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Bioflocculation: An alternative strategy for harvesting of microalgae overview

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HIGHLIGHTS

- Bioflocculation an efficient low cost technology for microalgal harvesting.
- Different flocculation strategies discussed.
- Auto-flocculation of algal cells is economically viable.

GRAPHICAL ABSTRACT

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ABSTRACT

algae based research has been extensively progressed for the production of value added products an iofuels. Potential application of microalgae for biofuel is recently gained more attention for possibilities of biodiesel and other high value metabolites. However, high cost of production of biomass associated with harvesting technologies is one of the major bottleneck for commercialization of algae based industrial product. Based on the operation economics, harvesting efficiency, technological possibilities, flocculation of algal biomass is a superior method for harvesting microalgae from the growth medium. In this article, latest trends of microalgal cell harvesting through flocculation are reviewed with emphasis on current progress and prospect in environmental friendly bio-based flocculation approach. Bioflocculation based microalgae harvesting technologies is a promising strategy for low cost microalgal biomass production for various applications.

1. Introduction

Use of fossil fuels leads to global climatic change, environmental pollution associated with health problems and energy crisis leads with the irreversible decrease of source of fossil fuels. Therefore many countries have focused their research for development of renewable and sustainable biofuels. One of such alternative biofuel source is algal biofuels. Oil-accumulating microalgae are reported to be a promising feedstock for biodiesel production. Microalgae are fast growing photosynthetic microbes capable of accumulating lipids, proteins and other high value products like

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DHA, EPA and pigments (Nurachman et al., 2015; Ummalyma and Sukumaran, 2015; Maki et al., 2014). Large-scale production of biofuels from microalgae is not yet economically viable. This is mainly due to the high-energy inputs required for harvesting of the algal cells (De Godos et al., 2011). Microalgal biomass production system includes growing microalgae in an environment that favors accumulation of target product and recovery of the microalgal biomass for downstream processing. However, due to the small size (5 ~ 50 µm) (Grima et al., 2003), the negative surface charge (about −7.5 ~ −40 mV) on the algae that results in dispersed stable algal suspensions especially during the growth phase (Packer, 2009), low biomass concentrations (0.5 ~ 5 g/L) and densities similar to that of water (Reynolds, 1984), prevent the harvesting as an extremely difficult task.

Harvesting microalgal biomass from growth medium is a significant challenge in many of the industries dealing with microalgal biomass production. In some commercial production systems, the culture broths have biomass densities below 0.5 kg/m^3, which mean that huge volumes need to be handled before algal oil extraction (Chen et al., 2014). Developing a cost effective harvesting method is one of the most challenging areas in the algal biofuel research (Greenwell et al., 2009) which is a key factor that limit the commercial use of microalgae. It has been reported that 20–30% of the total production cost is involved in the biomass harvesting (Mata et al., 2010; Grima et al., 2003). Other researchers have reported that the cost of the recovery process in their study contributed about 50% to the final cost of oil production (Greenwell et al., 2009; Chisti, 2007). Many of the studies on the microalgal biofuel production have been focused on the yield of lipids and composition of biomass rather than harvesting process. Therefore, it is necessary to develop effective and economic technologies for harvesting the biomass from the suspended water.

Current harvesting strategies includes mechanical, electrical, biological and chemical based methods (Christenson and Sims, 2011). In the case of mechanical methods, microalgal cells are harvested by centrifugation (Shelef et al., 1984), filtration (Vonshak and Richmond,1988), sedimentation ([illegible]), dissolved air flotation (Greenwell et al., 2009; Zhang et al., 2012), usage of attached algal biofilms and ultrafiltration membranes (Zhang et al., 2010). Electrical methods based on electrophoresis of the microalgae cells (Vandamme et al., 2011), presence of the negative charge on the surface of microalgal cells, they can be concentrated by being moved in an electric field (Zheng et al., 2012). Chemical methods generally refer to chemical flocculation induced by inorganic, organic flocculants, some electrolytes and synthetic polymers are typically utilized (Zheng et al., 2012). However, the limitation of this type of approach has contributed to very high cost associated with operation and maintenance because of energy requirement for the machinery especially for massive scale operation. Therefore, [illegible] production of microalgae is justified only in the case of production for costly products such as drug precursors and pharmaceutical products (Gong et al., 2011). Thus, the operational costs should be significantly reduced in order to make the commercial production of low value, bulk products such as for biofuels. Thus, to minimize the energy consumption of harvesting microalgae, an innovative technological approach is required. Bio-flocculation methods are flocculation induced by extracellular polymer compounds such as polysaccharides and proteins, derived from microalgae and other microorganisms (Ndikubwimana et al., 2015; Nie et al., 2011). Various latest technologies for harvesting of algal biomass are recently reviewed (Alam et al., 2016; Wan et al., 2015; Barros et al., 2015).

During flocculation, sizes of the floc cells are increased by aggregation of cells through flocculation process that can enhance the settling rate or flotation (Mata et al., 2010). Zeta potential is the apparent surface charge of the cells, which may affect the efficiency of flocculation (Henderson et al., 2008). Presence of negative charge on the surface of microalgae prevents them from self-aggregation within the suspension. The surface charge on the algae can be neutralized by the addition of chemicals known as flocculants. An ideal flocculant for microalgal harvesting should be inexpensive, nontoxic and effective at low concentrations (Grima et al., 2003). Multivalent inorganic metal salts like ferric sulphate, ferric chloride, aluminum sulphate and aluminum chloride which is popularly known as alums are frequently used for wastewater treatment to eliminate algae. Many reports have been suggested that inorganic flocculants can also have negative effect on the viability of algal cells and coloration, and they may [illegible] the growth medium preventing its recycling and reuse (Papazi et al., 2010; Schenk et al., 2008; Grima et al., 2003). Although alum and other inorganic flocculants are relatively cheap compared to organic flocculants, the higher dosage rates required can result in higher cost per unit of microalgal cells flocculated than more expensive organic flocculants (Mohn, 1988). In comparison to inorganic flocculants, the organic flocculants are reported to give an advantage in terms of lesser sensitivity to [illegible], non-toxic nature and wide range of applications and requirement of lower dosages for flocculation process. Chitosan is a naturally occurring flocculant commonly used in wastewater treatment for suspended solid separation. It's low cost and nontoxic nature make it one of the preferred flocculants in microalgae-based biotechnologies (Lersutthiwong et al., 2009). However for large scale algal biomass production, usage of chitosan will be luxury and the requirement of higher dosages compared to chemicals would appear to make it not viable for harvesting of microalgae for biofuel production (Kwon et al., 2014; Vandamme et al., 2011; Mohn, 1988). The type of organic flocculants chosen for flocculation is also depends upon the properties of the algal cultures like concentration of biomass, pH and charge of the algal broth. Table 1 represents the various flocculation methods applied for microalgae harvesting.

Bio-flocculants have emerged as a new research development in flocculation technology. Bio-flocculation process happens as a result of secretion of extracellular polymeric substances commonly known as EPS by living cells (Salehizadeh et al., 2000). The advantage of this strategy is that no addition of chemical flocculants is required and similar cultivation conditions can be used for the flocculating microalgae for harvesting of the target microalgae. This method is easy and cost-effective as chemical flocculation which is applied at industrial scale. It is also sustainable and economically viable since no costs are involved for pre-treatment of the biomass for oil extraction and medium before it can be re-used and it is eco-friendly process. Flocculation has been found as a promising strategy to harvest microalgae with low cost, and various novel flocculation technologies have been developed and many researches on these aspects are under progressing to reduce the harvesting cost of algal biomass for fuel application. However, there are still a lot of challenges in microalgae biomass concentration methods using efficient and cost effective flocculating technologies. In this article, the recent developments in bio- flocculation technology with special importance on the application of auto-flocculation for cost effective harvesting of microalgae biomass is discussed.

2. Bio-flocculation methods for microalgae

Flocculation process assisted with microorganisms or their polymer substances is known as bio-flocculation (Wan et al., 2015). Commonly bioflocculations are applied in waste water treatment systems (Van Den Hende et al., 2011; Zhang et al., 2009). Compared to other methods of flocculation, bioflocculations are cheap and environmental friendly and sustainable approach for the bulk harvesting of algal biomass. Recent exploitation of

Table 1
Comparison of microalgal harvesting using various flocculation methods.

Flocculation method	Advantages	Limitations	References
Chemical flocculants	Well known technology, Reliable	Metal accumulation in biomass, toxic nature	Ummalyma et al. (2016) Kwon et al. (2014) Gong et al. (2011) Papazi et al. (2010)
Biopolymers	Dosage is low, bio-based chemicals	Expensive	Vandamme et al. (2011) Lersutthiwong et al. (2009)
Magnetic coagulants	Separation is enhanced with magnetic force	Costly, established only on lab scale	Luo and Nguyen (2017) Vandamme et al. (2011)
Electrical method	Low energy requirement and reliable process	Contamination of biomass with metals	Wan et al. (2015) [illegible] al. (2012) Vandamme et al. (2011)
Bio-flocculation	Cheap and sustainable chemicals and contamination free	To be confirmed at scale up levels	Wan et al. (2015) Van Den Hende et al. (2011)

bioflocculation for harvesting algal biomass are categorized into four type (1) Plant based bioflocculation, (2) microbial flocculation, (3) Bio-flocculation by microalgal- fungal association and (4) autoflocculation.

2.1. Flocculation mediated by plant based product

Plant derivative as bio-flocculants are recently emerged as an attractive approach to polymeric flocculants their application in wastewater treatment was tested because of its biodegradability, non toxicity, wide availability from renewable resources and the methods is environmental friendly process. The application of plant derivatives such as biopolymers or proteins for treatment of various types of wastewater have been discovered and reported by many (Al-Hamadani et al., 2011; Anastasakis et al., 20[illegible]; Mishra and Bajpai, 2006). Flocculant derived from plant origin are recently gained much attention for the flocculation of algal biomass. Flocculation by natural plant based product is one of the possible, low cost alternatives for bio flocculation [illegible]. Studies on flocculation of *Chlorella* sp. with protein from *Moringa oleifera* seed found as an effective flocculant with flocculation efficiency of 90% (Hamid et al., 2014). Polysaccharide based cationic flocculants are alternative to the expensive synthetic flocculants because of their biodegradability and high flocculation efficiency (Pal et al., 2008). Cationic inulin is tried for the harvesting of *Botryococcus* sp. with 88.6% efficiency was obtained for 15 min at concentrations of 60 [illegible] (Rahul et al., 2015). The most predominant mechanism involved in flocculation by polymers is bridging mechanism (Pal et al., 20[illegible]). One of the possible mechanisms of this kind of flocculation is that the extracellular matrix of green algae are enriched with different types of sugars, polysaccharides and [illegible] like rhamnose, uronic acids, glucose, xylose, galactose, mannose, cellulose, pectin, pectic acids and ulvan along with other functional groups. Presence of functional group like carboxyl, sulphate, amino and other negatively charged atoms in the above extracellular matrix imparts an overall negative charge to the algal surface (Domozych et al., 2012). Flocculation by cationic inulin, leads to electrostatic interaction between the opposing charges 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 polysaccharides framework concurrently bridges many algal cells and this meshing-bridging action generates a structural complex in the form bulky flocs. The flocs once created settled down faster and eventually get separated from culture broth (Rahul et al., 2015). Bio-flocculation of two green algae *Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4 were evaluated at a concentration of 80 and 35 mg/L respectively is optimized dose for dewatering (Banerjee et al., 201[illegible]). Another report on the cationic guar gum based flocculant for microalgae *Chlamydomonas sp.* and *Chlorella sp.* showed flocculation efficiencies of 94% and 92% at concentration of 100 ppm and [illegible] ppm respectively (Banerjee et al., 2013). The strategy revealed that harvesting of *Chlorella vulgaris* by bioflocculation using seed powder of clearing nut, *Strychnos potatorum*. The maximum efficiency is achieved with this seed powder of 99.68% at a concentration of 100 mg/L for 150 agitation speed at 35 °C settled time of 30 min. The overall study expressed that seed powder from *S. potatorum* could probably by bioflocculant for microalgal biomass and a promising alternative for costly and unsafe chemical flocculants. Moreover, this kind of bioflocculant established their utility for harvesting microalgal cells economically, effectively and an eco-friendly way (Razack et al., 2015). Flocculation mediated by plant based polymers are less toxic, fast and low cost methods for harvesting of algal biomass but little concerns is cost associated with the addition of cationic quaternary amine group into some polymer. Table 2 present the different plant based product used for harvesting of micro algal cells.

2.2. Microbial based bioflocculation

Microbial flocculation of microalgae is caused by secreted biopolymers, especially by EPS or γ -glutamic acids (Zheng et al., 2012; Rawat et al., 2011). Flocculants produced by microorganisms can be a crucial cost effective step towards renewable microalgal based biofuel production. Microbial bioflocculation eliminates the need for chemical flocculants, which represent an expensive, non-feasible and toxic alternative. However, for this kind of flocculation technologies used co-culture of microalgae with bacteria results in microbiological contamination of biomasses that interfering final application of biomasses for food or feed (Vandamme et al., 2013). In the case of biofuel application of biomass, the added microorganisms may even contribute to the increase in lipid yields and fatty acids contents (Chen et al., 2012; Salim et al., 2011). Resulting culture media from these methods can also be effectively reused, therefore reducing biomass production cost (Zhou et al., 2012). The

Table 2
Bioflocculant from plants used for harvesting microalgae.

Plant product	Microalage	FE (%)	References
Moringa oleifera	*Chlorella* sp.	90	Hamid et al. (2014)
Guar gum	*Chlamydomonas* sp.	84	Banerjee et al. (2014)
Guar gum	*Chlorella* sp.	92	Banerjee et al. (2014)
Strychnos potatorum	*Chlorella vulgaris*	99.7	Razack et al. (2015)
Inulin	*Botryococcus* sp.	88.6	Rahul et al. (2015)

FE: Flocculation Efficiency.

success of microbial flocculation depends on the production of EPS/ γ -glutamic acids by the bacteria in high concentrations and the ability of microalgae to attach to them to form large flocs (Lee et al., 2010).

The mechanism of microbial bio-flocculants mediated flocculation is poorly understood and needs more research in this aspect. It has been proposed that charged functional groups presented in bio-flocculant could help in aggregation of microalgal cells along with either charge neutralization and electrostatic patch or bridging, which then helps the flocculation of algal cells (Wan et al., 2013). Table 3 represents the various bacterial cells used for flocculation of different microalgal cells. Application of poly γ -glutamic acids from *B. subtilis* is used for harvesting the biomass of microalgae *Nannochloropsis oculata* LICME 002, *Phaeodactylum tricornutum*, *C. vulgaris* LICME 001 and *Botryococcus braunii* LICME 003 gave no less than 90% flocculation efficiency and a concentration factor greater than 20. Images of the harvested microalgal cells showed that there is no damage to cell integrity, and hence no lipid loss during this process. The study revealed that flocculation with γ-PGA is feasible for harvesting microalgae for biodiesel production (Zheng et al., 2012). Ndikubwimana et al., (2014) reported that broth of *B. licheniformis* CGMCC 2876 rich in γ-PGA is used for the flocculation of microalgae *Desmodesmus* sp. F51 at an efficiency of 92%. They suggested that effective constituent γ-PGA, in the broth of *B. licheniformis* CGMCC 2876, can however be produced, purified and sold commercially for microalgae harvesting purposes.

Bacterial bio-aggregation is a natural phenomenon and is often observed in laboratory grown algal cultures. Several bacteria have been identified as bio-aggregating agents that can be used [illegible] aggregate algae (Wang et al., 2012; Nontembiso et al., 20[illegible] Gardes et al., 2011; Oh et al., 2001). Bio-flocculants obtained fro[illegible] *Pestalotiopsis* sp. are found to be exhibited biomass recovery effi[illegible] ciency at a concentration of 100 mg/L for harvestin[illegible] iomass of 0.3 g/L of *Botryococcus* (Lee et al., 1998). Cult[illegible] brot[illegible] f bio-flocculants from *Paenibacillus* sp. was used fo[illegible] e floccu[illegible] tion of mass culture of *C. vulgaris*. Flocculation efficie[illegible] wa[illegible] from 72 to 83% with the help of adding s[illegible] amo[illegible] of Ca^{2+} into the broth of bio-flocculant than the ch[illegible] cal floccula[illegible] (Oh et al., 2001). In addition to this such bio-f[illegible] ts from sam[illegible] cterial species revealed that high floccula[illegible] on effic[illegible] y (95%) for high cell density culture of *Scenedesmu*[illegible] . (3.5 g/L) in [illegible] presence of Ca^{2+} and Fe^{3+} ions, and reusin[illegible] upernatant as th[illegible] lture medium showed less than 8% d[illegible] ease in[illegible] e biomass production (Kim et al., 2011). It has been reported that presence of Ca^{2+} improved bio-flocculants produced by *Klebsiella pneumoniae* for the removal of cyanobacteria *Synecosystis* (Nie et al., 2011). Bio-flocculants produced from these organisms are a type of protein polysaccharides that assisted in the 95% precipitation of biomass. Another report revealed that biomass of *Nannochloropsis oceanica* successfully harvested by flocculation mediated by the bio-flocculants produced from *Solibacillus silvestris* without the addition of any extra addition of ions like of Ca^{2+} or Fe^{3+} during bio-flocculation process. Bio-flocculant from the culture broth of this bacterium showed 90% flocculating efficiency on this alga. Further chemical characterization of the purified bio-flocculant indi[illegible] it is a proteoglycans composed of 75.1% carbohyd[illegible] and 2[illegible] protein (w/w). This bio-flocculant does not af[illegible] the growth [illegible] algal cells and can be reused for econo[illegible] ical [illegible] esting of [illegible] *oceanica*. Thereby avoiding secondary c[illegible] minatio[illegible] d red[illegible] ng the cost of biomass harvesting pr[illegible] ss (Wan et [illegible] 3). This bio-flocculant is a significant [illegible] rovem[illegible] from ea[illegible] r ones, since this bio-flocculant can be recy[illegible] losing [illegible] ly 3% of flocculation efficiency and it is [illegible] toxic [illegible] croalga[illegible] ells.

MaB (microa[illegible] bacterial) [illegible] aggregations formed by microalgae an[illegible] um which c[illegible] elp the microalgae to settle faster than m[illegible] croalga[illegible] one (Van Den Hende et al., 2011). Both bacteria and algae can a[illegible] o produce EPS that are indistinguishable [illegible] e another. Fu[illegible] rmore, these polymers are responsible [illegible] cells to cells contact without cell stress or lysis over an ext[illegible] ded period [illegible] ime (Lee et al., 2009). However, it appears that the [illegible] sence of [illegible] se microbes is required for a predicting the floccu[illegible] n met[illegible] (Lee et al., 2013). Report showed that some bacteria [illegible] genera *Flavobacterium*, *Terrimonas* and *Sphingob*[illegible] *rium*, which are naturally associated with microalgal growth, h[illegible] n a combined role on harvesting *Chlorella vulgaris*. Flocs [illegible] med as results of xenic cultures presented diameters of about 100 mm, which resulted in higher sedimentation and flocculation ability when compared to axenic growth of *C. vulgaris* alone with diameter of flocs size is 20 mm. Also, the addition of the bacterial broth to the microalgal culture in a later growth stage showed greater flocculation efficiency than the axenic culture, which underlined that both bacterial cells and bacterial extracellular metabolites play an important role in the process of flocculation (Lee et al., 2013). *Escherichia coli* and *Rhodococcus* sp. are used for the bio-flocculation of two microalgae *Chlorella zofingiensis* and *Scenedesmus dimorphus* (Agbakpe et al., 2014). Their results showed that the UV irradiation and polyethylenimine (PEI) coated *E. coli* cells markedly increased the harvesting efficiencies from 23% to 83% for *S. dimorphus* when compared to uncoated *E. coli* cells.

Microbial flocculants associated bioflocculation involves the cultivation of microbes and the purification of bioflocculants. Drawback of this kind of flocculants are very less productivity and high dosage of flocculants are required that further leads to a high production cost of flocculants that consequently increase the high operation cost of bioflocculation driven cell harvesting. Moreover, the species-specific characteristics of bio-flocculants which might result from the special cell surface properties of microalgae have also limited their application (Oh et al., 2001). In order solve these issues efforts addressed by bioprocess engineering or genetic engineering approach to increase the productivity of bio-flocculants and to decrease their dosage by enhancing their affinity towards microalgal cells to accelerate the commercial application of bio-flocculants. Co-cultivation strategies are giving more preferences for the flocculation of microalgal biomasses. Although the problem with high cost, bio-flocculants still have open a novel methods of application in harvesting microalgae due to their uniqueness of being safe, biodegradable, eco- friendly and short span of time.

Table 3
Microbial mediat[illegible] occula[illegible] of micro[illegible]

Microorg[illegible] s	M[illegible] roalage	FE (%)	References
[illegible]	*C. vulgaris*	83	Oh et al. (2001)
Klebsiella pneumoni[illegible]	*Synecosystis*	95	Nie et al. (2011)
Paenibacillus sp.	*Scenedesmus* sp.	95	Kim et al. (2011)
B. subtilis (γ –PGA)	*Nannochloropsis oculata* LICME002	96	Zheng et al. (2012)
B. subtilis (γ –PGA)	*Phaeodactylum tricornutum*	97	Zheng et al. (2012)
B. subtilis (γ –PGA)	*C. vulgaris* LICME001	90	Zheng et al. (2012)
B. subtilis (γ –PGA)	*Botryococcus braunii* LICME 003	92	Zheng et al. (2012)
Solibacillus silvestris (proteoglycans)	*Nannochloropsis oceanic*	90	Wan et al. (2013)
Escherichia coli	*Chlorella zofingiensis*	83	Agbakpe et al. (2014)
B. licheniformis CGMCC 2876 (γ –PGA)	*Desmodesmus* sp. F51	92	Ndikubwimana et al. (2014)

2.3. Flocculation induced by fungus

Lichens are natural association exist between fungi, microalgae and cyanobacteria. In this coexisting mutual symbiotic communication, fungi utilize the sugars and other nutrients produced by the algae through photosynthetic process; in return, the fungi provides protection to the algae by holding water, serving as a larger capture area for mineral nutrients (Zhou et al., 2012; Zoller et al., 2003). This proposed that fungal-microalgal pellets can also function as a self-sufficient organization which can potentially improve the overall economics of a large scale integrated microalgal industry. Fungal self-pelletization has been observed for numerous filamentous strains and can be explained by either coagulative or non-coagulative machineries (Gultom and Hu, 2013; Liu et al., 2008). The coagulative method mediated by spores, which leads to the developments of aggregates/pellets. Fungus from the group *Aspergillus* sp., *Basidiomycete* sp. and *Phanerochaete* sp. produce dense spherical aggregates through this kind of mechanism (Gultom and Hu, 2013; Zhang and Hu, 2012). The non-coagulative process consists of the germinated hyphae from the spores, which then will interlinked to form pellets. This mechanism showed by the fungus *Rhizopus* sp., *Mucor* sp. and *Penicillium* sp. (Gultom and Hu, 2013; Zhang and Hu, 2012). Harvesting technology mediated by fungus does not needed any addition of toxic inorganic chemical compounds or energy and a number of reports showed that many algal cells are very effective with fungus for flocculation purposes (Xie et al., 2013; Zhou et al., 2012; Zhang and Hu, 2012). This method can be fitted to industrially important algal species it can offer a solution to one of the major hurdles associated with the energy demanding and costly biomass recovery processes. Table 4 [illegible] various fungal strains used for flocculation of different micr[oal]gal cells. The detailed mechanisms of algal-fungal interactions are [st]ill unknown. It has been suggested that algae have a negative sur[face] charge usually (−23.7 mV) due to the presence [of i]on-acti[ve] carboxylic, phosphoric, phosphodiester, hydro[xyl] and a[mi]ne functional groups (Gultom and Hu, 2013; Grim[a et] al., 20[illegible]). Fungal mycelia rich in polysaccharides that have be[en sh]o[wn to be posi]tively charged (+46.1 mV) and hence [it is] possi[ble to n]eutralize the negative charges present on the su[rfac]e of algae, e[nab]ling attachment to the fungal cell wall. Fu[ngal medi]ated algal [floc]culation is effective for both heterotrophic and p[hoto]trophic algal species. Fungal associated pelletiza[tion] is already [succ]essfully utilized for entrapping sludge solid[s d]uring waste wate[r tr]eatment process (Gultom and Hu, 201[illegible]). Furth[erm]ore, some fungal species were reported to have lipi[d con]te[nt o]f over 30% of total biomass, making them suitable for bio[illegible] feedst[ock] along with the microalgal biomass (Zho[u et al.,] 201[illegible]). Furth[er]more, this flocculation technique does not require different cultivation conditions and allows total medium reuse without further treatment (Zhou et al., 2012).

Reports showed that *C. vulgaris* cells cultivated with *Aspergillus* sp. spores was completely pelletized and that has a capability to remove the nitrogen and phosphate in wastewater efficiently (Zhou et al., 2012). Further, when *C. vulgaris* cells was co- cultivated with *Aspergillus niger* spores, total fatty acids production is improved better under heterotrophic mode of cultivation (Zhang and Hu, 2012). Similar observation is also observed when co-cultivation of oleaginous fungus *Cunninghamella echinulata* with *C. vulgaris* at a ratio of 1:2 for the harvesting of biomass (Xie et al., 2013).The study revealed t[illegible] of the biomass is removed from the culture medium w[ith]in two [day]s of incubation with fungus and co-cultivation ca[n be] controlled t[o ac]hieve continuous cultivation of algae. Co-cultur[e o]f fungus [wi]th algae are gaining more important in[illegible]ent sce[nario] for [im]proving lipids and other biochemical [pro]duction and [illegible]t harvesting of microalgal biomass (M[ura]dov et [al.], 2015). [The]ir studies revealed that mixing of *A. fumig[atus]* [pellet]s with [th]e high cell density culture of *C. protot[heco]ides* a[nd *T.*] *suecic[a]* [ce]lls. Results showed that up to 90% floc[cula]tion efficie[ncy w]as obtained in the both marine and freshw[ater alga]e after 24 h [of] co-cultivation. Another report showed tha[t] co-cu[lturi]ng of *Aspergillus niger* with *Chlorella vulgaris* that can helps in har[vesti]ng the algal biomass with 90% efficiency (G[ultom e]t al., 2014). C[o-c]ultivation of fresh water and marine a[lga]e with *A. fumigatus* cells showed that it has supplemented [illegible] beneficia[l e]ffects on biomass, lipid content and phyco-r[eme]diation of [w]astewater also improved. Analysis of fatty acids co[mpos]ition f[ro]m the fungal-algal pellet's suggested that it can be tu[ned to] specific fatty acids for specific applications and optimized through co-cultivating different algae and fungi without the [illegible] genetic modifications (Wrede et al., 2014). The inoculation of *C. sorokiniana* with spores produced from *I. fumosorosea* and co-cultivated under phototrophic conditions resulted formation of big lichen pellets which in turn increased the size of the biomass to 1–2 mm in diameter that helps recovery of biomass to 94–97% by filtration which subsequently reducing the costs of harvesting as well as significantly increasing yield of biomass (Mackay et al., 2015). Immobilization of microalgal cells to the fungal mycelium of *Aspergillus nomius* showed that around 94% precipitation of *Chlorella vulgaris* and 97% precipitation of cells were obtained in marine microalgae *Nannochloropsis* sp. (Talukder et al., 2014). Another report showed that proteins isolated from yeast *S. bayanus* var. *uvarum* during their fermentation and their ability to induce flocculation process was conducted. The result indicated that incorporation of 0.1 mg/ml of bio-flocculant proteins from yeast resulted in biomass recovery of 95% and 75% from *Chlamydomonas* sp. and *Picochlorum* sp. respectively (Diaz-Santos et al., 2015). Novel flocculation agent based on spent brewer's yeast *Saccharomyces pastorianus* from brewing industry was used for the harvesting of freshwater microalgae *Chlorella vulgaris*. Results showed that modified the yeast surface with positively charged functional group DEAE increased harvesting efficiency of 90% at concentration of 0.4 mg/g (Prochazkova et al., 2015).

The impressive performance on microalgae harvesting has enabled fungus assisted bioflocculation, a potential low-cost cell harvesting method. This might open a new door in the integration of microbial biomass conversion and autotrophic microalgae based biorefinery. This system can be used for the production of various chemical products like combination of poly unsaturated fatty acids, antioxidants and other nutraceuticals. Limitation of this approach of co-cultivation of fungi with microalgae demands organic substrates for the generation of fungal pellets as well as some function are limited to particular range of pH and species of microalgae which could restrict its application. Furthermore, a risk of fungal contamination in harvested biomass is also greatly concerned

Table 4
Flocculation e[fficienci]es of microalgae with fungus/yeast by co-cultivation.

Fungus/yeast	Microalage	FE (%)	References
Cunninghamella echinulata	*C. vulgaris*	99	Xie et al. (2013)
Aspergillus niger	*Chlorella vulgaris*	90	Gultom et al. (2014)
Aspergillus nomius	*Chlorella vulguris*	97	Talukder et al. (2014)
Aspergillus nomius	*Nannochloropsis sp.*	94	Talukder et al. (2014)
A. fumigatus	*T. suecica*	90	Muradov et al. (2015)
S. bayanus var. *uvarum*	*Chlamydomonas sp*	95	Diaz-Santos et al. (2015)
S. bayanus var. *uvarum*	*Picochlorum sp*	75	Diaz-Santos et al. (2015)
I. fumosorosea	*C. sorokiniana*	97	Mackay et al. (2015)
A. fumigatus	*C. protothecoides*	90	Muradov et al. (2015)
Saccharomyces pastorianus	*Chlorella vulguris*	90	Prochazkova et al. (2015)

when the microalgal biomass will be applied as food or feed supplements. Application of algal biomass for fuel (Biodiesel) application this methods of harvesting is one of the low cost choice to chemical based flocculation.

2.4. Autoflocculation/algae-algal flocculation

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. Auto-flocculation is the flocculation that can occur naturally in certain microalgae and microalgae may flocculate in response to some environmental stress, changes in nitrogen, pH, dissolved oxygen and amount of calcium and magnesium ions in the culture mediums (Uduman et al., 2010; Schenk et al., 2008).

Auto-flocculation does not occur in all microalgal species and the process can be slow and unreliable (Schenk et al., 2008). It has been reported that this process was associated with increased pH due to photosynthetic CO_2 consumption compared with precipitation of phosphate, magnesium, calcium and carbonate salts with algal cells (Sukenik and Shelef, 1984).

2.4.1. Autoflocculation by pH modulation

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 increased the formation of dense flocs which result in less settling times. However, not all the microalgae species flocculate with increased or decreased pH levels (Perez et al., 2017; Ummalyma et al., 2016; Liu et al., 2013; Wu et al., 2012). Harith et al., (2009) reported that at pH values less than 10.0, only slight separations between the microalgae *Chaetoceros calcitrans* cells but the separation was further increased from the pH 8.0 to 10.0 using NaOH and KOH increased the flocculation efficiency from 13 to 82% and from 35 to 78% in 4 h respectively. In order to boost with this pH of 10.5 resulted in 90% flocculation efficiency for the freshwater microalgae *Chlorella vulgaris*, *Scenedesmus* sp. and *Chlorococcum* sp. pH value of 9.0–9.3 resulted in 90% flocculation efficiency for the marine algae *Nannochloropsis* sp. and *Phaeodactylum tricornutum* (Wu et al., 2012). pH of 8.6–10.5 is successfully tried for the 90% biomass harvesting of the halo tolerant microalgae *Dunaliella tertiolecta* (Horiuchi et al., 2003). Ummalyma et al. (2016) reported that pH value of 11.0–12.0 leads to the flocculation of fresh water microalgae *Chlorococcum* sp. RAP[illegible]. Self-flocculating microalgae such as *C. nivale*, *C. ellipsoideum* and *Scenedesmus* sp. were used for flocculation potential, the maximum flocculation efficiencies of >90% is reported at pH 4.5 and stable within pH ranges of 4.5–1.5 (Liu et al., 2013). However, the self flocculating algae are used for the flocculation of the target microalgae (*C. zofingiensis* and *C. vulgaris*) w[illegible]5 μm), flocculated by the pH decrease-induced flocculation method (Liu et al., 2014). More research is needed for exploring the self flocculating microalgae for harvesting of non-flocculating oleaginous microalgae for various industrial applications.

Flocculation can also induced in some microalgae species naturally in the medium because of the changes in concentration of dissolved oxygen content in the broth (Uduman et al., 2010). Schenk et al., (2008) reported that dissolved oxygen stress can result in microalgae flocculation. Reports showed that increased dissolved oxygen in solution triggers 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 (Liao et al., 2011). They also showed that increased photosynthetic activity by microalgae also increases the dissolved oxygen content and the formation of dense flocs. The dissolved oxygen concentrations of 14–16 mg/L promoted flocculation in the system and high dissolved oxygen concentrations in the medium also stimulate the auto flocculation of microalgae (Wilen and Balmer, 1999).

2.4.2. Flocculation by nutrient stress and presence of metal ions

Self aggregation of microalgal cells may be triggered naturally as a result of environmental stimulus such as stress caused by nitrogen concentration in the suspended water (Uduman et al., 2010; Schenk et al., 2008). Some species of microalgae flocculated as a result of nitrogen stress in the media (Sukenik and Shelef, 1984).They reported that microalgae *Scenedesmus dimorphus* flocculation is an example of such kind of flocculation. Microalgae cells can also aggregated as a result of nitrate assimilation (Nurdogan and Oswald, 1995). Reasons for this aggregation of cells are assimilation of nitrate as nitrogen source, which increase the pH of the medium and promotes autoflocculation of [illegible] et al., 2012; Uusitalo, 1996). Report showed that nitrate concentration of 840.4 mg/L was sufficient [illegible]ating *Chlorella vulgaris* in MBB medium (Nguyen et al., 2014).

Addition of Ca^{2+} and Mg^{2+} [illegible] the culture media spontaneously induce flocculation of cells as a result of co-precipitation of calcium and magnesium which further induces the fluctuation in the pH of the medium which leads to effective flocculation of cells (Wang et al., 2014). Report on the evaluation of Mg^{2+}, Ca^{2+} and CO_3^{2-} ions for their flocculation potential and settling of microalgae cells, showed that Mg^{2+} ion with high pH levels resulted in effective flocculation and rapid sedimentation than the other ions (Smith and Davis, 2012). They got settling rates that were 10[illegible] higher than those obtained with natural sedimentation. The possible reason for this mechanism is that magnesium h[illegible] flocs are positively charged whereas calcium carbonate [illegible]s are negatively charged (Ayoub et al., 1986). Thus, destabilization of the negatively charged microalgae cells is greater when magnesium ions are added into the medium than calcium ions. Microalgae *Chlorella vulgaris* is auto-flocculated with efficiency of 90% by addition of Ca^{2+} and Mg^{2+} at concentrations of 120 mg/L and 1000 mg/L, respectively (Nguyen et al., 2014). Vandamme et al., (2012) reported that addition of Mg^{2+} in *Chlorella vulgaris* culture induced auto-flocculation. Table 5 represents the auto-flocculation of various algae.

2.4.3. Algae-algal interactions

Cells flocculation generally exists in microorganisms and several self-flocculating microalgae have also been identified such as *Chlorella vulgaris* JSC-7 (Alam et al., 2014), *Scenedesmus obliquus* AS-6-1 (Guo et al., 2013), *Ankistrodesmus falcatus* (SAG202-9) and *Ettlia texensis* (SAG79.80) (Salim et al., 2012 and Salim et al., 2011). Few reports are available on self flocculation of algal cells and exact mechanism of auto flocculation is still obscure. It is reported that water soluble extracts of marine microalga *Skeletonema marinoi* induced flocculation of *Nannochloropsis oculata*, with an efficiency of flocculation was 95% achieved after 6 h of settling time (Taylor et al., 2012). Alam et al., (2014) and Guo et al. (2013) had studied the biochemical basis of auto flocculation of two microalgae *C. vulgaris* JSC-7 and *S. obliquus* AS-6-1.They found that the polysaccharides biosynthesized by these two strains were responsible for self-flocculation. Another recent report proposed that glycoprotein is involved in cell flocculation of green microalgae *E. texensis* SAG79.80 (Salim et al., 2014). More supporting to this another report state that cell wall polysaccharides enriched with phosphodiester group of self flocculating *Chlorella vulgaris* JSC-7 can acts as flocculating agent for flocculation of freely suspended microalgae *C. vulgaris* CNW11 and *Scenedesmus obliquus* FSP. This report showed that flocculation efficiency of 80% was achieved with this process (Alam et al., 2014). Therefore,

Table 5
Auto-flocculation of microalgae.

Auto-flocculation	Microalgae	FE (%)	References
Microalgae			
Ettlia texensis	*Chlorella vulgaris*	55	Salim et al. (2011, 2012)
Scenedesmus obliquus	*Chlorella vulgaris*	34	Salim et al. (2011, 2012)
Ankistrodesmus falcatus	*Chlorella vulgaris*	50	Salim et al. (2011, 2012)
Tetraselmis suecica	*Neochloris oleoabundans*	72	Salim et al. (2011, 2012)
Skeletonema marinoi	*Nannochloropsis oculata*	95	Taylor et al. (2012)
Scenedesmus obliquus AS-6-1	*S. obliquus*	80	Guo et al. (2013)
Scenedesmus obliquus AS-6-1	*Chlorella vulgaris*	85	Guo et al. (2013)
Chlorella vulgaris JSC-7	*C. vulgaris CNW11*	80	Alam et al. (2014)
pH modulation			
pH 8	*Chaetoceros calcitrans*	85	Harith et al. (2009)
pH 10.2	*Chaetoceros calcitrans*	90	Harith et al. (2009)
pH 10.5	*Chlorella vulgaris,*	>90	Wu et al. (2012)
pH 10.5	*Scenedesmus* sp.	>90	Wu et al. (2012)
pH 9	*Nannochloropsis* sp.	90	Wu et al. (2012)
pH 9	*Phaeodactylum tricornutum*	90	Wu et al. (2012)
pH 12	*Chlorococcum sp.RAP-13*	94	Ummalyma et al. (2016)
pH 4.5	*Chlorococcum nivale*	>90	Liu et al. (2013)
pH 4.5	*Chlorococcum ellipsoideum*	>90	Liu et al. (2013)
pH 4.5	*Scenedesmus* sp.	>90	Liu et al. (2013)

microalgal auto-flocculation may occur when the flocculating agents such as polysaccharides or glycoprotein produced [illegible] microalgal cells surface that themselves patch [illegible] cells or may be due to development of bridges among the cells through neutralization of charge in the bro[illegible] stim[illegible] self-flocculation. More research is needed in this [illegible] understand the exact mechanism of self flocculation of microalgal cells and exploration of self flocculating microalgae for successful production of algae based biorefineries. Microalgal cell self-flocculation, differing from the flocculation induced [illegible] pH modulation can occur naturally via interaction of adjacent [illegible] without the addition of acid, alkaline or metal ion in medium. Moreover, harvesting microalgae using self-flocculation which requires no extra investment in cultivation [illegible] microalgae and purification of bio-flocculants, hence it is a [illegible]ble alternative method for low-cost harvesting [illegible]s for [illegible]food, feed and nutraceutical applications.

3. Future perspectives

Microalgae are important future bioresources for various industrial applications. So there is a need to develop suitable technologies for harvesting the algal biomass from the huge volume of culture broth. Bio-flocculation is the alternative strategies for harvesting the biomass. Future algal bio-refineries should focus on self-flocculating microalgae resistant to attack by algal predators or feeders. Attention should be given to algal organic matters released into the media that have properties to assist the microalgae to flocculate. Studies should be focused on the understanding mechanisms of how this algal organic matter is responsible for flocculating the microalgae. All microalgal bio-flocculation is conducted in the lab scale only but should also investigate strategies for scaled up applications rather than improving bio-flocculation efficiencies under specific conditions. Algal self-flocculation is low cost methods, more research is needed in this area to understand the exact mechanism of self-flocculation of microalgal cells. Microalgal self-flocculation, differing from the flocculation stimulated by pH modulation, can occur naturally via interaction of adjacent cells without the addition of acid, alkali, or metal ions in medium. Moreover, harvesting microalgae using self-flocculation which requires no extra expenditure in cultivation of microalgae or purification of bio-flocculant is a promising method for low-cost harvesting. So far only few self-flocculation microalgae are reported which itself cannot meet the commercial demands for application as harvesting technology for microalgae. Genetic modification of microalgae is needed by in[illegible] genes responsible for flocculation into microalgae without compromising their high biomass productivity of spec[illegible] metabolites [illegible] high flocculation efficiency. Therefore, efficient cost-effective qualities have made bio-flocculation is a [illegible] method of harvesting microalgal species.

4. Conclusion

Development of economically [illegible] flocculation strategies for microalgae harvesting significantly contributes to cost reduction and energy saving [illegible] mass production of micro algal biomass. Chemical flocculation [illegible] high efficiency but may cause contamination in algal biomass and environment. Bio-based flocculation using microorganisms and purified bio-flocculants is promising [illegible] microalgae harvest since it is safe and eco-friendly process. Limitations of microbes based bio-flocculation are only applicable for [illegible]s not suitable for food/ feed applications. Auto-flocculation of cells through the flocculating substances synthesized by microalgae is the most promising method for [illegible]ective and eco-friendly harvesting method for various biorefinery applications.

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