et al, 2016). Nevertheless, cultivating bacteria in combination with microalgae requires a carbon source in the medium; in case of wastewater, a carbon source is generally present which facilitates cultivation of microalgae and bacteria. Another disadvantage of this process is that there is a potential danger of unwanted bacterial contamination of the microalgal production plant (Alam et al, 2016). Bacterial flocculants are important low cost method towards renewable microalgal based biofuel production (Ummalyma et al, 2017) being ecofriendly, less expensive, and, feasible, they do not generate toxic waste.

Microalgae get attached to the bacterial EPS or glutamic acid and form larger flocs promoting flocculation; e.g Polyglutamic acid from Bacillus subtilis is used for harvesting of biomass of microalgae e.g Nanochloropsis oculata LICME 002, Phaeodactylum tricornutum, C. vulgaris LICME 001 and Botryococcus braunii LICME 003; further, Bacillus licheniformis CGMCC 2876 (γ – PGA) is used for harvesting of microalgae Desmodesmus sp. F51 and flocculation efficiency is 92% (Alam et al, 2016). According to Van Den Hende et al. (2011), the microalgal bacterial flocs or MaB-flocs are aggregation of microalgae and bacteria; these because of their larger size, settle quickly by gravity. Essentially, EPS polymers are responsible for cell to cell contact without cell stress or lysis over an extended period of time (Ummalyma et al, 2017).

### 8. Actinomycetes as Flocculants

Apart from bacteria, reports suggest the utilization of bioflocculant from an actinomyctes Streptomyces sp. hsn06 for harvesting microalgae C. vulgaris (Li et al, 2018), wherein best flocculation was observed using 5 mM CaCl2 (5 min).

### 9. Yeast Flocculants

Co-cultivation of algae with yeast promotes biomass and lipid production, as well as, effective flocculation harvesting (Matter et al, 2019). Reports suggest the co-culturing of yeast Rhodotorula glutinis and alga Scenedesmus. obliquus which leads to synergistic increase in biomass production (40 to 50%) and lipid content (60 to 70%), as compared with single cultures (Yen et al, 2015). Diaz-Santos et al. (2015) observed the bio-flocculation efficiencies of freshwater alga Chlamydomonas reinhardtii and marine alga Picoclorhum sp. using whole cells and extracellular proteins of anaerobically grown yeast Saccharomyces bayanus. In their study, while the whole yeast cells showed only moderate harvesting efficiencies (80 and 60% respectively) for C. reinhardtii and Picoclorhum sp., treatment with a relatively low concentration of extracellular yeast proteins (0.1 g/L), led to significant increase in the flocculation efficiencies (95% and 75% respectively). Soluble cell wall proteins from Saccharomyces bayanus var. varum are reported to yield a flocculation efficiency of 75% using a dose of 1 mg. 10/mL (Díaz-Santos et al, 2016). Chemically modified autolysates of S. cerevisiae (a by-product of the brewing industry) are also reported to exhibit high harvesting efficiency (>90%) for C. vulgaris at a dosage of 0.4 mg /g cell (Matter et al, 2019). However, the use of either yeast whole cells or their extracellular proteins appears to be environmentally friendly, but the practicality of their availability and their low-cost requires examination.

### 10. Factors influencing bioflocculation:

Production of bioflocculants (especially microbial flocculants) is highly subjective to the culture medium composition and other physicochemical parameters. Although certain factors influencing algae-algae flocculation have been detailed before, factors affecting other aspects of bioflocculation are elaborated below.

#### 10.1. Abiotic Factors:

##### 10.1.1. Effect of Carbon and Nitrogen Sources on Bioflocculant Production

Okaiyeto et al. (2016) describe carbon sources to play a substantial role in enhancing the secretion of bioflocculants by microorganisms, while Salehizadeh and Yan (2014) acknowledged the significance of carbon and nitrogen sources in the production of bioflocculants. Lee et al. (2001) reported that Bacillus licheniformis XI4 favored ethanol, sucrose, and starch as appropriate carbon sources for the secretion of ZS–7 bioflocculant, whereas ammonium chloride was preferred as a nitrogen source of choice. Glucose was the preferred carbon source among other sources investigated for bioflocculant production by Virgibacillus sp., while sucrose, corn starch, glycerol, and glucose as appropriate substrates for bioflocculant production by Apergillus parasiticus, exhibiting a high flocculating activity above 80% at 72 h of fermentation (c.f. Okaiyeto et al, 2016). Further, Klebsiella sp. bioflocculant requires maltose and urea as carbon and nitrogen sources, respectively; however, sodium carbonate and tryptone were most favorable for bioflocculant production by Oceanobacillus sp. (c.f. Okaiyeto et al, 2016). The optimum conditions for bioflocculant production, flocculating activity, chemical composition, and yields are known to vary with the organism (c.f. Okaiyeto et al, 2016).

##### 10.1.2. Temperature:

© 2020 JETIR October 2020, Volume 7, Issue 10 www.jetir.org ( ISSN-2349-5162)
Temperature influences the production of exopolymeric substances. Although the optimal temperature range for bioflocculant production would vary with species, the cultivation temperature has a great impact on bioflocculant production in microorganisms (Li et al. 2009). Enzymes responsible for bioflocculant production are activated at an optimum temperature (Zhang et al. 2007). In fact the proteins and peptides in an exopolymeric substance are generally thermally labile. Nevertheless, the thermal stability is an important property when it comes to commercial utility of a bioflocculant; Okaïyeto et al. (2016) describe thermal stability of few bioflocculants with a characteristic of their polysaccharide backbone. Uduman et al. (2010) observed that the percent of recovery of microalgae increases as temperature increases; they explained this phenomena by a collision theory. At higher temperatures mobility of cellular particle increases, therefore, as the temperature increases there is a greater probability of the polymer and microalgal cell to collide. Increase in a number of possible interactions, would indicate that the number of collisions increased; this in turn shows the flocculation rate had improved. When density difference is greater, the temperature increases, and, consequently settling rates are improved. Li et al. (2018) evaluated the Actinomycete Streptomyces for its bioflocculation of C. vulgaris observing that the flocculation activities under different algal temperatures including 10, 20, and 30 °C were significantly (p<0.01) lower than that in positive control, which were 18.6, 81.9, and 80.8% of positive control, respectively. They observed high flocculation efficiency under 40 °C of algal temperature, compared to positive control. According to Li et al. (2018) most reported bioflocculants generally are either proteins, polysaccharides, or other extracellular polymeric substances; amongst these, proteins always lose bioactivity under high temperature with thermal instability e.g when treated under different temperatures the flocculation activity of mycelial pellets of the Actinomycete Streptomyces sp. hsn06 on C. vulgaris cells is reported to declined.

10.1.3. **pH**:

pH comprise one of the most influential external factors that impacts flocculating activity of bioflocculants (Salehizadeh & Yan, 2014, Zaki et al. 2013), any alteration in pH may changes the bioflocculant charge and surface characteristics of suspended particles thereby changing its flocculating ability (Zhang et al. 1999). Perhaps bioflocculants show distinct electric states at different pH values, which in turn influences the flocculation capability of the bioflocculants (Okaïyeto et al. 2016). Ugbenyen et al. (2014) reported that pH influences the stability of suspended particles and the formation of flocules. Some bioflocculants are active in acidic conditions (G. impudicum KG03; pH 3 to 6), while others prefer basic pH values (Streptomyces flocculant shows highest flocculation at pH 9 to 11 and at pH 7; Li et al. 2018). Nevertheless, certain bioflocculant demonstrate a wide range of flocculating activity indicating its applicability in numerous fields, viz. *F. eli* bioflocculant (showed 80% flocculation at pH from 3 to 11; Li et al. 2013) and Ruditapes philippinarum bioflocculant (pH range from 1 to 13; Gao et al. 2009). Essentially, the initial pH of the fermentation medium is key in producing bioflocculant as it influences flocculating efficiency; it determines the electrification of the cells and oxidation–reduction potential (which could influence the absorption of nutrients in the production medium and enzymatic reaction) (Okaïyeto et al. 2016). Optimum pH for bioflocculant production varies with species, for example Halomonas sp. prefers pH 7, while acidic conditions are preferred for synthesis Aspergillus parasiticus bioflocculant; Klebsiella sp. TG–1 bioflocculant production occurs at pH 8, while bioflocculant is secreted by Halobacillus sp. at pH 7 (Okaïyeto et al. 2016).

According to Maji et al. (2018) in case of *C. vulgaris*, pH of 3.5 and 9.5 resulted in highest flocculation, whereas pH of 4.0 and 9.0 had produced maximum flocculation in *S. obliquus*; furthermore, cells of Chlorococcum sp flocculated at higher levels at pH 3.5 and 9.0. Their results indicated that flocculation efficiency is pH, species and initial biomass concentration dependent.

10.1.4. **Effect of cations and/or metal ions**

Certain ions exhibit varying effects on different bioflocculants; bioflocculation activity is determined by the property and structural components of bioflocculants (its origin) and the valency and concentration of the ions (Wu & Ye, 2007). Usually a cation is used as coagulant aid in achieving high flocculation activity by neutralizing the negatively charged functional groups on the bioflocculant and suspended particles (Okaïyeto et al. 2016); this thereby encourages the formation of bridges between particles and the bioflocculant, and this further increases the adsorption of bioflocculant to the suspended particles. In other words cation play a vital role in stimulating the adsorption of flocules on suspended particles by decreasing the distance between them and increasing the electrostatic attraction between the bioflocculant molecules and the suspended particles. Li et al. (2007) observed that the addition of cations to a suspension increased the floc size, resulting in enhanced sedimentation. According to Cosa et al. (2013) found that calcium chloride and aluminum chloride are most stimulating cations in case of bioflocculant secreted by marine bacteria, Oceanobacillus sp. Pinky. Ca$^{2+}$ and Mg$^{2+}$ cations have synergistic effects on bioflocculant produced by *Serratia ficaria*, whereas Al$^{3+}$ and Fe$^{3+}$ showed a negative effect (Gong et al. 2008). Okaïyeto et al. (2016) mentioned that bioflocculant produced by Halomonas sp. and Micrococcus sp. are cation dependent; they reported improved flocculation activity in the presence of Al$^{3+}$, Ca$^{2+}$, and Mn$^{2+}$ and inhibited by Ba$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, Na$^+$, Li$^+$, and K$^+$. Monovalent cations (Na$^+$, Li$^+$, K$^+$) and the trivalent cation Fe$^{3+}$, showed little effect on flocculation activity on bioflocculant produced by Virgibacillus sp. Rob, whereas divalent cations (Ca$^{2+}$, Mn$^{2+}$, Mg$^{2+}$) and Al$^{3+}$ are known to greatly improve flocculating efficiency. Monovalent cations at times show weak stimulation of flocculation by their respective bioflocculants (Okaïyeto et al. 2016). Brachybacterium sp. bioflocculant in fact required Ca$^{2+}$, Mg$^{2+}$, and Mn$^{2+}$ for effective flocculation (Nwodo et al. 2012); similarly bioflocculant produced by Bacillus velezensis is reported to be stimulated in the presence of Ca$^{2+}$, Zn$^{2+}$, and Na$^+$ and inhibited in the presence of Al$^{3+}$, Fe$^{3+}$, and Mg$^{2+}$ (Zaki et al. 2012). Ummalyma et al. (2017) particularly evaluated effect of magnesium ion, calcium ion and carbonate ion on flocculation potential and settling of microalgal cells, stating that magnesium ion with high pH levels has an effective flocculation and rapid sedimentation in compare to other ions. According to them, under basic conditions, elevated concentration of di-and tri-valent ions could cause enhanced flocculation.

10.1.5. **Salinity**:

Sukenik et al. (1988) reported that salinity of brackish water and seawater requires high flocculant dosages and renders flocculation less effective than in freshwater algal media; their study on marine microalgae Isochrysis galbana and Chlorella stigmatophora showed that the flocculant dosages required were found to increase linearly with salinity as expressed in ion strength. In another study, Zheng et al. (2012) reported that microalgal flocculation in which there is cationic polymers are inhibited by sea water having high ion strength. These facts need to be considered in the utilization of bioflocculants for microalgal harvesting.
11. Biotic Factors:

11.1. Effect of Inoculum Size on Production of Bioflocculants

Okaiyeto et al. (2016) reported that among various physiological properties, inoculum size plays a substantial role in metabolic processes as it has a significant effect on cell growth and the production of secondary metabolites. While a small inoculum size prolongs the stagnant growth phase, a large inoculum size causes the niche of the microorganism to overlap excessively, thereby suppressing bioflocculant production. Bioflocculant production by *Oceanobacillus* sp. Pinky requires 2% inoculum size, whereas 3% (v/v) inoculums size was preferred for the production of bioflocculant by *Bacillus* sp.

11.2. Biomass Concentration and Bioflocculant Dosage

The bioflocculant concentration plays a vital role in any bioflocculation process; it is essential to maintain an optimum biomass concentration to achieve maximum flocculation activity in case of microalgae harvesting too. On the other hand, the bioflocculant dose or concentration is tremendously important (Ndikubwimana et al. 2014). According to Ndikubwimana et al. (2016), bioflocculant *Desmodesmus sp.* F51 bioflocculation efficiency varies with bioflocculant dosage when *Bacillus. licheniformis* was used; effect of bioflocculant dosage was investigated by keeping biomass concentration constant (0.5 g/L) and the bioflocculant dosage of 1, 2, 2.5, 3, 4 and 5 mL/L was added to aliquots of microalgal suspension. When bioflocculant dosage is 2.5 mL/L, 99.5±0.4% flocculation efficiency achieved, and when γ –PGA dosage was 20 mL/L and concentration factor is 15.1 they observed 82% flocculation efficiency.

Conclusion and perspectives

Utility of algal biomass for fuels, food, feed, and value-added products has escalated worldwide. However the energy-intensive microalgae harvesting is one of the biggest challenges that researchers and industrialists face during downstream processing. Biological harvesting of microalgae has emerged as a promising technology that is economically viable and environmentally friendly. Bio-flocculation of microalgae has several unique advantages; moreover, its strategies could be scaled-up for commercial applications. The use of purified bioflocculants and microorganisms does not involve heavy investments; however, it is essential to understand the variegated approaches, the potential utilization of a variety of bioflocculants available and the factors influencing bioflocculation processes for microalgal harvesting. This review serves to address these issues for the benefit of researchers and industrialists.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The author Dr. K. Suresh Kumar grateful to UGC-BSR (No.F.30-373/2017 (BSR)), New Delhi for providing financial assistance.

References


