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Review

Flocculation Harvesting Techniques for Microalgae: A Review

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Abstract: Microalgae have been considered as one of the most promising biomass feedstocks for various industrial applications such as biofuels, animal/aquaculture feeds, food supplements, nutraceuticals, and pharmaceuticals. Several biotechnological challenges associated with algae cultivation, including the small size and negative surface charge of algal cells as well as the dilution of its cultures, need to be circumvented, which increases the cost and labor. Therefore, efficient biomass recovery or harvesting of diverse algal species represents a critical bottleneck for large-scale algal biorefinery process. Among different algae harvesting techniques (e.g., centrifugation, gravity sedimentation, screening, filtration, and air flotation), the flocculation-based processes have acquired much attention due to their promising efficiency and scalability. This review covers the basics and recent research trends of various flocculation techniques, such as auto-flocculation, bio-flocculation, chemical flocculation, particle-based flocculation, and electrochemical flocculation, and also discusses their advantages and disadvantages. The challenges and prospects for the development of eco-friendly and economical algae harvesting processes have also been outlined here.

Keywords: microalgae; harvesting; flocculation; biomass; biorefinery; biofuel

1. Introduction

Microalgal biomass has attracted much attention in the academic and industrial fields due to its various industrial applications such as animal/aquaculture feeds, food supplements, nutraceuticals, and pharmaceuticals [1,2]. Recently, petroleum-fuel scarcity as well as global warming associated with greenhouse gas emissions (e.g., CO_2) are obliging scientists and engineers to actively look for new and renewable sources of transportation fuels [3]. Various liquid and gaseous biofuels, such as diesel, aviation fuel, ethanol, butanol, hydrogen, and methane, can be produced from algal biomass through biological and thermochemical transformation technologies [4,5].

Microalgae can utilize CO_2 as an inorganic carbon substrate using light energy and can be grown using diverse water resources, including freshwater, seawater, and even industrial/domestic wastewater. They can be also cultivated at a large-scale using different bioreactor systems such as open ponds and photobioreactors [6,7]. However, due to the low concentration (~5 g/L) in culture, small size (~5 μm) and negative surface charge (~−20 mV) of algal cells, external energy and/or

chemicals are generally required to accelerate their recovery from base water [8,9]. Furthermore, other morphological and physiological characteristics of algal cells such as shape, cell wall structure, and extracellular organic matter (EOM) change significantly depending on the nutritional and environmental conditions including medium composition, light, temperature, pH, culture duration, and bioreactor type [10]. The algae harvesting costs are generally estimated at 20–30%, with the occasional rise to 60%, of the total biomass production cost, depending on the algal species and culture process used [11,12]. Therefore, the development of a high-efficiency and cost-effective harvesting process is key to achieving commercial scale algae-based process.

Algal biomass harvesting has been extensively studied with particular focus on centrifugation, gravity sedimentation, screening, filtration, air flotation, and flocculation techniques. However, there is no single universal harvesting method for all algal species and/or applications, which is both technically and economically viable [13,14]. For instance, centrifugation is based on a mechanical gravitational force that allows for efficient harvesting of suspended cells in a short time. However, due to the intensive energy requirement, it is recommended only for high-value algal products such as in foods and pharmaceuticals [14,15]. In the filtration process, micro-sized algal cells can be passed through a suitable membrane under high pressure to obtain a thick paste of algal biomass [16]. This size-exclusion method may be useful and scalable for algae harvesting only if problems in membrane blocking can be minimized or prevented [17,18]. The air flotation (or inverted sedimentation) harvesting process is based on the generation of up-rising gas bubbles that bind to algal cells and induce their flotation to the liquid surface [19]. However, due to differences in the surface hydrophobicity of algal cells, harvesting efficiency varies greatly depending on the species of algae [20]. It should also be noted that the high operation cost for producing small air bubbles can limit large-scale commercialization.

Flocculation refers to the aggregation of unstable and small particles through surface charge neutralization, electrostatic patching and/or bridging after addition of flocculants. Flocs formation allows for separation (or recovery) by simple gravity-induced settling or any other conventional separation method [21,22]. The flocculation process is simple and efficient, and has been extensively investigated as a promising strategy for harvesting various algal species [9,23]. Figure 1 shows the flocculation harvesting process of algal cells for algal biorefinery.

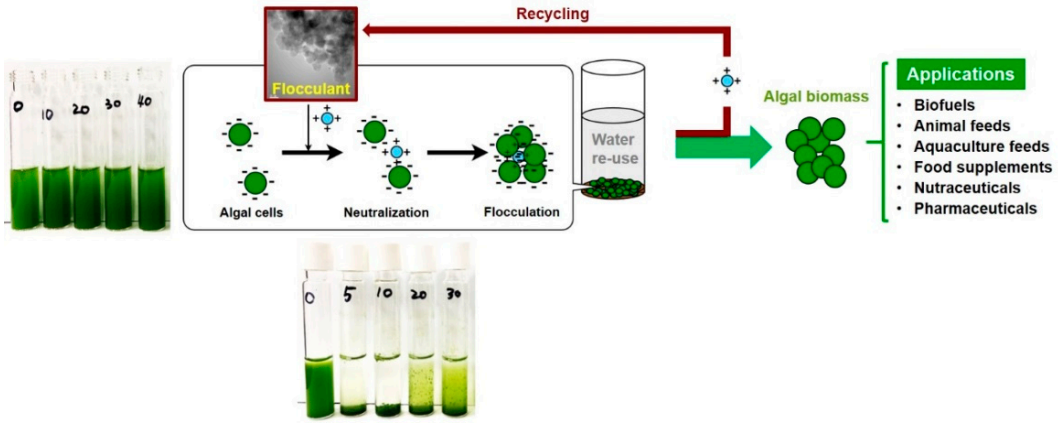

Figure 1. Schematic diagram of the flocculation harvesting process of algal cells with a recyclable flocculant.

As the flocculant plays a major role in the flocculation harvesting process, the discovery of a highly efficient and cost-effective flocculant has forever remained a challenge in most studies. Nowadays, the use of conventional inorganic metal salts such as aluminum sulfate and ferric sulfate has been reduced due to high dosage and biomass contamination [9]. Various natural and synthetic organic flocculants have been designed and developed to improve flocculation efficiency. However, the former have high production cost and a short shelf-life while the latter have adverse effects on harvested biomass and non-biodegradability, due to their petroleum origins [24]. Metal cations

released from the electrode under direct electric current condition are able to electrostatically attract almost all types of algal cells, resulting in efficient flocculation. Significant efforts are being directed to prevent electrode/biomass fouling and to reduce systemic/electric cost for large-scale algae harvesting. Nanoparticles in either single or hybrid forms decorated with various cationic chemicals have been employed for rapid algae separation and/or multi-functionalities such as cell disruption and lipid extraction [25]. This approach although highly efficient, is expensive and is mostly limited to laboratory-scale studies. Spontaneous aggregation of algal cells under specific conditions and the use of a self-flocculating microorganism can be considered as sustainable and environment-friendly [22,26]. However, species-specific reactivity, availability of low-cost bio-flocculant-microorganisms, and process scale-up should be properly considered for practical applications. Ideally, in addition to excellent harvesting efficiency and promising scalability, the industrial flocculant should satisfy the demands for recyclability, low toxicity, low-cost material, and massive production process.

Many review articles are available on the use and mechanism of specific flocculants/techniques [9,19,25,27–30], but a comprehensive comparison of various flocculation-based methods including classical inorganic chemicals and newly engineered nanoparticles, is limited. We herein classify the flocculation techniques that have been studied and applied in the algal biorefinement process, into five categories which are auto-flocculation, bio-flocculation, chemical flocculation, particle-based flocculation, and electrochemical flocculation. The main objective of this review is to introduce the readers to these flocculation techniques and the key issues in their commercialization. The review thus provides an updated overview of the recent technical developments and progress in this field. Further research directions have also been proposed to direct the development of flocculation harvesting processes suitable for large-scale algae applications.

2. Auto-Flocculation

In auto-flocculation, suspended algal cells spontaneously aggregate, forming large flocs, which induce their simple gravitational sedimentation (Figure 2). This phenomenon has been observed in various algal species particularly under non-ideal culture conditions such as change in pH and cultural aging, as summarized in Table 1. Both alkaline and acidic conditions have been reported to reduce the intensities of the negative surface charge of algal cells, thereby promoting their self-aggregation [31]. Under alkaline conditions above pH 9, the changes in the surface charge of algal cells are mainly attributable to significant secretion of protective extracellular polymers [32]. Under acidic conditions, fluctuating dissociations of carboxyl and amine groups in the algal cell wall can cause changes in surface charge.

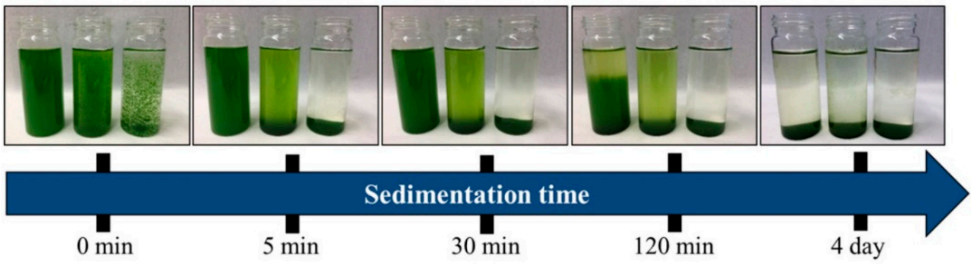

Figure 2. Auto-flocculation harvesting of algal biomass. Three vials contain algal samples cultured in different nitrate concentrations: (**left**) 0.5×; (**middle**) 1× (original); and (**right**) 2×. Reprinted from Reference [33], distributed under the terms of the Creative Commons Attribution License.

Table 1. Comparison of auto-flocculation techniques for algae harvesting.

Condition		Alga (Cell Density)	Optimal Harvesting	Ref.
Acidic pH	pH 4.0	*C. ellipsoideum* (4.38 g/L)	95% @ 15 min	[31]
	pH 4.0	*C. nivale* (4.17 g/L)	94% @ 15 min	[31]
	pH 4.0	*Scenedesmus* sp. (6.94 g/L)	98% @ 15 min	[31]
Alkaline pH	pH 11.5	*C. muelleri* #862 (0.42 g/L)	100% @ 30 min	[34]
	pH 11.0	*C. vulgaris* (0.5 g/L)	95% @ 60 min	[35]
	pH 12.0	*Chlorococcum* sp. R-AP13	94% @ 10 min	[36]
	pH 12.5	*Ettlia* sp. YC001 (1.2 g/L)	94% @ 30 min	[37]
	pH 10.4	*N. oculate* (2.27×10^5 cells/mL)	90% @ 10 min	[38]
	pH 11.6	*S. quadricauda* #507 (0.54 g/L)	95% @ 30 min	[34]
Culture aging	16 days	*S. obliquus* AS-6-1 (2.25 g/L)	80% @ 30 min	[39]

It should be noted that auto-flocculation efficiency resulting from pH manipulation is largely species-dependent [32]. There is a sigmoidal relationship between increase in cultural pH and flocculation efficiency for the freshwater alga *Ettlia* sp. YC001. The harvesting efficiency is very low at ~11% in the pH range of 6.5–8.5, while it dramatically increases to 83% and 94% with increase in pH to 10.5 and 12.5, respectively [37]. Similarly, a high harvesting efficiency of 94% was reported for *Chlorococcum* sp. R-AP13 at pH 12 [36]. However, in the case of *Scenedesmus obliquus* NRCIbr1, a slight improvement in auto-flocculation efficiency from 10.4% to 33.2% was observed despite a pH increase from 7 to 10 [40]. In another study, high flocculation efficiency of ~90% was reported for three freshwater algal species, *C. nivale*, *C. ellipsoideum*, and *Scenedesmus* sp., under acidic conditions (pH 4) [31]. Self-flocculating activity can also be affected by cell density. At pH 11.5, the harvesting efficiency of *S. quadricauda* was as high as 94.7% for a low cell density of 0.54 g/L, but it was significantly decreased to 71.7% when applied at a high cell density of 2.7 g/L [34].

Aging of algal cultures is usually combined with the release of EOM from algal cells (mainly composed of proteins and polysaccharides) into the aqueous environment [28]. EOM is thought to play an important role in the self-flocculation of algal cells, specifically by forming biofilms and changing the surface charge of cells, presumably via neutralization [41,42]. The amount and composition of EOM is largely dependent on species, growth stages, and culture conditions (culture duration, pH, light, and temperature) [43]. The auto-flocculation efficiency of *S. obliquus* did not exceed 5.5% at the early stationary growth-phase under neutral pH, but was considerably increased to 24.4% (for the same culture and pH range) at the late stationary growth-phase [40]. Similarly, by increasing the cell aging time, the auto-flocculation efficiency of *Chlorococcum* sp. R-AP13 increased considerably from 62% in one-week-old culture to 75% in the three-week-old culture [36].

The composition and concentration of the nutrients in the medium such as nitrogen sources and minerals can influence auto-flocculation efficiency as well as algal biomass production. In this context, relatively higher auto-flocculation efficiency for *Chlorella vulgaris* has been reported when nitrate (NO_3^-) was used as the nitrogen substrate instead of ammonium (NH_4^+). It has been proposed that this enhancement may be attributed mainly to the co-precipitation of Ca^{2+} and Mg^{2+} ions originally dissolved in the medium with algal cells, under a high pH environment resulting from the nitrate assimilating metabolic activities of algal cells [44]. Similarly, in a *Nannochloropsis oculata* culture at pH 10.4, the self-aggregation efficiency (~90%) of algal cells could be enhanced by the co-precipitation of Ca^{2+} and Mg^{2+} [38]. 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 [13]. In another study, the auto-flocculation efficiency of *Chlorococcum* sp. was improved considerably from 63% to 84% by increasing the ammonium concentration from 10 to 50 mg/L, possibly due to the enhanced extracellular protein secretion at the higher ammonium concentration [42].

Auto-flocculation-based harvesting of algae is a potentially low-cost and eco-friendly biomass recovery strategy, because no chemical flocculant is used [8,37]. Additionally, the medium used can be effectively recycled after harvesting, for subsequent algal cultivations [36]. However, it should be noted that this harvesting process is generally slow and highly species-specific [30,40].

3. Bio-Flocculation

Bio-flocculation is performed by adding a self-flocculating microorganism (or its extracellular biopolymer) to the culture broth to harvest non-flocculating, target algae (Figure 3). Bio-flocculants include bacteria, fungi, yeasts, or self-flocculating algae as well as their exudate-rich culture supernatants, as shown in Table 2. Since no chemical is required in this process similar to the case of auto-flocculation, the bio-flocculation method can also be considered as a sustainable and environmentally friendly technique for algal biomass harvesting [45,46]. A bio-flocculant-microorganism can be prepared by co-culturing with target algae or culturing separately in a different bioreactor, before performing the intended use [22,30]. Although the mechanism of bio-flocculation has not been clearly elucidated, it is believed that it is mainly a function of the reactivity of the extracellular biopolymer and/or the direct adsorption of the self-flocculating microorganisms on the target algae [23,27].

3.1. Algal–Fungal Bio-Flocculation

Many filamentous fungi have been known to possess self-pelletizing abilities by which algal cells can be entrapped and adsorbed [46]. Fungal biomass is usually produced separately in a fermenter, after which its pellets are applied to algae harvesting (called co-pelletization) [27]. Sometimes bio-flocculations are carried out by the co-cultivation of fungi and algae. However, during the co-culture, fungal cells could have a positive or negative impact on algal growth, depending on their species and culture conditions. Interestingly, co-existing fungi can protect algal culture from external microbial contamination [13]. Harvested fungal–algal biomass (co-pellets) has been successfully utilized as feedstock for various biofuels such as biodiesel, alcohols, and biogas through fermentation and thermochemical methods [47].

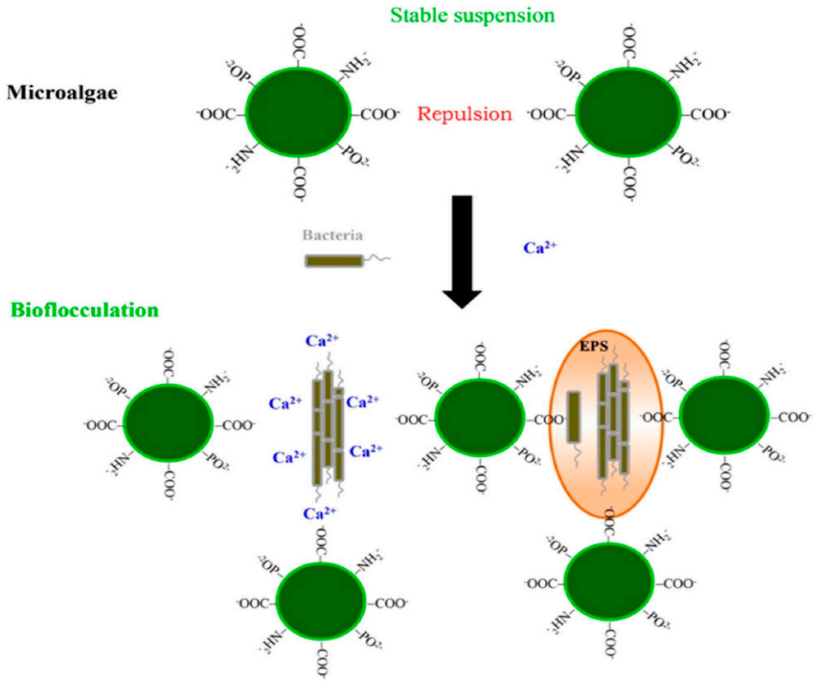

Figure 3. A schematic diagram of bio-flocculation harvesting of algal biomass using bacteria. EPS, extracellular polymeric substances. Reprinted from Reference [27] with permission from Springer Nature.

The co-pelletizing abilities of four fungal species (*Aspergillus lentulus*, *A. terreus*, *Polyporus* sp., and *Rhizopus oryzae*) have been evaluated in a bio-flocculation study, by co-culturing with alga *Chroococcus* sp. in a photosynthetic mineral salt medium augmented with glucose (5 g/L) as an organic

carbon substrate. Remarkably, all the tested fungal strains were able to effectively flocculate the targeted alga with high harvesting efficiency of ~92% [48]. Similarly, *A. fumigatus* has been recommended for algae harvesting through co-pelletization, among several self-pelletizing oleaginous fungal strains. This fungal strain showed the highest harvesting efficiency (>90%) for both freshwater alga *C. protothecoides* and marine alga *Tetraselmis suecica* during co-cultivation in swine wastewaters within 24 h. It should be noted that, during the co-cultures, the total biomass and lipid production increased synergistically [47]. *A. Fumigatus* was also reported for successful co-cultivation with the heterotrophic alga *Thraustochytrid* sp. in an anaerobically digested swine wastewater.

An efficient harvesting efficiency (~90%) was obtained through the co-pelletization of fungal mycelium and algal cells. Furthermore, they could obtain enhanced biomass and lipid productivity as well as effective nutrient removal from the wastewater [49]. Interestingly, the processing time required for the fungal pellet-based harvesting is much shorter than that needed for spore-based harvesting. For example, harvesting of *Chlorella* sp. based on co-pelletization with *Penicillium* sp. require only 2.5 h, while 28 h was required in the case of the spores-based method to obtain the same harvesting efficiency (>98%) [50].

The algal–fungal bio-flocculation process can be considered to be highly efficient and sustainable. However, environmental contamination by fungal species and/or spores as well as process scale-up should be properly considered for practical applications [26].

Table 2. Comparison of bio-flocculation techniques for algae harvesting.

	Flocculant (Dosage)	Alga (Cell Density, Volume or Amount)	Optimal Harvesting	Ref.
Fungus	*A. fumigatus*	*C. protothecoides*	~90% @ 24 h	[47]
	A. fumigatus (1.5–2.0 × 10^7 spores/L)	*S. quadricauda* (5–8 × 10^8 cell/mL)	~97% @ 48 h	[49]
	A. fumigatus	*T. suecica*	~90% @ 24 h	[47]
	A. lentulus (1.0 × 10^6 spores/mL)	*Chroococcus* sp. (1.58 g/L)	~100% @ 24 h	[48]
	Penicillium cells (1.92 g)	*Chlorella* sp. (3.84 g)	~98% @ 2.5 h	[50]
	Penicillium spores (1.1 × 10^4 cells/mL)	*Chlorella* sp. (3.84 g)	~99% @ 28 h	[50]
Yeast	Extracellular protein of *S. bayanus* (0.1 g/L)	*C. reinhardtii* (10 mL)	95% @ 3 h	[51]
	Extracellular protein of *S. bayanus* (0.1 g/L)	*Picochlorum* sp. (10 mL)	75% @ 3 h	[51]
	S. bayanus (1:1, *v/v*)	*C. reinhardtii* (10 mL)	80% @ 6 h	[51]
	S. bayanus (1:1, *v/v*)	*Picochlorum* sp. (10 mL)	60% @ 6 h	[51]
	S. pastorianus (0.4 mg/g cell)	*C. vulgaris* (5 g/L)	90% @ 70 min	[45]
Bacterium	*Flavobacterium*, *Terrimonas*, and *Sphingobacterium*	*C. vulgaris* (6 × 10^6 cells/mL)	94% @ 24 h	[52]
	Bio-flocculant secreted from *S. silvestris* W01 (3:1, *w/w*)	*N. oceanica* DUT01	90% @ 10 min	[53]
Alga	*S. obliquus* AS-6–1 (1%, *v/v*)	*S. obliquus* FSP-3 (10 mL)	83% @ 30 min	[39]
	Exudates-rich spent media of *C.* cf. *pseudomicroporum* (1:1, *v/v*)	*S. ellipsoideus* (15 mL)	97% @ 4 h	[54]
	Phormidium sp.	*Chlorella* sp.	100% @ 5 min	[14]

3.2. Algal–Yeast Bio-Flocculation

Co-cultivation of algae with yeast to promote both biomass and lipid production as well as effective flocculation harvesting, has also been reported. Co-culturing of yeast *Rhodotorula glutinis* and alga *S. obliquus* led to synergistic increase in biomass production (40–50%) and lipid content (60–70%), compared with single cultures [55]. The bio-flocculation efficiencies of freshwater alga *Chlamydomonas reinhardtii* and marine alga *Picochlorum* sp. were investigated using whole cells and extracellular proteins of anaerobically grown yeast *Saccharomyces bayanus*. The yeast whole cells showed only moderate harvesting efficiencies of 80% and 60% for *C. reinhardtii* and *Picochlorum* sp., respectively, after 90 min treatment, albeit at high mixing ratios (1:1). Interestingly, treatment with a relatively low concentration of extracellular yeast proteins (0.1 g/L), led to significant increase in the flocculation efficiencies to 95% and 75%, respectively, for the former and latter algal species [51]. Chemically modified autolysates of *S. cerevisiae* (a by-product of the brewing industry) could exhibit high harvesting efficiency of over 90% when applied to *C. vulgaris* at a dosage of 0.4 mg/g cell [45].

The algae harvesting process using either yeast whole cells or their extracellular proteins appears to be environmentally friendly. However, extensive research on the technical feasibility of the use of other biofuel algal strains and the availability of low-cost yeast products is required for practical process development.

3.3. Algal–Bacterial Bio-Flocculation

Bacteria generally co-exist during conventional algae cultivation under reactor-scale and/or outdoor conditions. Their interactions are species-specific and can be either mutually benefiting or inhibiting according to the nutritional and environmental conditions [56,57]. Interestingly, some algae-associated bacteria such as *Flavobacterium*, *Terrimonas*, and *Sphingobacterium* have been proven to promote the formation of large flocs which can settle thereby enhancing algae flocculation. A xenic culture of *C. vulgaris* with bacteria exhibited high harvesting efficiency of ~94%, while an axenic (bacteria-free) *Chlorella* culture showed only 2% flocculation efficiency [52]. Bacterium *Solibacillus silvestris* isolated from activated sludge has been reported to secrete a less-toxic bio-flocculant (composed of 75.1% proteoglycan and 24.9% protein), inducing electrostatic bridging and patching between algal cells [53]. However, this bio-flocculant (dosage, 0.2 g/L) showed only a relatively low harvesting efficiency of 76.3% for marine alga *N. oceanica*.

Fast growth rates of bacteria can be beneficial relative to other microbial flocculants such as yeast and fungus. However, species-specific reactivity and environmental safety issues should be carefully considered and resolved for large-scale applications [58].

3.4. Algal–Algal Bio-Flocculation

The use of a self-aggregating alga (or its extracellular polymeric substance, EPS) to harvest non-flocculating, target alga is known as algal–algal bio-flocculation. Significant research has been conducted in this field so far. Self-aggregation has been observed for many algal species such as *Ankistrodesmus falcatus*, *C. vulgaris* JSC-7, *E. texensis*, *S. obliquus* AS-6-1, *Scenedesmus* sp. BH, and *T. Suecica* [39,46,59]. A self-aggregation-inducing EPS was purified from *S. obliquus* AS-6-1 and identified as a cell-wall-associated polysaccharide (consisting of glucose, mannose, galactose, rhamnose, and fructose) [39]. The auto-flocculating ability of *E. texensis* could be attributed to the glycoprotein-type EPS, based on scanning electron microscopy (SEM) and cell surface property analyses. EPS is also considered to play an important role in the harvesting of non-self-aggregating *Chlorella* spp. [46]. Similarly, the EPS-rich culture of the self-aggregating *Coelastrum* cf. *pseudomicroporum* resulted in a high harvesting efficiency of 96.8% for *Scenedesmus* sp. after treatment for 4 h [54]. It should also be noted that algal–algal bio-flocculation is species-specific. For instance, the self-flocculating *S. obliquus* AS-6-1 resulted in a harvesting efficiency of 80–85% for two freshwater algae, *C. vulgaris* and *S. obliquus*. However, when applied to the marine alga *N. oceanica* DUT01, the bio-flocculation efficiency was significantly reduced to 60% [39]. The algal–algal bio-flocculation process could be enhanced by optimization of environmental conditions such as pH; for example, maintaining the pH at 4.5 enabled a harvesting efficiency of over 90% for *C. zofingiensis* and *C. vulgaris*, using three self-aggregating algae, namely *C. nivale*, *C. ellipsoideum*, and *Scenedesmus* sp. [59].

The utilization of self-aggregating algae in the bio-flocculation process can have operational advantages over bacterial and fungal methods in terms of microbial contamination and post-purification processes. The biochemical and physiological mechanisms need to be clearly elucidated to improve the sustainability of the algal–algal bio-flocculation process. In addition, if a low-cost algae mass-cultivation process is realized, this bio-flocculation technique can be applied in order to commercialize the algal bio-refinement.

4. Chemical Flocculation

Chemical flocculation of algae occurs due to charge neutralization and electrostatic bridging between the suspended algal cells and the applied flocculant(s), resulting in floc formation and subsequent sedimentation (Figure 4). Multivalent inorganic chemicals, biopolymers, or inorganic–

organic hybrid polymers have been extensively used as algae-harvesting flocculants. Aluminum sulfate and ferric chloride are of the most popular inorganic flocculants for wastewater clarification and algal biomass recovery [60]. Chitosan, cationic starches, modified tannins, and polyacrylamides are examples of organic polymers that are widely used [32,61]. The harvesting efficiency of both organic and inorganic flocculants depends largely on their physicochemical properties such as solubility and electronegativity, as well as the operating conditions, such as dosage and algal solution characteristics (e.g., cell density, pH, and ionic strength) [62,63]. It should be noted that the sizes of the flocs formed through charge neutralization with conventional inorganic chemicals are generally small, requiring high dosage for algae flocculation. On the other hand, the bridging and sweeping reactions between polymeric flocculants and algal cells can lead to the formation of larger sized flocs, thereby promoting efficient biomass recovery at a relatively low dosage [24]. Table 3 briefly compares the different inorganic and organic chemical flocculants for algae harvesting.

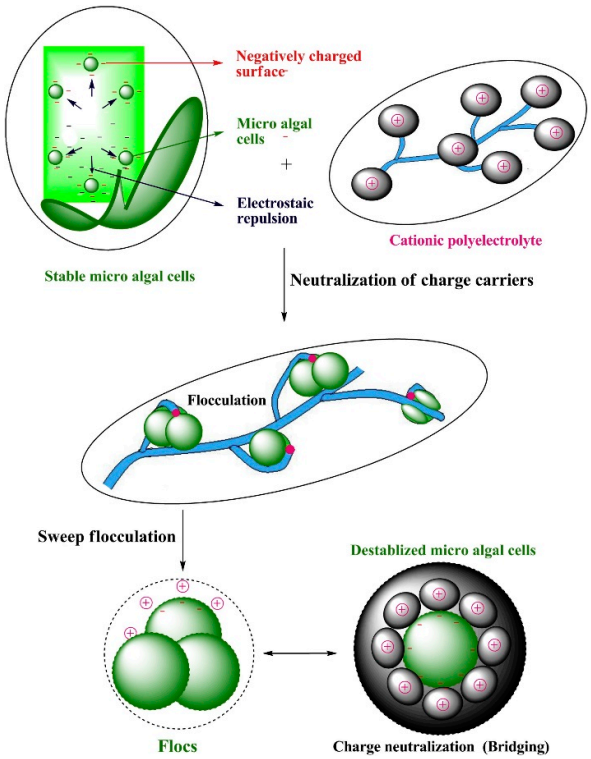

Figure 4. A schematic diagram of chemical flocculation harvesting of microalgae using a cationic polyelectrolyte. Reprinted from Reference [29], distributed under the terms of the Creative Commons Attribution License.

Table 3. Comparison of inorganic and organic chemical flocculants for algae harvesting.

	Flocculant (Dosage)	Alga (Cell Density or Volume)	Optimal Harvesting	Ref.
Inorganic flocculant	$Al_2(SO_4)_3$ (1.2 g/L)	*Tetraselmis* sp. KCTC12236BP (3 g/L)	86% @ 30 min	[64]
	$Al_2(SO_4)_3$ (152 mg/L)	*Chlorella* sp. (0.12 g/L)	100% @ 60 min	[65]
	$Al_2(SO_4)_3$ (180 mg/L)	*Scenedesmus* sp. (0.20 g/L)	90% @ 20 min	[66]
	$Al_2(SO_4)_3$ (20 mg/L)	*C. reinhardtii* (0.31 g/L)	90% @ 20 min	[66]
	$Al_2(SO_4)_3$ (438.1 μM)	*N. oculata* (1.7 g/L)	92% @ 320 min	[32]
	$Al_2(SO_4)_3$ (50 mg/L)	*S. limacinum* (0.93 g/L)	90% @ 20 min	[66]
	CaO (60 mg/L)	*C. vulgaris* (1.5 g/L)	85% @ 5min	[67]
	$CaCO_3$-rich eggshell (80 mg/L)	*C. vulgaris* (2.3 g/L)	99% @ 20 min	[68]
	$FeCl_3$ (0.4 g/L)	*N. oculata* (50 mL)	94% @ 180 min	[69]
	$FeCl_3$ (143 mg/L)	*Chlorella* sp. (0.12 g/L)	100% @ 40 min	[65]
	$FeCl_3$ (438.1 μM)	*N. oculata* (2.2 g/L)	78% @ 320 min	[32]
	$Fe_2(SO_4)_3$ (0.6 g/L)	*N. oculata* (50 mL)	87% @ 180 min	[69]
	$Fe_2(SO_4)_3$ (1.0 g/L)	*Chlorella* sp. KR-1 (1.52 g/L)	98% @ 30 min	[70]

	$Mg(OH)_2$ (1 mM)	*Chlorella* sp. (0.1 g/L)	90% @ 30 min	[71]
Organic flocculant	Cationic inulin (60 mg/L)	*Botryococcus* sp.	89% @ 15 min	[72]
	Cationic starches (0.01 g/L)	*S. dimorphus* (0.12 g/L)	95% @ 90 min	[73]
	Cationic starches (1.4:1, w/w)	*S. obliquus*	90% @ 60 min	[74]
	Cationic starches (119 mg/g cell)	*B. braunii* (0.62 g/L)	94% @ 20 min	[75]
	Cationic starches (50 mg/L)	*S. limacinum* (0.93 g/L)	90% @ 20 min	[66]
	Cationic starches (7.1 mg/L)	*C. vulgaris* (0.75 g/L)	90% @ 120 min	[76]
	Cationic starches (89 mg/g cell)	*C. pyrenoidosa* (1.02 g/L)	96% @ 20 min	[75]
	Chitosan (10 mg/g cell)	*C. sorokiniana*	99% @ 45 min	[77]
	Chitosan (120 mg/L))	*C. vulgaris* (1 g/L)	99% @ 3 min	[78]
	Chitosan (40 mg/L)	*Scenedesmus* sp. A1	82% @ 60 min	[12]
	Chitosan (30 mg/L)	*Chlorella* sp. (3×10^7 cells/mL)	97% @ 60 min	[61]
	Chitosan (30 mg/L) + sodium alginate (40 mg/L)	*S. obliquus*	86% @ 60 min	[40]
	Epichlorohydrin-n,n-diisopropylamine-dimethylamine (8 mg/L)	*Scenedesmus* sp. (100 mL)	>90% @ 30 min	[79]
	Modified tannin (10 mg/L)	*M. aeruginosa* (1×10^9 cells/L)	97% @ 120 min	[80]
	Modified tannin (210 mg/L)	*Scenedesmus* sp.	97% @ 40 min	[81]
	Modified tannin (10 mg/L)	*N. oculate* (400 mg/L)	98% @ 30 min	[82]
	Poly-L-lysine (70–150 kDa, 0.5 mg/L)	*C. ellipsoidea* (1 g/L)	98% @ 75 min	[83]

4.1. Inorganic Flocculant

Iron and aluminum salts form positively charged hydroxides in aqueous media, that destabilize negatively charged algal cells, inducing algae flocculation and subsequent precipitation [71]. Aluminum sulfate ($Al_2(SO_4)_3$), commercially known as "alum", is widely used in algae harvesting processes and wastewater treatments (Table 3). It was reported that 1.2 g/L alum enables a harvesting efficiency of 85.6% for marine alga *Tetraselmis* sp. of 3 g/L cell density [64]. Despite the high doses required for algae harvesting, the relatively low cost of alum makes it a potential choice for bulk biomass recovery in biofuel production [84]. However, it should be noted that this method is generally not recommended for algal biorefinery processes that utilize whole cells for multiple bio-products including biofuel and biochemicals, since excess aluminum is a well-known carcinogen and also known to cause Alzheimer's disease [19,64].

Ferric sulfate ($Fe_2(SO_4)_3$) and ferric chloride ($FeCl_3$) have been used for algae harvesting as well. Harvesting efficiencies of 93.8% and 87.3% were reported for marine alga *N. oculata* using 0.4 g/L $Fe_2(SO_4)_3$ and 0.6 g/L $FeCl_3$, respectively, after treatment for 180 min [69]. However, $Fe_2(SO_4)_3$ is not preferred for large-scale applications due to its strong and corrosive reactivity. [64]. This challenge could be resolved if $Fe_2(SO_4)_3$ is efficiently removed and recycled after being used for algae harvesting. Recently, Kim et al. [70] reported that $Fe_2(SO_4)_3$ could be reused three times for harvesting *Chlorella*, and very high flocculation efficiencies of ~98% biomass could be achieved each time; further, the biomass could be used directly in the post-lipid extraction process. $Fe_2(SO_4)_3$ from algae flocs was effectively released under low-pH conditions by adding sulfuric acid.

Positively charged calcium salts and magnesium hydroxide are able to neutralize negatively charged algal cells in addition to their pH-increase effect, thus inducing algae flocculation [35,85]. For instance, 60 mg/L CaO was reported to harvest a *C. vulgaris* biomass of 1.5 g/L cell density with an efficiency of 85% [67]. In addition, 80 mg/L of $CaCO_3$-rich eggshell (dissolved in 1% HCl solution) has been reported as an effective flocculant (99% efficiency) for harvesting *C. vulgaris* of 2.3 g/L after treatment for 20 min at pH 6 [68]. $Mg(OH)_2$ at 145 mg/L has been reported to induce 90% precipitation in *Chlorella* sp. at a pH of 10.7. Interestingly, $Mg(OH)_2$ can be effectively be recovered (up to 95%) from harvested biomass if the pH is properly controlled (reduced to 8) [71].

Various inorganic flocculants have been frequently used in algae biofuel applications, but the release of such chemicals into the environment is a critical environmental and health concern.

4.2. Organic Flocculant

Some organic polymers have been utilized for algae harvesting and wastewater treatment (Table 3). Generally, anionic polymers are not considered as ideal flocculants, due to poor bridging reactivity between them and negatively charged algal cells [76]. The cationic polymers are more suitable for inducing neutralization (or reduction) of the negative surface charges of algal cells, which results in the destabilization and flocculation of algal cells [75].

Natural biopolymers are generally regarded as renewable, less toxic, and environmentally friendly [86]. However, technical obstacles such as high production cost, narrow operating pH range, and low efficiency for some algal species (e.g., marine types) could limit commercial applications for algae harvesting [65]. Chitosan (deacetylated chitin) is a cationic polyelectrolytic polymer composed of linear poly-amino-saccharide chains. It is less toxic, biodegradable, and relatively inexpensive (e.g., when manufactured from abundant chitin waste such as crab and shrimp shells) [77,78]. Accordingly, this polymer has been widely applied in the food, biomedicine, and cosmetics industries, and has been used as an immobilizing resin for bacteria and enzymes for various biocatalytic reactions. In addition, chitosan has been used for algae harvesting as well [87]. The flocculation capability of chitosan is attributed to various interactions between chitosan monomers and algal cells, such as adsorption, bridging, sweeping, and charge neutralization [61,86]. Some commercial chitosan products such as ChitoVan ™ are available elsewhere [86].

The efficiency and dosage of chitosan are largely dependent on various culture parameters such as pH, temperature, cell density, ionic strength, and growth stage as well as algal species [12,77]. In particular, pH is regarded as the main factor affecting the polyelectrolytic property of the chitosan biopolymer. Under acidic conditions, chitosan tends to exist in a linear arrangement surrounded with positively charged deacetylated units, resulting in effective destabilization of algae, mainly by charge neutralization. Meanwhile, the structure of chitosan changes into a coiled arrangement under alkaline conditions and flocculation occurs primarily due to the bridging mechanism [61,77]. Accordingly, chitosan flocculation operation is generally recommended for acidic conditions [78]. When the pH of chitosan solution was reduced below 7, a high harvesting efficiency of 99% was obtained for *C. sorokiniana* at a very low dose of 10 mg chitosan/g biomass [76]. However, under alkaline conditions, relatively high chitosan dosages were required to achieve effective flocculation [12,40].

Cationic starch can be synthesized through chemical modifications (e.g., etherification) from an abundant natural polymer, starch. The formulation degree of functional cationic groups is responsible for flocculation capability [73,88]. Considering its relatively low cost and environmental safety, cationic starch has often been used for wastewater treatments and algae harvesting. The reported cationic starch concentrations for 1 g algal biomass recovery were 89 and 119 mg for *C. pyrenoidosa* and *Botryococcus braunii*, respectively [75]. Harvesting efficiencies of 90 and 85% could be obtained for *S. obliquus* using corn and potato-based cationic starches, respectively, at the same flocculant/algae ratio of 1.4:1 (*w*/*w*) [74]. Cationic starch can be used in combination with other biopolymers such as chitosan in order to increase harvesting efficiency [75]. Compared with chitosan flocculant, cationic starch has been reported to be cost-effective, particularly under alkaline conditions [22,76]. Unmodified (original) starch has also been tested for algae harvesting but showed a very low efficiency (15% in case of *S. dimorphus*) [73]. To reduce the dosages of other chemical flocculants, original starch has been suggested as an auxiliary flocculant. The addition of autoclaved rice starch (120 mg/L) has been reported to reduce the amount (up to 54%) of aluminum salts required for *Chlorella* harvesting [89]. In addition, the residual starch components after harvesting can be used as organic nutrients for subsequent algae cultivations.

Alginate is an anionic polysaccharide that is extracted mainly from brown seaweed [90]. Similar to unmodified starch, this material can be used also as a second flocculant to improve flocculation efficiency and reduce the dosage of the main chemical flocculant. Matter et al. [40] observed a low harvesting efficiency of less than 70% for *S. obliquus* when 10 mg/L chitosan was used alone at pH 6. Interestingly, after adding 10 mg/L alginate to the chitosan-treated algal solution (called the dual flocculation process), the flocculation efficiency could be significantly improved to over 85%, even under slightly alkaline conditions. This improvement might be attributable to the formation of efficient polyelectrolytic complexes in the presence of both chitosan and alginate.

Soluble poly-L-lysine (PLL) is a cationic, linear biopolymer charged with hydrophilic amine moieties, and is secreted by various *Streptomycetaceae* bacteria as well as few filamentous fungi [91,92]. This biopolymer is reportedly highly efficient for algae harvesting and can be used as an antimicrobial agent for reducing biomass contamination [93]. Application dosage and its efficiency are mainly dependent on its molecular weight (MW). PLLs with MWs of 4–15 and 70–150 kDa have been applied to harvest *C. ellipsoidea*. A dosage of 5 mg/L of low MW PLL resulted in a harvesting efficiency of 89%, but only 1 mg of high MW PLL was required to achieve a very high harvesting efficiency of 98.9%. The investigators claimed that the quality and shelf-life of the PLL-treated algal biomass might have been enhanced by the prevention of external microbial contamination [83].

Tannins (plant phenols) are less-toxic, biodegradable, anionic branched polyelectrolytes, which can be extracted from many plants such as *Acacia mearnsii*. To use tannin as a flocculant for negatively charged particles such as algal cells, cationic moieties should be added to its original structure via chemical modifications such as the Mannich reaction [80]. A high harvesting efficiency of 99% was recorded for the marine alga *N. oculata* at pH 6 by using only 10 mg/L of Tanfloc, a commercial product obtained by cationization of natural tannin, under bench-scale experimental conditions [82]. Remarkably, its good algae harvesting performance could be maintained in a pilot-scale process. However, efficacy of Tanfloc efficacy is species-specific. A higher dosage of 210 mg/L was required to achieve a 96.7% harvesting efficiency for freshwater alga *Scenedesmus* sp. at a pH of 7.8 [81]. Inulin (a plant-based polysaccharide) has also been tried as a flocculant after the cationization reaction, using etherifying reagents such as 3-chloro-2-hydroxypropyl trimethyl ammonium chloride. This flocculant showed a harvesting efficiency of 88.6% for *Botryococcus* sp. within 15 min, at a dosage of 60 mg/L. Bridging between particles was posited as the main flocculation mechanism here [72].

Synthetic polyelectrolytic (cationic and anionic) polymers in linear or branched forms including acrylamide, acrylic acid, diallyl dimethyl ammonium chloride, and epichlorohydrin diisopropylamine dimethylamine have been reported for harvesting of both freshwater and marine algae [24,79]. For example, high harvesting efficiency of over 90% was observed for *Scenedesmus* sp. using only 8 mg/L polyamine polymer. In comparison, relatively high amounts of chitosan (80 mg/L) and alum (250 mg/L) were required to reach similar harvesting efficiencies [79]. However, it should be noted that synthetic polymers cannot be regenerated and have adverse effects on harvested biomass and aquatic environments due to their petroleum origins [94].

5. Particle-Based Flocculation

Particle-based flocculation can potentially circumvent some drawbacks of conventional chemical flocculation such as bio-toxicity and difficulties related to chemical recovery. For these purposes, particle-based flocculants should be designed to be more efficient, recoverable, and/or have multi-functionalities other than algae recovery, such as cell disruption and lipid extraction [9]. Therefore, numerous research efforts have devoted effort towards the development of new and optimal nano/micro-particle-based flocculants. This section summarizes the recent progress in algae harvesting using the nano/micro-particle-based flocculants, namely aminoclay (AC)-based particles, magnetic particles (Table 4), and more advanced multi-functional or recyclable particles.

5.1. Aminoclay-Based Nanoparticle

Various ACs manufactured with cationic metals (Al^{3+}, Ca^{2+}, Mg^{2+}, etc.) and organo-functional materials such as 3-aminopropyltriethoxysilane (APTES) have been applied to several biological and environmental applications. Such biological applications include catalysis, bio-inorganic composites, DNA transformation, gene expression, antimicrobial performance, cytotoxicity, and bio-imaging. Environmental applications of ACs include algal bio-refinement, colorimetric sensing, control of algal blooms, and water/air purification [95]. Some ACs (e.g., magnesium aminoclay (Mg-AC)) cannot prevent light penetration due to their transparency and solubility in aqueous solutions [96]. They also have high CO_2 availability at primary amine groups via CO_2 conversion into carbonate ions [97].

The ability of ACs to cause flocculation of negatively charged algal cells is attributed to the abundance of amine groups on their surfaces. In one study, Mg-AC was reported to flocculate the

freshwater alga *C. vulgaris* within 5 min at a dosage of 1.0 g/L, with a harvesting efficiency of more than 90% in a wide range of pH from 5 to 12. The same study also showed that the harvesting efficiency using ACs largely depends on the algal species, biomass concentration, and dosage. At low biomass concentrations, flocculation occurs mainly due to charge neutralization and electrostatic bridging between algal cells and AC particles. On the other hand, the sweep flocculation mechanism plays an important role in algae harvesting at high biomass concentrations [98].

The harvesting efficiency obtained by using ACs is reported to be almost proportional to the oxidation number of the cations. Lee et al. [99] studied the harvesting efficiencies of different ACs and compared them with alum for the flocculation of *Chlorella* sp. KR-1. They found that aluminum aminoclay (Al-AC) and Mg-AC had superior harvesting efficiencies even at low concentrations. Almost 100% of the algal cells (1.7 g cell/L) could be harvested within 30 min using 0.6 g/L Al-AC and Mg-AC. The order of harvesting efficiency was Al-AC > Mg-AC > Ca-AC (calcium aminoclay) > alum. ACs can also be applied to marine media but with lower efficiency. For example, the marine alga *N. oculata* could be harvested within 120 min using 0.25 g/L Fe-AC (iron aminoclay) and Mg-AC, while only 5 min were required to harvest freshwater alga *C. vulgaris* [98].

Table 4. Comparison of particle-based flocculants for algae harvesting.

Kind	Flocculant	Dosage	Alga (Cell Density)	Optimal Harvesting	Ref.
Aminoclay-based nanoparticle	Al-AC	0.6 g/L	*Chlorella* sp. KR-1 (1.7 g/L)	100% @ 30 min	[99]
	AC-conjugated TiO_2	3.0 g/L	*Chlorella* sp. KR-1 (1.5 g/L)	~85% @ 10 min	[100]
	AC-induced humic acid	5.0 g/L	*Chlorella* sp. (1.3 g/L)	~100% @ 30 min	[101]
	AC-templated nZVI	19.1 g/L	*Chlorella* sp. KR-1 (1.5 g/L)	~100% @ 3 min	[102]
	APTES-coated $BaFe_{12}O_{19}$	2.3 g/g cell	*Chlorella* sp. KR-1	99% @ 3 min	[103]
	MgAC-Fe_3O_4 hybrid composites	4.7 g/L	*Chlorella* sp. KR-1 (1.75 g/L)	99% @ 10 min	[104]
	MgAC-Fe_3O_4 hybrid composites	4.3 g/L	*S. obliquus* (2.0 g/L)	99% @ 10 min	[104]
	Mg-APTES	0.6 g/L	*Chlorella* sp. KR-1 (1.7 g/L)	100% @ 30 min	[99]
	Mg-APTES	1.0 g/L	*C. vulgaris* (1.0 g/L)	97% @ 125 min	[98]
	Mg-AC and Ce-AC	0.2 g/L	Cyanobacteria	~100% @ 60 min	[105]
Magnetic particle	Fe_3O_4 nanoparticle	55.9 mg cell/mg particles	*B. braunii*	98% @ 1 min	[106]
	Fe_3O_4 nanoparticle	5.8 mg cell/mg particles	*C. ellipsoidea*	98% @ 1 min	[106]
	Fe_3O_4 nanoparticle	0.12 g/L	*N. maritima*	95% @ 4 min	[107]
	Fe_3O_4 magnetic particle	10 g/g cell	*Chlorella* sp. KR-1	99% @ 1 min	[108]
	Fe_3O_4-embedded carbon microparticle	~25 g/L	*Chlorella* sp. KR-1 (~2 g/L)	99% @ 1 min	[109]
	Fe_3O_4–PEI nanocomposite	0.02 g/L	*C. ellipsoidea* (0.75 g/L)	97% @ 2 min	[110]
	PEI-coated Fe_3O_4	0.2 g/L	*S. dimorphus* (1.8 g/L)	82.7% @ 3 min	[111]
	Fe_3O_4-carbon-microparticle	10 g/L	*Chlorella* sp. KR-1 (2.0 g/L)	99% @ 1 min	[109]
	Chitosan–Fe_3O_4 composite	1.4 g/L	*Chlorella* sp. KR-1 (1.0 g/L)	~99% @ 5 min	[112]
	Chitosan-coated Fe_3O_4-TiO_2	0.07 g/g cell	*C. minutissima* (3.0 g/L)	98% @ 2 min	[113]

The AC-based flocculation processes have been improved by forming composites with other materials. Mixing Mg-AC and cerium aminoclay (Ce-AC) resulted in 100% efficiency for cyanobacteria harvesting after 1 h with only 10% of the required concentration for the individual AC [105]. The introduction of nZVI (nanoscale zero-valent iron) nanoparticles as the core of the AC-nZVI composites can create synergy in terms of time and efficiency. The AC-nZVI composite can remarkably reduce the harvesting time (3 min) for *Chlorella* sp. compared with that (30 min) using original Mg-AC. The former's harvesting efficiency (around 100%) was also higher than that of the latter (70%) [102]. Humic acid and nZVI composites with AC also showed fast floc formation and very high harvesting efficiency (~100%) relative to only AC [101]. However, a bottleneck for using ACs for algae harvesting applications is its expensive manufacturing, which can limit their utility in scaled-up processes [99]. A second bottleneck is the uncontrollable size and water dispersibility of synthesized ACs [99,114], which could be overcome by material engineering efforts such as a template-assisted AC synthesis process [114].

5.2. Magnetic Particle

Magnetic nanoparticles (MNPs) have played innovative roles in different biomedical, biotechnological, and environmental applications. Solid–liquid separation using MNPs is one of the most promising strategies in wastewater treatment [115]. Regarding algae separation, MNPs were first applied to remove harmful algae in lakes [116]. Later, as algal biomass became a more popular feedstock for various applications, including biofuels production, they were used for the recovery of algae from mass-culture systems [117]. Magnetic-particle-based flocculation has a powerful advantage in terms of separation time as MNP-attached flocs can be easily manipulated by an external magnetic field (Figure 5). Since 2010, varieties of freshwater and marine algal species have been successfully harvested via flocculation processes with magnetic particles for biofuel production (see Table 4). Despite the high manufacturing costs of MNPs, the reuse of culture supernatant (after magnetic separation) as the culture medium and the recycling of MNPs was attempted to decrease the cost of biomass and biofuel production [107,118].

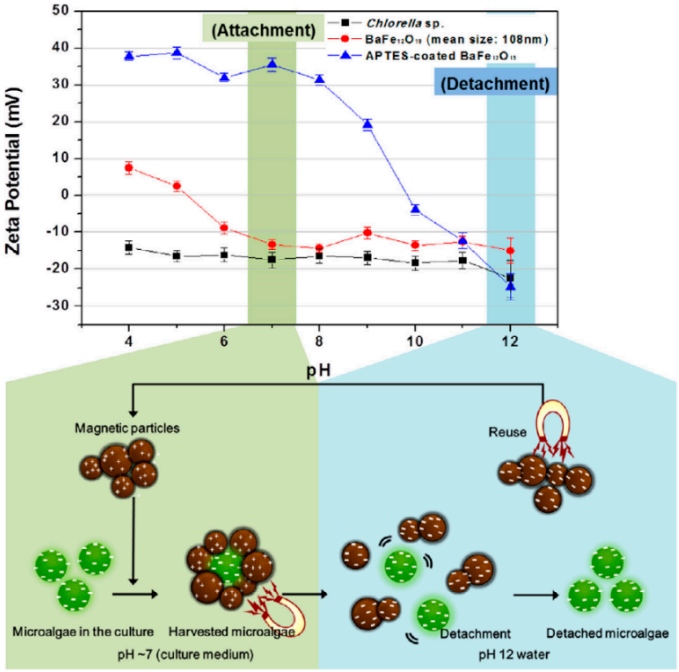

Figure 5. Schematic illustration of magnetic algae harvesting and sequential detaching of $BaFe_{12}O_{19}$ particles. Reprinted from Reference [103] with the permission of Elsevier.

The magnetic particles most commonly utilized for algae separation are iron oxide (Fe_3O_4) and yttrium iron oxide ($Y_3Fe_5O_{12}$) nanoparticles. It was reported that 120 mg/L of Fe_3O_4 nanoparticles could be applied to harvest marine alga *N. maritima* within 4 min with 95% efficiency [107]. Interestingly, $Y_3Fe_5O_{12}$ is superior in terms of reuse after detachment from algal aggregates via pH control. However, it should be noted that, at high pH values, Fe_3O_4 was more easily separated from the flocs than $Y_3Fe_5O_{12}$ [119].

Harvesting efficiency can be significantly enhanced using "hybrid magnetic nanoparticles", which are magnetic particles coated with positively charged materials. These can be applied easily and quickly, to harvest negatively charged algal cells in relatively low flocculant doses [110,120]. Lee et al. [112] reported the successful use of a chitosan–Fe_3O_4 nanoparticle composite to harvest *Chlorella* without the need for pH adjustment. They obtained better than 99% harvesting efficiency using a 1.4 g/L flocculant dosage within 2–5 min with the aid of a permanent magnet. This harvesting method strongly facilitates the recycling of the spent medium for further culture cycles after harvesting. Similarly, Fe_3O_4 coated with polyethylenimine (PEI-Fe_3O_4) nanoparticles was successfully utilized for harvesting algae. In a harvesting study for *C. ellipsoidea*, the required nanoparticle concentration was

only 20 mg/L, which was sufficient to harvest around 97% of the algal biomass within 2 min [110]. Another study attributed the superiority of PEI-coated Fe_3O_4 over non-coated Fe_3O_4 in significantly increasing the zeta potential from −7.9 to +39 mV, respectively. They also found that the algal biomass had been successfully detached from the PEI-Fe_3O_4–algae slurry by only sonication for 5 min (500 W), without the addition of HCl or NaOH. In addition, the recovered PEI-Fe_3O_4 nanoparticles (after detachment) could maintain their harvesting efficiency. In the same study, UV irradiation was used to enhance the recovery and harvesting performance of the magnetic flocculant, which was attributed to changes in the nature of the surface of the algal cells [111].

In the magnetic-flocculation-based algae harvesting process, flocculant recyclability is one of the most important challenges which needs to be resolved in order to reduce operating costs [103,111]. Acid treatment, sonication, UV irradiation, and hydrophobicity shifting are usually used to facilitate separation of flocculants from harvested algal biomass [121]. Sonication has been proven to be an efficient treatment method, as mentioned above [111]. Acid treatment is also considered to be a successful flocculant-floc detaching method. An earlier study [106] demonstrated the ability of Fe_3O_4 nanoparticles to harvest both *B. braunii* and *C. ellipsoidea* with a harvesting efficiency above 98% within 1 min. The harvested cells could be detached from the algae–Fe_3O_4 flocs using HCl treatment, and the magnetic Fe_3O_4 nanoparticles could be reused 5 times with almost the same harvesting efficiency. Hydrophobicity shifting was reported as a tool for flocculant-biomass detachment using the photocatalytic reaction. To shift the particle hydrophobicity, Fe_3O_4 nanoparticles were coated with steric acid (SA) as a target molecule that could be altered by photocatalytic reaction. The changes in hydrophobicity for the SA-Fe_3O_4-ZnO composite gradually increased the number of algal cells migrating into the bulk suspension from the flocs, as a function of UV irradiation [121].

Development of low-density flocculant of sizes comparable to algal cells is ideal for easy detachment. Seo et al. [103] reported successful detachment of $BaFe_{12}O_{19}$ from algae–$BaFe_{12}O_{19}$ flocs based on pH control and the re-usability of the magnetic flocculant. According to their results, the detachment efficiency of *Chlorella* sp. KR-1 was sharply increased from 13% to 85% after increasing the flocculant size from about 100 nm to 1.2 μm. The same algal species could be harvested and detached by commercial Fe_3O_4 microparticles (Sigma-Aldrich, St. Louis, MO, USA) through only pH control [108], with pH 7 being used for harvesting and pH 12 for detaching. In addition, the supernatant of the spent culture medium was suitable for a second cultivation cycle after supplementation with 50% fresh medium.

Despite the short time required for algae harvesting using magnetic particles, the manufacturing costs could be the main limiting factor for its scalability to biofuel production. Therefore, more studies are needed to reduce these costs through the utilization of low-cost materials as well as to enable the reusability of the flocculants after biomass recovery.

5.3. Multi-Functional Particle

Production of biodiesel from algal biomass requires several subsequent post-harvesting downstream processes such as cell disruption, lipid extraction, and conversion to fatty acid methyl ester (FAME) [5]. Therefore, particle-based flocculants have recently been developed for additional functions required for those subsequent steps. Their integrated use can be categorized into three types: harvesting/cell disruption, harvesting/lipid extraction, and harvesting/lipid conversion (Figure 6).

Figure 6. A schematic illustration of utilization of multi-functional nanoparticles in algal biorefinery process development. Reprinted from Reference [122] with permission from Springer International Publishing.

To couple harvesting and disruption of algal cells for biofuel production, it is essential to use flocculant composites that have dual cationic and photocatalytic properties. For instance, chitosan-coated Fe_3O_4-TiO_2 composites were able to electrostatically attract algal cells causing floc formation [113]. In addition, this composite showed sufficient photocatalytic activity (due to the TiO_2 shell) in disrupting the cell wall of harvested algae after exposure to UV irradiation or visible light. The dosage of 0.07 g particle/g biomass from chitosan-coated Fe_3O_4-TiO_2 resulted in a harvesting efficiency of over 98% for *C. minutissima* within 2 min (due to the magnetic property of Fe_3O_4). This dosage was significantly lower than that reported for chitosan only (0.11 g particle/g biomass) under the conditions of that study [113]. High percentage of lipid recovery at 96% and 97% could be obtained after exposure to UV for 2 h and visible light for 3 h, respectively, relative to lipid recovery in 10 min of ultrasonic treatment. In another study, Lee et al. [100] used Mg-AC conjugated with TiO_2 for simultaneous harvesting and wet-disruption of algae. They reported that Mg-AC played a role in algae flocculation while TiO_2 facilitated the direct disruption of cells under UV radiation. Around 85% of the cells could be harvested within 10 min of injection of the Mg-AC-TiO_2 composite into the *Chlorella* culture (1.5 g/L). Subsequently, the harvested biomass was irradiated by 365 nm UV light for 3 h, leading to 95% cell disruption.

In terms of harvesting and lipid extraction, the multi-functional properties of Fe_3O_4-embedded carbon microparticles could be utilized for algae flocculation and hydrophobic-interaction-based lipid extraction. These composites have cationic and lipophilic properties derived from carbonized polyvinylpyrrolidone as well as magnetic properties due to the embedded Fe_3O_4 nanoparticles. A dosage of 2 g/L was able to harvest 99% of *Chlorella* within 1 min via electrostatic interaction. Moreover, with a high dosage (10 g/L) of Fe_3O_4-embedded carbon microparticles, the extracted lipid droplets could be recovered after 10 min of ultrasonic treatment [109]. Integration of algae flocculation harvesting and conversion of lipid into biodiesel have been effectively performed using Fe_3O_4@SiO_2 nanoparticles functionalized with a strong base, such as triazabicyclodecene. This nanocomposite has been reported to effectively flocculate *C. vulgaris* within 1 min (by the adsorption attraction between algal cells and the nanoparticles) and stimulate the conversion of algal lipid into FAME with a high conversion efficiency of 97.1%. This was an outstanding yield compared to those obtained using other catalysts such as H_2SO_4, $AlCl_3$, Amberlyst-15, and $CuCl_2$ [123]. Despite the high manufacturing costs for magnetic particle-based flocculants, their multi-functional applications, including cell disruption and lipid extraction/conversion as well as flocculant recovery, might render them suitable for large-scale applications.

6. Electrochemical Flocculation

Electrochemical algae harvesting is generally carried out by passing a direct electrical current through electrodes into a culture broth wherein algal cells act as negatively charged colloids (Figure 7). There are two types of electrodes, "sacrificial electrodes", whose metal ions are released into the aquatic environment, and "non-sacrificial electrodes" with non-reactive anodes and cathodes (Table 5). The electrical current in aqueous solution can cause a water-electrolysis reaction through either the sacrificial or non-sacrificial electrodes, which would release hydrogen and oxygen gases from the cathode and anode electrodes, respectively [124,125]. In this review, the electrochemical flocculation (ECF) process is discussed for the following three aspects: the sacrificial electrode, the non-sacrificial electrode, and electro-flotation.

6.1. Sacrificial Electrode

The use of a sacrificial metal anode (usually iron and aluminum, or possibly magnesium, copper, zinc or brass) in an electrochemical system causes the release of positively charged metal cations. These dissolved metal cations are able to electrostatically attract negatively charged algal cells, resulting in flocculation [84,126]. It should be noted that ECF processes do not produce counter ions such as chloride and sulfate (generally observed in the conventional chemical flocculation processes), since metal salts are not added here. In ECF, the water electrolysis reaction at the cathode can generate H_2 microbubbles that can attach to the algal cells and further promote electro-flotation (see Section 6.3. for details). The main disadvantages of the ECF process are power consumption, the short lifetime of electrodes due to their dissolution, and contamination of harvested biomass with metal oxides [127]. The performance of the ECF-based algae harvesting process is affected by various operating parameters such as electrode material, inter-electrode distance, current density, processing time, cultural pH, salt content, and temperature.

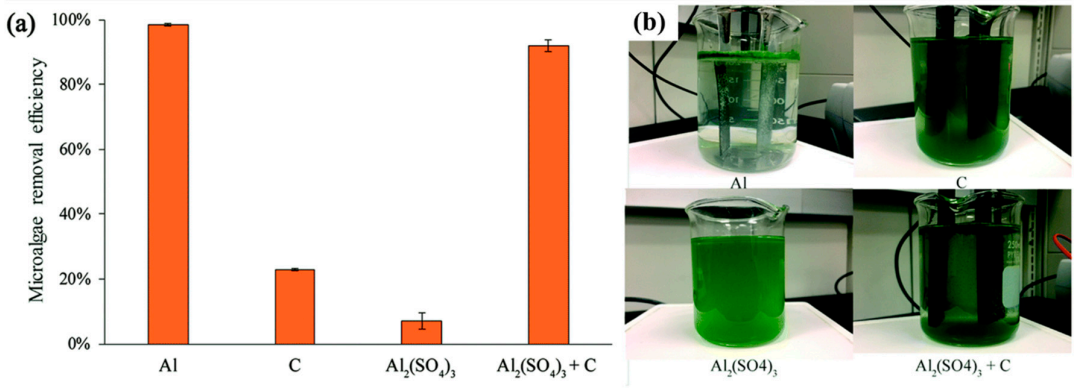

Figure 7. Electrochemical flocculation harvesting of microalgae using aluminum electrodes (Al), graphite electrodes (C), aluminum sulfate ($Al_2(SO_4)_3$), and graphite electrodes with aluminum sulfate ($Al_2(SO4)_3$ + C): (**a**) microalgae removal efficiency; and (**b**) photographs of the harvesting processes. Reprinted from Reference [128], distributed under the terms of the Creative Commons Attribution License.

Table 5. Comparison of sacrificial and non-sacrificial electrodes for algae harvesting.

Electrode		Alga (Cell Density)	Optimal Harvesting (Energy Requirement)	Ref.
Sacrificial	Al	*C. pyrenoidosa*	95.8% @ 1 min (0.3 kWh/kg cell)	[126]
	Al	*C. vulgaris*	98% @ 4 min (0.3 kWh/kg cell)	[129]
	Al	*M. aeruginosa* (1.3×10^9 cells/mL)	100% @ 45 min (0.4 kWh/m^3)	[130]
	Al	*Nannochloropsis* sp. (2.5 g/L)	97% @ 10 min (0.06 kWh/kg cell)	[131]
	Al	*Scenedesmus* sp.	~98.5% @ 20 min (2.3 kWh/kg cell)	[128]
	Al	*P. tricornutum*	80% @ 30 min (0.2 kWh/kg cell)	[132]
	Fe	*C. vulgaris*	80% @ 30 min (2.1 kWh/kg cell)	[132]
	Fe	*Chlorococcum* sp.	96% @ 15 min (9.2 kWh/kg cell)	[133]

	Fe	Green algae mixture (*Scenedesmus*, *Kirchneriella*, and *Microcystis*)	~95.6% @ 24 h (4.4 kWh/kg cell)	[134]
	Fe	*Tetraselmis* sp.	94% @ 15 min (4.4 kWh/kg cell)	[133]
Non-sacrificial	C	*C. sorokiniana*	~95% @ 15 min (1.6 kWh/kg cell)	[124]
	C	*C. pyrenoidosa*	79.2% @ 1 min (0.3 kWh/kg cell)	[126]

Among various metals, aluminum is most commonly used as the sacrificial electrode because of its promising algae harvesting efficiency. For instance, ECF using aluminum electrodes for the harvesting of *Nannochloropsis* sp. resulted in 97% harvesting efficiency at a current density of 8.3 mA/cm^2 when treated for 10 min. It should be noted that the properties related to biofuel quality (e.g., total lipid content and fatty acid composition) of the harvested algal biomass after ECF treatment did not show any considerable changes [131]. In ECF, the processing time required for harvesting could be reduced by increasing the current density. This was clearly observed when the current density was increased from 2.2 to 6.7 mA/cm^2 during ECF processing of *C. vulgaris* with considerably decrease in processing time from 7 to 4 min. The energy consumption under the reduced current density was estimated to be 2.94 × 10^{-4} kWh/g biomass [129]. Fayad et al. [127] compared aluminum and iron electrodes in batch mode harvesting of *C. vulgaris*. Further, they investigated several operating parameters including current density, processing time, stirring speed, initial pH, and inter-electrode distance. Optimal conditions for electrochemical harvesting of *C. vulgaris* were reported to be aluminum electrodes, 60 min treatment with 2.9 mA/cm^2 of current density, pH 4, stirring speed of 250 rpm, and an inter-electrode distance of 1 cm. Compared with the cases for aluminum, the algae harvesting performance using iron electrodes was generally lower due to the low current efficiency [129,135]. The flocculation capability of iron hydroxide formed from the iron electrode was also lower than that of the aluminum hydroxide formed from an aluminum electrode [136].

The salt content in the medium affects the electrical conductivity of the algal culture broth. Accordingly, significant difference in electrochemical harvesting efficiency between fresh and marine algal species has been reported. The minimum power required for effective harvesting of the freshwater alga *C. vulgaris* was 2.1 kWh/kg at a current density of 1.5 mA/cm^2. While, the power could be significantly reduced to 0.2 kWh/kg at 0.6 mA/cm^2 under salty conditions in case of the marine alga *Phaeodactylum tricornutum* [132]. In the same context, the addition of 1.5 g/L NaCl to freshwater alga *C. vulgaris* medium has been recommended to improve the harvesting performance of the ECF process using aluminum electrodes [127]. At the same time, the additional NaCl cost should be properly considered for large-scale applications.

Temperature is one of the factors that can significantly affect ECF harvesting efficiency. Electrical conductivity increased with increasing temperature, which allowed for stronger current flow through the system thereby enhancing biomass recovery. For instance, the ECF harvesting efficiency of the two marine algae *Chlorococcum* sp. and *Tetraselmis* sp. were as low as 5% and 68%, respectively, at a temperature of 5 °C. However, when the operating temperature was increased to 60 °C, the harvesting efficiency of the former and latter species were significantly increased to 96% and 94%, respectively, as indicative of power requirements of 9.16 and 4.44 kWh/kg after treatment for 15 min [133]

Current density is another important factor that affects both harvesting efficiency and energy consumption [58]. Increasing the current density usually shortens processing time but increases the energy consumption for electrochemical flocculation [130]. Therefore, a proper balance should be maintained between time and energy cost [58]. Medium acidity should also be properly considered for operation of the ECF process. For example, at an acidic and neutral pH range of 4 to 7, negatively charged algal cells efficiently react with aluminum oxides, whereas under alkaline conditions over pH 8, formation of algal flocs might be inhibited by undesirable monomeric hydroxo-aluminum ions [58]. It should be noted that the iron electrode-based ECF process can induce formation of a brown-colored algae–iron slurry [58]. Al^{3+} ions from the aluminum electrode can also contaminate the algal biomass and supernatant effluent, which might seriously limit its large-scale application [64,134]. Therefore, there is a need to develop effective strategies to reduce environmental and health toxicity

as well as alternative efficient, environmentally-friendly materials to replace the current metal electrodes.

6.2. Non-Sacrificial Electrode

When non-sacrificial electrodes such as carbon are operated under direct current conditions, negatively charged algal cells move towards the anode electrodes. Once the algal cells reach the oppositely charged electrode, they lose their negative charges through neutralization, resulting in flocculation [58]. Algae harvesting efficiency largely depends on operating conditions such as current conductivity and salt content in the culture broth. For instance, using carbon electrodes significantly increased the harvesting efficiency of *C. sorokiniana* from 66.0% to 94.5% with increasing NaCl concentration from 2 to 6 g/L under 1.6 kWh/kg biomass electric current conditions [124]. A chitosan-assisted ECF process incorporating carbon-graphene electrodes was developed for harvesting of *Chlorella* sp., in a remarkable pilot-scale (1000 L) study by Zhou et al. [137]. In this study, various operating factors such as chitosan dosage, pH value, cell density, stirring rate, mixing time, voltage, current intensity, and inter-electrode distance were extensively investigated. One kilogram of algal biomass could be harvested with a high harvesting efficiency of 90% under optimized conditions (specifically, 0.2 L sulfuric acid, 23.7 g chitosan, and 0.42 kWh of electricity). If both high-efficient biomass recovery and low energy consumption are guaranteed, non-sacrificial carbon electrodes can be considered to be one of the more attractive solutions for large-scale electrochemical harvesting processes [124].

6.3. Electro-Flotation

If the ECF-based harvesting process is properly operated either with sacrificial or non-sacrificial electrodes, well-distributed and dense gas bubbles of micro-scale size can be generated from water electrolysis in the aqueous solution. The bubbles can attach efficiently to the suspended algal cells, thus inducing the flotation of cells to the liquid surface [138–141]. It should be noted that, in the cases of conventional air-pressure flotation processes, the gas bubbles generated are of comparatively large size and lack good distribution [137]. Rahmani et al. [126] developed an integrated photovoltaic electro-flotation system utilizing various electrode materials such as aluminum, iron, zinc, copper, and carbon for harvesting of *C. pyrenoidosa*. They reported that the aluminum electrode exhibited highest harvesting efficiency of over 95%, compared with the other types of electrode (efficiency order: Zn > 93%, Cu > 83%, C = 79%, Fe > 70%). The electrical power for 1 kg algal biomass harvesting were estimated to be 0.28 and 0.34 kWh for aluminum and carbon electrodes, respectively.

Electrochemical algae harvesting processes are applicable to almost all types of algal species, and also offer technical advantages such as high efficiency, continuous operation, and relatively short-processing time. In addition, effective recycling of spent media (e.g., from the non-reactive electrode case) can reduce overall algal biomass production cost [142]. However, the complex electrochemical system, power consumption, and/or biomass contamination (e.g., by sacrificial electrode use) must be carefully considered for practical, large-scale algae biofuel applications [143].

7. Conclusions and Future Perspectives

The importance of microalgae research is increasing in parallel with increasing demands for food, animal feeds, pharmaceuticals, and biofuels. However, moving from lab-scale to commercial-scale applications still requires extensive developments for reliable, cheap, and eco-friendly algae cultivation and harvesting processes. Table 6 summarizes the mechanism and advantages/disadvantages of the five different flocculation methods mentioned above. The specific flocculation process should be carefully selected and optimized comprehensively in consideration of various key factors such as efficiency, environmental impact, operating cost, value-added utilization of whole biomass, characteristics of algal species, and culture conditions.

Table 6. Comparison of five flocculation techniques for algae harvesting.

Technique	Mechanism	Advantages	Disadvantages
Auto-flocculation	Spontaneous aggregation and sedimentation under stress conditions	•Cheap •Eco-friendly •No chemical flocculant is required	•Limited to certain algal species •Time-consuming •Low efficiency
Bio-flocculation	Co-pelletization of target algal species using bio-flocculants (fungi, bacteria, yeast, algae and their extracellular polymers)	•Renewable •No chemical flocculant is required	•Species-specific •Biomass contamination •Environmental concern due to flocculant release
Chemical flocculation	Charge neutralization, bridging, and sweeping of algal cells with charged chemicals	•Fast •Effective •Scalable	•Biomass contamination •Environmental concern due to flocculant release •Efficiency is sensitive to culture conditions
Particle-based flocculation	Charge neutralization and electrostatic bridging with functional nano/macro-particles	•Rapid (e.g., magnetic separation) •Multi-functionalities for post-processing •Reusability of flocculant	•Expensive manufacturing •Limited to laboratory-scale studies
Electrochemical flocculation	Floc formation using metal ions and charge neutralization by passing direct electrical current through electrodes	•Fast •Suitable for almost all types of algae •No chemical is required	•Fouling and short life-time of electrodes •Biomass contamination by metal ions •Electrical energy demand

Along with process optimizations such as scale-up, detailed understanding of the genetic, biochemical, and physiological characteristics of auto-flocculating algae is essential for development of fast and efficient auto-flocculation harvesting processes. Genetic and metabolic engineering of algal cells can be considered to improve their auto-flocculation capability without adverse effects on growth or target metabolites (e.g., lipid and carbohydrate). The expression and temporal regulation of specific cell wall protein(s) causing cell aggregation in response to measurable environmental triggers (e.g., pH) and/or after reaching the desired growth stage, could also be important targets for further investigation.

Utilization of other microorganisms for algae harvesting (called bio-flocculation) can be considered to be one of the most promising and potentially sustainable approaches. Use of fungi, yeasts, bacteria, and/or self-flocculating algal species to harvest target non-flocculating algal species has been reported to have synergistic effects on overall biomass and lipid productivity. More studies are needed to elucidate the mechanism of microbial floc (or pellet) formation along with continued screening for efficient bio-flocculants from natural environments. The biological flocculation process should be further optimized to both maximize harvesting efficiency and reduce operation cost. Potential contamination of the bio-flocculant on the algal product(s) and the aquatic environment should be carefully considered for large-scale applications.

Albeit competitive in terms of cost and performance, the use of conventional inorganic chemical flocculants has not yet been recommended for food, feed or pharmaceutical applications. However, it might be considered for large scale applications such as biofuel and wastewater treatment if the adverse impacts of the applied chemical flocculants on the final products and water resources are avoided or minimized. The discovery of new organic flocculants (e.g., from natural sources) with low cost, high efficiency, and less toxicity should also be a focus of future research.

Recently, algae harvesting using various functional nano/micro particles based on AC and magnetic materials has been intensively investigated. However, more studies are needed to reduce the high manufacturing costs of particle-based flocculants. In addition, to facilitate effective recycling, the design and engineering of flocculants with high-efficiency and multi-functionality for post-harvesting processes, such as lipid extraction and fuel conversion, can be the basis for overcoming the high cost of artificial particle use.

For practical application of electrochemical flocculation processes, extensive studies should be carried out to develop optimal electrodes with efficient, non-fouling, and less-toxic properties. Scale-up and process optimization for reducing both equipment and electric costs should also be investigated further. Finally, synergistic combinations of different flocculation techniques could provide a powerful means of maximizing biomass recovery for large-scale algae-based process while minimizing operating costs and environmental impacts.

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Abbreviations

AC	Aminoclay
Al-AC	Aluminum aminoclay
APTES	3-Aminopropyltriethoxysilane
Ca-AC	Calcium aminoclay
Ce-AC	Cerium aminoclay
ECF	Electrochemical flocculation
EOM	Extracellular organic matter
EPS	Extracellular polymeric substances
FAME	Fatty acid methyl ester
Fe-AC	Iron aminoclay
Mg-AC	Magnesium aminoclay
MNP	Magnetic nanoparticle
MW	Molecular weight
nZVI	Nanosacle zero-valent iron
PEI	Polyethylenimine
PLL	Poly-L-lysine
SA	Steric acid
SEM	Scanning electron microscopy

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