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), bio-flocculation 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 1produce 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 flanged with certain drawbacks; e.g utilizing 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.

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

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 (Pragya 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 reducing 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. Flotation

Flotation is a more advance and effective technique as compared to sedimentation (Chen et al, 1998). This is a physiochemical 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 flotation; 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 flotation (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 flocculants 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 flocculants (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). Flocs 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 flocculants. 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 microalgae within the suspension; therefore, flocculants 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).

Microalgal flocculation methods include: Spontaneous and forced alkaline flocculation, chemical flocculation with addition of flocculants, 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 flocculant 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 flocculant dose per biomass weight (Branyikova et al, 2018). Microalgal growth phase also influences flocculation; moreover, the pH, dissolved CO_2, 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 flocculants have been tested for microalgal harvesting. These include a variety of salts, inorganic chemicals, organic/polyelectrolyte flocculant, etc. However, despite being economical, these chemicals could prove to be hazardous and often contaminate the algal biomass (Lee et al, 2009). Inorganic chemical flocculants 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 flocculation in water treatment. Ferric chloride ($FeCl_3$), aluminium sulfate ($Al_2(SO_4)_3$) and ferric sulfate ($Fe_2(SO_4)_3$), 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 flocculation, but they are not eco–friendly, contaminate biomass, alter the growth medium, and cause color change.

Organic flocculants 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 microalgae flocs due to electro-repulsion. The flocculating power of the polyelectrolyte hangs on the properties such as charge and functional groups on the surface of microalgae, growth medium pH and density of the algal culture (Chen et al, 2011). Barros et al. (2015) reported that cationic polyelectrolytes with high charge density are more effective flocculants to harvest microalgae and the effective dose decreases with an increase in molecular weight of coagulant whereas anionic polyelectrolyte 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 flocculant 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 microalgal 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 polyethylenimine 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 microalgal 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 microalgal 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 microalgal aggregates or flocs (Wan et al, 2015). In this process, the bubbles produced at the anode rise to the surface entrapping microalgal 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_2O_3) nanoparticles adsorb directly on the microalgal 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 microalgal 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 (Salim 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.
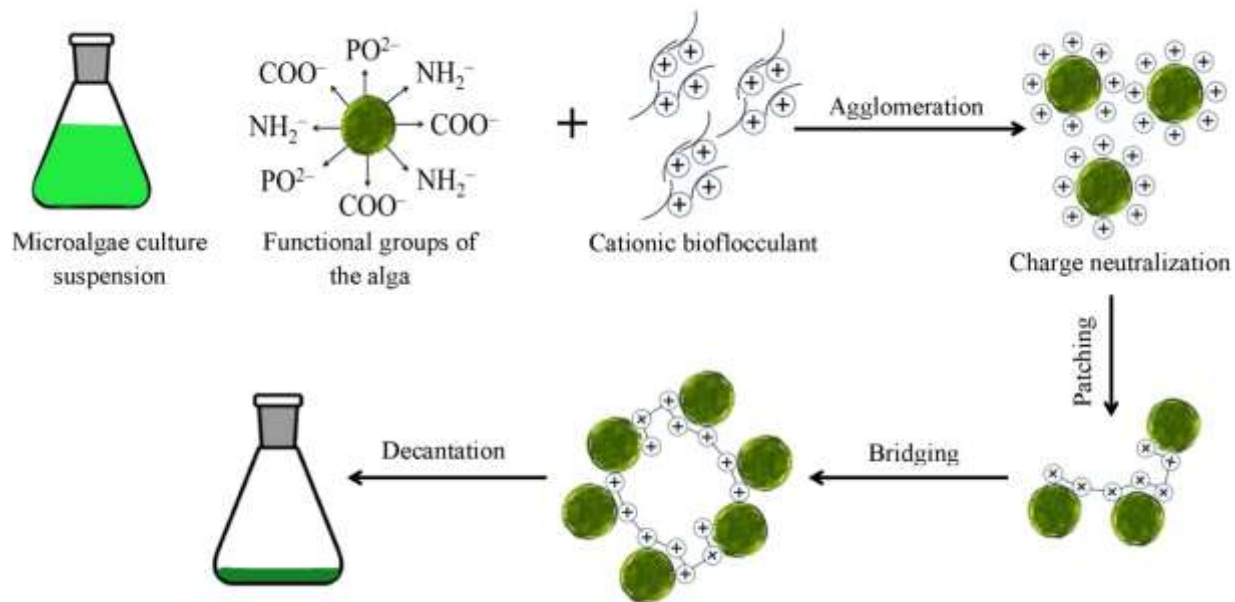

Figure shows the possible mechanisms of microalgae bioflocculation

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 Ummalayma 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 microalgae 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 CO_2 supply through photosynthesis, microalgae remove CO_2 dissolved in the culture medium, increasing its pH value. Autoflocculation is an actively investigated harvesting strategy of microalgae (Umamalyma et al, 2016). Table 2 demonstrates auto-flocculation of microalgae.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 microalgae *C. vulgaris CNW11* and *S. obliquus FSP* with 80% flocculation efficiency. Nonetheless, the use of excessive volumes of the self–flocculating microalga *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 Al^{+3}, Fe^{+3}, or Ca^{+2} (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 microalgae *C. vulgaris* JSC–7 and *S. obliquus* AS–6–1 (Alam et al,

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 MJk/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* CNW11 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 microalgae 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 to10.5 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 calcitrants* 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* NRCIbr1. High flocculation efficiency (90%) in case of three freshwater algal species, *C. nivale, C. ellipsoideum*, and *Scenedesmus* sp. occur under acidic conditions (pH 4).

Table 2. autofloculation of microalgae

Plant based biofloulant	Microalgae sp.	Types of microalgae	Harvesting efficiency (%)	Reference
Chitosan	*Thallesiosira pseudomonas*	Marine	>90	Heasman et al., 2000
Poly-γ-glutamic acid	*Nonochloropsis occulata* *Chlorella vulgaris, Nannochloropsis oculata, and* *Phaeodactylum Tricornutum*	Marine	>90	Zhang et al., 2012
Cationic starch	*Scendesmus dimorphus*	Fresh water	70-95	Hensel, Riefler and Stuart 2014
Guar gum	*Chlamydomonas* sp. *Chlorella* sp.	Fresh water	84 92	Banergee et al 2014
Inulin	*Botryococcus* sp.	Marine	88.6	Rahul et al. (2015)
Moong bean protein extract	*Nanochloropsis* sp.	Marine	>90	Kandasamy and Shaleh, 2016

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 (Umamalyma 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.

In 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.

RAP13, the auto-flocculation efficiency is reported to increase from 62 to 75% respectively (Ummalyma et al, 2016).

Medium composition, ions and nutrient stress:

Matter et al. (2019) state that the composition and concentration of nutrients in the medium (nitrogen sources and minerals) influence auto-flocculation efficiency and algal biomass production. Microalgae can also aggregate as a result of nitrate assimilation (where aggregation of cells occur 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_3^-) was used as the nitrogen substrate instead of ammonium (NH_4^+); this is because of the fact that, under a high pH environment, there is co-precipitation of Ca^{+2} and Mg^{+2} ions originally dissolved in the medium with algal 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^{+2} and Mg^{+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^{+2} and Mg^{+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^{+2} and Mg^{+2} are used to autoflocculate microalgae *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^{+2} in *C. vulgaris* culture induced auto-flocculation. Among Mg^{+2}, Ca^{+2} and CO_3^{2-} ions, at high pH levels the ion Mg^{+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 microalgae cells is greater when magnesium ions are added into the medium than calcium ions.

Self-aggregation of microalgae could 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 (Ummalyma 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 (Ummalyma 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 autoflocculation

Some microalgae (described as self-flocculating microalgae) flocculate spontaneously; e.g *C. vulgaris JSC–7*, *S. obliquus AS–6–1*, *Ankistrodesmus falcatus* and *Ettlia 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 *Ettlia 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 *Scendesmus 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 occulata* (Taylor et al, 2012). However, in the three self–flocculating microalgae *C. nivale*, *C. ellipsoideum* and *Scendesmus* sp., 90% flocculation efficiencies were observed at pH 4.5 (Ummalyma 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. nivale, 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 microalgae 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 (Umamalyma 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 & Shaleh, 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 Scendesmus (Vandamme et al, 2009).

Table 3. plant-based biofloulant for harvesting of microalgae.

Autoflocculation	Microalgae sp.	Flocculation efficiency (%)	Reference
pH 8	*Chaetoceros calcitrants*	85	Harith et al., 2009
pH 10.2	*Chaetoceros calcitrants*	90	Harith et al., 2009
pH 10.2	*Chaetoceros*	90	Harith et al., 2009
Scendesmus obliquus	*Chlorella vulgaris*	34	Salim et al., 2012
Skeletonema marinoi	*Nonochloropsis occulata*	90	Taylor, Rand and Caldwell 2012
Ettlia texensis	*Chlorella vulgaris*	55	Salim et al.,2011
pH 10.5	*Scendesmus* sp.	90	Wu et al. 2012
pH 9	*Ellipsoideum Phaeodactylum Tricornutum*	90	Wu et al. 2012
pH 9	*Nanochloropsis* sp.	90	Wu et al. 2012
pH 10.5	*Chlorella vulgaris*	>90	Wu et. al., 2012
Scendesmus obliquus AS 6-1	*C. vulgaris*	85	Guo et. al., 2013
Scendesmus obliquus AS 6-1	*S. obliquus*	80	Guo et. al., 2013
pH 4.5	*Chlorococcum ellipsoideum*	>90	Liu et. al., 2013
pH 4.5	*Scendesmus* sp.	>90	Liu et. al., 2013
Chlorella vulgaris JSC-7	*Chlorella vulgaris* CNW-11	80	Alam et al., 2014
pH 12	*Chlorococcum* sp.RAP13	94	Ummalyma et al. (2016)
Ankistrodesmus falcatus	*Chlorella vulgaris*	50	Ummalyma 2017
Tetraselmis suecica	*Neochloris oleoabundans*	72	Ummalyma 2017

Noor et al. (2016) reported flocculation efficiency of *Moringa oleifera* after oil extraction (MOAE) and non-extracted *Moringa oleifera* (MOWE) for algae *Nannochloropsis 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 *Nannochloropsis 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 algal biomass; however, there are certain concern 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 algae 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 microalgal 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

Algae	Fungi	Flocculation efficiency (%)	Reference
T. suecica	*A. fumigates*	90	Muradov 2015
C. protothecoides	*A. fumigates*	90	Muradov 2015
C. vulgaris	*Aspergillus niger*	80.3	Alam 2016
C. vulgaris UMN 235	*A. oryzae*	100	Alam 2016
Chlamydomonas reinhardtii	*S. bayanus var. uvarum*	65	Alam 2016
Picochlorum sp. HM1	*S. bayanus var. uvarum*	50	Alam 2016
C. vulgaris	*Saccharomyces postorianus*	90	Ummalayma 2017
Nanochloropsis	*Aspergillus nomius*	94	Ummalayma 2017
C. vulgaris	*Cunnighamella echinulata*	99	Ummalayma 2017
C. vulgaris	*A. nomius*	97	Ummalayma 2017
Chlamydomonas sp.	*S. bayanus var. uvarum*	95	Ummalayma 2017
Picochlorum sp.	*S. bayanus var. uvarum*	75	Ummalayma 2017

According to Branyikova et al. (2018), specific consortia of positively charged fungi could induce bioflocculation of microalgae; 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 pelletization 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, straws 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 pelletization 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-pelletization, that can be explained by either coagulative or non-coagulative mechanisms (Ummalyma et al, 2017).

The conditions for cell pelletization 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 pelletization/granulation/aggregation (attaching on each other).

Overall the fungal pelletization 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 *Phanerochaete* 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 fugal 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 $1.2x\ 10^4$ spores /mL, 20 g/L glucose, and pH 4.0 to 5.0. Zhou et al. (2012) have also reported fungal bioflocculation of *C. vulguris*. Talukder et al. (2014) reported that both marine (e.g *Nannochloropsis sp.*) and freshwater (e.g. *Chlorella vulguris*) microalgae were almost completely (94-97%) precipitated using the mycelium of *Aspergillus nomius CCK–PDA7#6*. 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 harvesting 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 (Gutzeit 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 *Shinella 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 5. bacteria-based flocculation of microalgae

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

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 *Desmodesmus sp. F51* was dependent on the initial culture pH, and, the flocculation efficiency increased when the initial culture pH was changed from 7.2 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 Ca^{+2} 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 $CaCl_2$ and 0.2 mM $FeCl_3$ 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–flotation, wherein, the flotation efficiency of 92.7% was observed using 20 mg/L bioflocculant in the presence of 5mM $CaCl_2$. 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 flocculant activity for *C. vulgaris*

(Oh et al, 2001), but this usually requires chemical flocculants ($CaCl_2$) to achieve optimal performance.

Contrarily, 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 to 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 (Ndikubwimana 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 microalgae 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 *Nanochlropsis occulata* 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 actinomycete Streptomyces sp. hsn06 for harvesting microalgae *C. vulgaris* (Li et al, 2018), wherein best flocculation was observed using 5 mM $CaCl_2$ (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 *Scendesmus. 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). Díaz-Santos et al. (2015) observed the bio-flocculation efficiencies of freshwater alga *Chlamydomonas reinhardtii* and marine alga *Picochlorum* 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 *Picochlorum* 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. uvarum* 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 biofloccullaton:

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 X14* 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:

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; Okaiyeto 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 (Okaiyeto et al, 2016). Ugbenyen et al. (2014) reported that pH influences the stability of suspended particles and the formation of floccules.

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. *P. elgii* 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 the production of 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) (Okaiyeto 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 (Okaiyeto 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 (Okaiyeto 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 plays a vital role in stimulating the adsorption of flocculants 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). Okaiyeto et al. (2016) mentioned that bioflocculant produced by *Halomonas* sp. and *Micrococcus* sp. are cation dependent; they reported improved flocculating 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 (Okaiyeto 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 ionic 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 ionic 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 biomass 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 mlL^{-1} 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 mgL^{-1} 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.

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References

[1] Agbakpe, M. Ge, S. Zhang, W. Zhang, X. Kobylarz, P. 2014. Algae harvesting for biofuel production: influences of UV irradiation and polyethylenimine (PEI) coating on bacterial biocoagulation. Journal of BioresourTechnology, 166:266-272.

[2] Alam, M.A. Vandamme, D. Wan, C. Zhao, X. Foubert, I. Wang, Z. Muylaert, K. and Yuan, Z. 2016. Bioflocculation as an innovative harvesting strategy for microalgae. Journal of Rev Environ SciBiotechnology 15(4):573-583.

[3] Al-Hothaly, K.A. 2018. An optimized method for the bio–harvesting of microalgae, *Botryococcusbraunii*, using *Aspergillus* sp. in large-scale studies. Journal of MethodsX 5:788-794.

[4] Anastasakis, K. Kalderis, D. and Diamadopoulos, E. 2009. Flocculation behavior of mallow and okra mucilage in treating wastewater. Journal of Desalination 249(2):786–791.

[5] Baharuddin, N.N. Azizi, N.S. Sohif, H.N. Karim, W.A. Al-Obaidi, J.R. and Basiran, M.N. 2016. Marine microalgae flocculation using plant: the case of *Nannochloropsis oculata* and *Moringa oleifera*. Journal of Pak J Bot 48(2):831-840.

[6] Banerjee, C. Ghosh, S. Sen, G. Mishra, S. Shukla, P. and Bandopadhyay, R. 2014. Study of algal biomass harvesting through cationic cassia gum, a natural plant based biopolymer. Journal of Bioresour Technology 151:6-11.

[7] Banerjee, C. Ghosh, S. Sen, G. Mishra, S. Shukla, P. and Bandopadhyay, R. 2013. Study of algal biomass harvesting using cationic guar gum from the natural plant source as flocculant. Journal of Carbohydr Polym 92(1):675-681.

[8] Barros, A. Goncalves, A. Simoes, M. and Pires, J. 2015. Harvesting techniques applied to microalgae: a review. Journal of Renew Sustain Energy Rev 41:1489-1500.

[9] Bosma, R. Van Spronsen, W.A. Tramper, J. and Wijffels, R.H. 2003. Ultrasound, a new separation technique to harvest microalgae. Journal of Appl Phycol 15(2–3):143-153.

[10] Branyikova, I. Prochazkova, G. Potocar, T. Jezkova, Z. and Branyik, T. 2018. Harvesting of microalgae by flocculation. Journal of Fermentation 4(93) :1-12.

[11] Cerff, M. Morweiser, M. Dillschneider, R. Michel, A. Menzel, K. and Posten, C. 2012. Harvesting fresh water and marine algae by magnetic separation: screening of separation parameters and high gradient magnetic filtration. Journal of BioresourTechnol 118:289–295.

[12] Chen, C–Y. Yeh, Ki.L. Aisyah, R. Lee, D–J. and Chang, J–S. 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Journal of BioresourTechnol 102(1):71-81.

[13] Chen, G. Zhao, L. Qi, Y. and Cui, Y–L. 2014. Chitosan and its derivatives applied in harvesting microalgae for biodiesel production: an outlook. Journal of Nanomat, 2014:1-9.

[14] Chen, Y.M. Liu, J.C. and Ju, Y.H. 1998. Flotation removal of algae from water. Journal of Colloid Surf B, 12:49–55.

[15] Chen, Y.M. Liu, J.C. and Ju, Y.H. 1998. Flotation removal of algae from water. Journal of Colloid Surf B 12:49–55.

[16] Christenson, L. and Sims, R. 2011. Production and harvesting of microalgae for wastewater treatment, biofuels and bioproducts. Journal of Biotechnol Adv 29(6):686-702.

[17] Cosa, S. Ugbenyen, M.A. Mabinya, L.V. Rumbold, K. Olaniran, A.O. Aghdasi, F. Okoh, A.I. 2013. A marine bacterium, *Oceanobacillus* sp. pinky, isolated from Algoa Bay sediment produces a thermostable glycoprotein flocculant. Afr J Biotechnol 12(26):4135-4146.

[18] Danquah, M.K. Gladman, B. Moheimani, N. and Forde, G.M. 2009. Microalgal growth characteristics and subsequent influence on dewatering efficiency. Chem Eng J 151(1–3):73-78.

[19] Díaz-Santos, E. Vila, M. De la Vega, M. León, R. and Vigara, J. 2015. Study of bioflocculation induced by *Saccharomyces bayanus* var. *uvarum* and flocculating protein factors in microalgae. Journal of Algal Res, 8:23-29.

[20] Díaz-Santos, E. Vila, M. Vigara, J. and León, R. 2016. A new approach to express transgenes in microalgae and its use to increase the flocculation ability of *Chlamydomonas reinhardtii*. Journal of Appl Phycol 28:1611-1621.

[21] Domozych, D.S. Ciancia, M. Fangel, J.U. Mikkelsen, M.D. Ulvskov, P. and Willats, W.G. 2012. The cell walls of green algae: a journey through evolution and diversity. Journal of Front Plant Sci 3:1-8.

[22] Feofila, E.P. 2010. The fungal cell wall: modern concepts of its composition and biological function. Journal of Microbiology, 79(6):723-733.

[23] Ferraro, A. 2017. Microalgae as source of biofuel: Technology and prospective. Journal of Physics Conf, series, 939:1-5.

[24] Gao, Q. Zhu, X.H. Mu, J. Zhang, Y. and Dong, X.W. 2009. Using *Ruditapesphilippinarum* conglutination mud to produce bioflocculant and its applications in wastewater treatment. Journal of BioresourTechnol, 100(21):4996-5001.

[25] Gong, W.X. Wang, S.G. Sun, X.F. Liu, X.W. Yue, Q.Y. and Gao, B.Y. 2008. Bioflocculant production by culture of *Serratiaficaria* and its application in wastewater treatment. Journal of BioresourTechnol, 99: 4668-4674.

[26] Gultom, S.O. and Hu, B. 2013. Review of microalgae harvesting via co-pelletization with filamentous fungus. Journal of Energies, 6(11):5921-5939.

[27] Guo, S.L. Zhao, X.Q. Wan, C. Huang, Z.Y. Yang, Y.L. Alam, M.A. Ho, S.H. Bai, F.W. and Chang, J.S. 2013. Characterization of flocculating agent from the self-flocculating microalga *Scenedesmus obliquus* AS-6-1 for efficient biomass harvest. Journal of BioresourTechnol 145:285-289.

[28] Gutzeit, G. Lorch, D. Weber, A. Engels, M. and Neis, U. 2005. Bioflocculent algal–bacterial biomass improves low-cost wastewater treatment. Journal of Water Sci Tech, 529(12):9-18.

[29] Haldar, C. Ram, R. Kumar, S. and Menna, L.L. 2018. Microalgae as a Potential Source of Biofuels and its Current Advances. Journal of Mar. bio oceano, 2(1):1-5.

[30] Hansel, P.A. Riefler, R.G. and Stuart, B.J. 2014. Efficient flocculation of microalgae for biomass production using cationic starch. Journal of Algal Res. 5:133-139.

[31] Harith, Z.T. Yusoff, F.M. Mohamed, M.S. Din, M.S.M. and Ariff, A.B. 2000. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve mollusks—a summary. Journal of Aquacult Res, 31(8–9):637-659.

[32] Harith, Z.T. Yusoff, F.M. Mohamed, M.S. Din, M.S.M. and Ariff, A.B. 2009. Effect of different flocculants on the flocculation performance of microalgae, Chaetoceroscalcitrans, cells. Jornal of Afr J Biotechnol, 8(21):5971-5978.

[33] Heasman, M. Diemar, J. O'Connor, W. Sushames, T. and Foulkes, L. 2000. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve mollusks - a summary. Journal of Aquacult Res, 31:637-659.

[34] Heng, L. Jun, N. Wen-jie, H. and Guibai, L. 2009. Algae removal by ultrasonic irradiation-coagulation. Journal of Desalination, 239:191-197.

[35] Horiuchi, J.I. Ohba, I. Tada, K. Kobayashi, M. Kanno, T. and Kishimoto, M. 2003. Effective cell harvesting of the halotolerant microalgae *Dunaliella tertiolecta* with pH control. Journal of Biosci Bioeng 95(4):412-415.

[36] Kandasamy, G. and Shaleh, S.R.M. 2017. Harvesting of the microalga *Nannochloropsis* sp. by bioflocculation with mung bean protein extract. Jornal of Applied biochemistry and biotechnology, 182(2):586–597.

[37] Kim, D.G. La, H.J. Ahn, C.Y. Park, Y.H. and Oh, H.M. 2010. Harvest of *Scenedesmus* sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. Jornal of BioresourceTechnol, 102(3):3163–3168.

[38] Kim, J. Yoo, G. Lee, H. Lim, J. Kim, K. Kim, C.W. Park, M.S. and Yang, J.W. 2013. Methods of downstream processing for the production of biodiesel from microalgae. Journal of Biotechnol Adv, 31(6):862–876.

[39] Lago, C. Caldés, N. Lechón, Y. (Eds.). 2018. The role of bioenergy in the emerging bioeconomy, resources, technologies, sustainability and policy. Cambridge, MA: Academic Press.

[40] Lee, A. Lewis, D. and Ashman, P. 2009. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. Journal of Appl Phycol, 21(5):559–567.

[41] Lee, A.K. Lewis, D.M. and Ashman, P.J. 2010. Energy requirements and economic analysis of a full-scale microbial flocculation system for microalgal harvesting. Journal of Chem Eng Res Des, 88:988–996.

[42] Lee, S.H. 2001. Improvement of water treatment performance by using polyamine flocculants. Journal of Environ. Technol, 22:274–278.

[43] Lei, X. Chen, Y. Shao, Z. Chen, Z. Li, Y. Zhu, H. Zhang, J. Zheng, W. and Zheng, T. 2015. Effective harvesting of the microalgae *Chlorella vulgaris* via flocculation-flotation with bioflocculant. Journal of BioresourTechnology, 198:922-925.

[44] Li, O. Lu, C. Liu, A. Zhu, L. Wang, P.M. Qian, C.D. Jiang, X.H. and Wu, X.C. 2013. Optimization and characterization of polysaccharide-based bioflocculant produced by *Paenibacilluselgii* B69 and its application in wastewater treatment. Journal of Bioresour Technol, 134:87-93.

[45] Li, X. Yang, Q. Huang, K. Zeng, G. Liao, D. Liu, J. and Long, W. 2007. Screening and characterization of a bioflocculant produced by *Aeromonas* sp. Journal of BIOMED ENVIRON SCI, 20(4):274-278.

[46] Li, Y. Xu, Y. Song, R. Tian, C. Liu, L. Zheng, T. and Wang, H. 2018. Flocculation characteristics of abioflocculant produced by the Actinomycete *Streptomyces* sp. *hsn06* on microalgae biomass. Journal of *BMC biotechnology*, *18*(1):1-10.

[47] Li, Z. Zhong, S. Lei, H.Y. Chen, R.W. Yu, Q. and Li, H.L. 2009. Production of a novel bioflocculant by *Bacillus licheniformis X14* and its application to low temperature drinking water treatment. Journal of Bioresour Technology, 100:3650-3656.

[48] Liao, B.Q. Lin, H.J. Langevin, S.P. Gao, W.J. and Leppard, G.G. 2011. Effects of temperature and dissolved oxygen on sludge properties and their role in bioflocculation and settling. Journal of Water Res, 45(2):509-520.

[49] Linder, M.B. 2009. Hydrophobins: proteins that self assemble at interfaces. Journal of CurrOpin Colloid Interface Sci, 14:356-363.

[50] Liu, J.X. Zhu, Y. Tao, Y.J. Zhang, Y.M. Li, A.F. Li, T. Sang, M. and Zhang, C.W. 2013. Freshwater microalgae harvested via flocculation induced by pH decrease. Journal of Biotechnol Biofuels, 6(98):1-11.

[51] Luo, L.Q. and Nguyen, A.V. 2017. A review of principles and applications of magnetic flocculation to separate ultrafine magnetic particles. Journal of Sep Purif Technology, 172:85-99.

[52] Luo, S. Wu, X. Jiang, H. Yu, M. Liu, Y. Min, A. Li, W. and Ruan, R. 2019. Edible fungi-assisted harvesting system for efficient microalgae bio-flocculation. Journal of Bioresour Technology, 282:325-330.

[53]   Lv, J. Wang, X. Liu, W. Feng, J. Liu, Q. Nan, F. Jiao, X. and Xie, S. 2018. The performance of a self-flocculating microalga *Chlorococcum* sp. GD in wastewater with different ammonia concentrations. Journal of Int. J. Environ. Res. Public Health, 15(434):1-13.

[54]   Mackay, S. Gomes, E. Holliger, C. Bauer, R. and Schwitzguébel, J.P. 2015. Harvesting of *Chlorella sorokiniana* by co-culture with the filamentous fungus *Isariafumosorosea*: a potential sustainable feedstock for hydrothermal gasification. Bioresour Technology, 185:353-361.

[55]   Maji, G.K. Choudhury, S. Hamid, S. Prashanth, R. Arjunan, S. and Sibi, G. 2018. Microalgae harvesting via flocculation: impact of pH, algae species and biomass concentration. Journal of Methods Microbiol Mol Biol, 1(2):1-5.

[56]   Matis, K.A. Gallios, G.P. and Kydros, K.A. 1993. Separation of fines by flotation techniques. Sep Technol, 3:76-90.

[57]   Matter, I.A. Bui, V.K.H. Jung, M. Seo, J.Y. Kim, Y.E. Lee, Y.C. and Oh,Y.K. 2019. Flocculation harvesting techniques for microalgae: a review. Journal of ApplSci, 9(3069):1-27.

[58]   Matter, I.A. Darwesh, O.M. and Eida, M.F. 2018. Harvesting of microalgae *S. obliquus* using chitosan-alginate dual flocculation system. Journal of Biosci. Res, 15(1):540-548.

[59]   Mohn, F.H. 1988. Harvesting of microalgal biomass. In: Borowitzka, M.A. Borowitzka, L.J. eds. *Micro-Algal Biotechnology.* NY: Cambridge University Press; 395-414.

[60]   Molina Grima E, Belarbi EH, Acien Fernandez FG, Robles Medina A, and Chisti, Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. Journal of Biotechnol Adv 20(7–8):491-515.

[61]   Molina Grima, E. Belarbi, E.H. Acien Fernandez, F.G. Robles Medina, A. and Chisti, Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. Journal of Biotechnol Adv 20:491-515.

[62]   Muradov, N. Taha, M. Miranda, A.F. Wrede, D. Kadali, K. Gujar, A. Stevenson, T. Ball, A.S. and Mouradov, A. 2015. Fungal-assisted algal flocculation: application in wastewater treatment and biofuel production. Journal of Biotechnol Biofuels, 8(24):1–23.

[63]   Ndikubwimana, T. 2016. Harvesting of freshwater microalgae with microbial bioflocculant: a pilot-scale study**.** Journal of Biotechnol Biofuels, 9:1-11.

[64]   Ndikubwimana, T. Zeng, X. He, N. Xiao, Z. Xie, Y. Chang, J.S. Lin, L. and Lu, Y. 2015. Microalgae biomass harvesting by bioflocculation-interpretation by classical DLVO theory. Journal of BiochemEng J, 101:160-167.

[65]   Ndikubwimana, T. Zeng, X. Liu, Y. Chang, J.S. and Lu, Y. 2014. Harvesting of microalgae *Desmodesmus* sp. F51 by bioflocculation with bacterial bioflocculant. Journal of Algal Res, 6:186–193.

[66]   Nguyen, T.D.P. Frappart, M. Jaouen, P. Pruvost, J. and Bourseau, P. 2014 Harvesting *Chlorella vulgaris* by natural increase in pH: effect of medium composition. Journal of Environ Technol, 35(11):1378–1388.

[67]   Nie, M. Yin, X. Jia, J. Wang, Y. Liu, S. Shen, Q. Li, P. and Wang, Z. 2011. Production of a novel bioflocculant MNXY1 by *Klebsiellapneumoniae* strain *NY1* and application in precipitation of cyanobacteria and municipal wastewater treatment**.** Journal of Appl Microbiol, 111**:** 547–558.

[68]   Nwodo, U.U. Green, E. and Okoh, A.I. 2012. Bacterial exopolysaccharides functionality and prospects. IntJ Mol13:14002–14015.

[69]   Oh, H.M. Lee, S.J. Park, M.H. Kim, H.S. Kim, H.C. Yoon, J.H. Kwon, G.S. and Yoon, B.D. 2001. Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. *AM49*. Journal of BiotechnolLett 23:1229–1234.

[70]   Okaiyeto, K. Nwodo, U.U. Okoli, S.A. Mabinya, L.V. and Okoh, A.I. 2016. Implications for public health demands alternatives to inorganic and synthetic flocculants: bioflocculants as important candidates. Journal of MicrobiologyOpen 5(2):177–211.

[71]   Pal, S. Mal, D. and Singh, R. 2005. Cationic starch: an effective flocculating agent. Journal of CarbohydrPolym 59(4):417–423.

[72]   Pal, S. Sen, G. Karmakar, N.C. Mal, D. and Singh, R.P. 2008. High performance flocculating agents based on cationic polysaccharides in relation to coal fine suspension. Journal of Carbohydr Polym, 74:590-596.

[73]   Peer, M. Skye, R. Thomas, H. Evan, S. Ute, C. Jan, H. Clemens, P. Olaf, K. and Ben, H. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Journal of Bioenergy Res, 1:20–43.

[74]   Pérez, L. Salgueiro, J.L. Maceiras, R. Cancela, A. and Sanchez, A. 2017. An effective method for harvesting of marine microalgae: pH induced flocculation. Journal of Biomass Bioenergy, 97:20–26.

[75]   Pragya, N. Pandey, K.K. and Sahoo, P.K. 2013. A review on harvesting, oil extraction and biofuels production technologies from microalgae. Journal of Renew Sustain Energy Rev, 24:159–71.

[76]   Pugazhendhi, A. Shobana, S. Bakonyi, P. Nemestóthy, N. Xia, A. Banu, J.R. and Kumar, G. 2019. A review on chemical mechanism of microalgae flocculation via polymers. Journal of Biotechnol Reports 21:1–10.

[77]   Rahul, R. Kumar, S. Jha, U. and Sen, G. 2015. Cationic inulin: a plant based natural biopolymer for algal biomass harvesting. Int J Biol Macromol, 72:868–874.

[78]   Razack, S.A. Duraiarasan, S. Shellomith, A.S. and Muralikrishnan, K. 2015. Statisticaloptimization of harvesting *C. vulgaris* using a novel bio-source. Journal of *Strychnospotatorum*.Biotechnol. Rep, 7:150–156.

[79]   Rebah, F.B. Mnif, W. Siddeeg, S.M. 2018. Microbial Flocculants as an Alternative to Synthetic Polymers for Wastewater Treatment: A Review. Symmetry, 10(11):1–19.

[80]   Salehizadeh, H. and Yan, N. 2014. Recent advances in extracellular biopolymer flocculants. Journal of Biotechnol. Adv, 32:1506–1522.

[81]   Salim, S. Bosma, R. Vermuë, M.H. and Wijffels, R.H. 2011. Harvesting of microalgae by bio-flocculation. Journal of ApplPhycol, 23:849–855.

[82]   Salim, S. Kosterink, N.R. Wacka, N.D.T. Vermue, M.H. and Wijffels, R.H. 2014. Mechanism behind autoflocculation of unicellular green microalgae *Ettliatexensis*. Journal of Biotech, 174:34–38.

[83]   Salim, S. Vermuë, M.H. and Wijffels, R.H. 2012. Ratio between autoflocculating and target microalgae affects the energy-efficient harvesting by bio-flocculation. Journal of BioresourTechnol 118:49–55.

[84]   Salim, S. Harvesting microalgae by bio-flocculation and autoflocculation, in Agrotechnology& Food Science Group; Bioprocess Engineering2013, Wageningen University: Wageningen, Nederland. p. 152

[85]   Schlesinger, A. Eisenstadt, D. Bar-Gil, A. Carmely, H. Einbinder, S. and Gressel, J. 2012. Inexpensive non-toxic

flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. Journal of Biotechnology Adv, 30(5):1023–1030.

[86] Scholz, M. Hoshino, T. Johnson, D. Riley, M.R. and Cuello, J.L. 2011. Flocculation of wall-deficient cells of *Chlamydomonasreinhardtii* mutant *cw15* by calcium and methanol. Journal of Biomass Bioenergy, 35:4835–4840.

[87] Singh, G. and Patidar, S.K. 2018. Microalgae harvesting techniques: A review. Journal of Environ Manag, 217:499–508.

[88] Sukenik, A. Bilanovic, D. and Shelef, G. 1988. Flocculation of microalgae in brackish and sea waters . Biomass, 15:187-199

[89] Sukenik, A. and Shelef, G. 1984. Algal autoflocculation-verification and proposed mechanism. Journal of Biotechnol Bioeng, 26:142–147.

[90] Sun, J. Zhang, X.H. Miao, X.J. and Zhou, J.T. 2012. Preparation and characteristics of bioflocculants from excess biological sludge. Journal of Bioresour Technology, 126:362–366.

[91] Sutherland, I.W. 2001. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology, 147:3–9.

[92] Talukder, M.M.R. Das, P. and Wu, J.C. 2014. Immobilization of microalgae on exogenous fungal mycelium: a promising separation method to harvest both marine and freshwater microalgae. BiochemEng J, 91:53–57.

[93] Taylor, R.L. Rand, J.D. and Caldwell, G.S. 2012. Treatment with algae extracts promotes flocculation, and enhances growth and neutral lipid content in *Nannochloropsis oculata*—a candidate for biofuel production. Mar Biotechnol, 14:774–781.

[94] Tran, N-AT. Seymour, J.R. Siboni, N. Evenhuis, C.R. and Tamburic, B. 2017. Photosynthetic carbon uptake induces autoflocculation of the marine microalga *Nannochloropsis oculata*. Journal of Algal Res 26:302–311.

[95] Uduman, N. Qi, Y. Danquah, M.K. Forde, G.M. and Hoadley, A. 2010 a. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. J Renew Sustain Energy 2(1):1-15.

[96] Uduman, N. Qi, Y. Danquah, M.K. and Hoadley, A.F.A. 2010 b. Marine microalgae flocculation and focused beam reflectance measurement. ChemEng J 162(3):935–940.

[97] Ugbenyen, A.M. and Okoh, A.I. 2014. Characteristics of a bioflocculant produced by a consortium of *Cobetia* and *Bacillus* species and its application in the treatment of wastewaters. Water SA, 40:139–144.

[98] Ummalyma, S.B. Gnansounou, E. Sukumaran, R.K. Sindhu, R. Pandey, A. and Sahoo, D. 2017. Bioflocculation: an alternative strategy for harvesting of microalgae–an overview. Journal of Bioresour Technol, 242:227-235.

[99] Ummalyma, S.B. Mathew, A.K. Pandey, A. and Sukumaran, R.K. 2016. Harvesting of microalgal biomass: efficient method for flocculation through pH modulation. Journal of Bioresour Technol. 213:216–221.

[100] Van den Hende S, Vervaeren H, Desmet S, Boon N (2011a) Bioflocculation of microalgae and bacteria combined with flue gas to improve sewage treatment. New Biotechnol 29(1):23–31

[101] Van Den Hende, S. Vervaeren, H. Saveyn, H. Maes, G. and Boon, N. 2011b. Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio. Journal of Biotechnol Bioeng, 108(3):549-558.

[102] Vandamme, D. Foubert, I. Fraeye, I. Meesschaert, B. and Muylaert, K. 2012. Flocculation of Chlorella vulgaris induced by high pH: role of magnesium and calcium and practical implications. Journal of Bioresource Technol, 105**:** 114-119.

[103] Vandamme, D. Foubert, I. Meesschaert, B. and Muylaert, K. 2010. Flocculation of microalgae using cationic starch. Journal of Appl Phycol 22(4):525–530.

[104] Vandamme, D. Foubert, I. and Muylaert, K. 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends Biotechnol, 31(4):233-239.

[105] Vandamme, D. Pontes, S.C.V. Goiris, K. Foubert, I. Pinoy, L.J.J. and Muylaert, K. 2011. Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. Journal of Biotechnol Bioeng, 108(10):2320–2329.

[106] Wan, C. Alam, M.A. Zhao, X.Q. Zhang, X.Y. Guo, S.L. Ho, S.H. Chang, J.S. and Bai, F.W. 2015. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. Journal of BioresourTechnology 184:251–257.

[107] Wan, C. Zhao, X.Q. Guo, S.L. Alam, M.A. and Bai, F.W. 2013. Bioflocculant production from *Solibacillus silvestris W01* and its application in cost-effective harvest of marine microalga *Nannochloropsis oceanica* by flocculation. Journal of Bioresource Technology, 135:207–212.

[108] Wang, Z. Hou, J. Bowden, D. and Belovich, J.M. 2013. Evaluation of an inclined gravity settler for microalgae harvesting. J ChemTechnol Biot 89:714–720.

[109] Wijffels, R.H. and Barbosa, M.J. 2010. An outlook on microalgal biofuels. Science, 329(5993):796–799.

[110] Wilén, B. and Balmér, P. 1998. Short term effects of dissolved oxygen concentration on the turbidity of the supernatant of activated sludge. Wat. Sci. Tech, 38(3)**:** 25–33.

[111] Wilen, B.M. and Balmer, P. 1999. The effect of dissolved oxygen concentration on the structure, size and size distribution of activated sludge flocs. Journal of Water Res, 33(2): 391–400.

[112] Wrede, D. Taha, M. Miranda, A.F. Kadali, K. Stevenson, T. Ball, A.S. and Mouradov, A. 2014. Co-cultivation of fungal and microalgal cells as an efficient system for harvesting microalgal cells, lipid production and wastewater treatment. PloS ONE, 9(11):1-22.

[113] Wu, J.Y. Ye, H.F. 2007. Characterization and flocculating properties of an extracellular biopolymer produced from a *Bacillus subtilis DYU1* isolate. Process Biochem, 42:1114-1123.

[114] Wu, Z. Zhu, Y. Zhang, Y. Zhang, C. Huang, W. Li, T. and Li, A. 2012. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. Journal of BioresourceTechnol, 110**:**496-502.

[115] Xie, S. 2013. Efficient coagulation of microalgae in cultures with filamentous fungi. Algal Res, 2(1):28-33.

[116] Yen, H.W. Chen, P.W. and Chen, L.J. 2015. The synergistic effects for the co-cultivation of oleaginous yeast-*Rhodotorul aglutinis* and microalgae-*Scenedesmus obliquus* on the biomass and total lipids accumulation. Jornal of Bioresour Technology, 184:148-152.

[117] Zaki, S. Elkady, M.F. Farag, S. and Abd-El-Haleem, D. 2012. Characterization and flocculation properties of a carbohydrate bioflocculant from a newly isolated *Bacillus velezensis* 40B. J Environ Biol, 34:51–58.

[118] Zaki, S. Farag, S. Elreesh, G.A. Elkady, M. Nosier, M. and Hallem, D.A.E. 2011. Characterizationof bioflocculants

produced by bacteria isolated from crude petroleum oil. Int J Environ SciTechnol, 8(4):831-840.

[119]   Zhang, J. and Hu, B. 2012. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. Journal of Bioresour Technol, 114:529–535.

[120]   Zhang, T. Lin, Z. and Hui-Ian, Z. 1999. Microbial flocculant and its application in environmental protection. J Env Sci, 11:1-12.

[121]   Zhang, Z.Q. Lin, B. Xia, S.Q. Wang, X.J. and Yang, A.M. 2007. Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. J Environ Sci China, 19(6):667-673.

[122]   Zheng, H. Gao, Z. Yin, J. Tang, X. Ji, X. and Huang, H. 2012. Harvesting of microalgae by flocculation with poly (gamma-glutamic acid). BioresourceTechnol 112: 212-220.

[123]   Zhou, W. Cheng, Y.Li.Y. Wan, Y. Liu, Y. Lin, X. and Ruan, R. 2012. Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. Appl Biochem Biotechnol, 167(2):214-228.

[124]   Zhou, W.G. Min, M. Hu, B. Ma, X.C. Liu, Y.H. Wang, Q. Shi, J. Chen, P. and Ruan, R. 2013. Filamentous fungi assisted bio-flocculation: a novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. Sep Purif Technol 107:158-165.