# Flocculation as a low-cost method for harvesting microalgae for bulk biomass production

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**The global demand for biomass for food, feed, biofuels, and chemical production is expected to increase in the coming decades. Microalgae are a promising new source of biomass that may complement agricultural crops. Production of microalgae has so far been limited to high-value applications. In order to realize large-scale production of microalgae biomass for low-value applications, new low-cost technologies are needed to produce and process microalgae. A major challenge lies in the harvesting of the microalgae, which requires the separation of a low amount of biomass consisting of small individual cells from a large volume of culture medium. Flocculation is seen as a promising low-cost harvesting method. Here, we overview the challenges and possible solutions for flocculating microalgae.**

## Microalgae: a promising new source of biomass

As a result of the growing world population and an increase in living standards in developing economies, demand for biomass for food and animal feed is expected to increase by >50% in the next two decades [1]. At the same time, initiatives are being taken to move from a fossil-fuel-based economy to a biobased economy in which biomass replaces petroleum as a source of transport fuel and as a feedstock for the chemical industry [2]. It is unlikely that agricultural biomass production can meet the growing demand, thus, there is an urgent need for new sources of biomass that do not compete with agriculture. Microalgae are today considered to be the most promising new source of biomass [3–5] (Box 1). Using technology available today, the energy demand is too high for bulk microalgal biomass production for food, feed, bulk chemicals, or biofuels [6–8]. For microalgal biomass to become a commodity like most agricultural crops, the yield has to be increased and the cost of production reduced. Recent years have seen an explosion in research and development in increasing the yield of microalgal biomass production through photobioreactor design [9], selection of strains [10], and genetic engineering of metabolic pathways [11]. Much less progress has been made on research and innovation in downstream processing, although this is essential to reduce the cost of the production process [12,13]. Today, microalgal production is rapidly moving from laboratory to pilot scale and commerical-scale demo installations [11], prompting the need for cost- and energy-efficient downstream processing technologies.

## The challenge of harvesting microalgae

A major challenge in downstream processing of microalgae lies in separating the microalgae from their growth medium, that is, the harvesting process. A high biomass concentration leads to mutual shading of the microalgal cells and thus a reduction in productivity, therefore, biomass concentrations in microalgal cultures are usually low: from 0.5 g/l in open pond reactors to about 5 g/l in photobioreactors. This means that a large volume of water has to be removed to harvest the biomass. As a result of the small size of the microalgal cells (2–20 $\mu$m) and their colloidal stability in suspension (Box 2), harvesting by means of sedimentation or simple screening is not feasible, except perhaps for larger species such as *Arthrospira*. When microalgae are produced for high-value products, harvesting is done by centrifugation. Centrifugation is however too expensive and energy-intensive if biomass is to be used for low-value products such as biofuels due to the large volumes of culture medium that need to be processed. Finding an alternative technology that is capable of processing large volumes of culture medium at a minimal cost is essential to reduce the cost and increase the scale of microalgal biomass production [14–17].

The cost and energy demand for harvesting microalgae could be significantly reduced if the cells could be preconcentrated by flocculation [18,19]. During flocculation, single cells form larger aggregates that can be separated from the medium by simple gravity sedimentation. When flocculation is used for harvesting microalgae, it is part of a two-step harvesting process. Flocculation is used during the first step to concentrate a dilute suspension of 0.5 g/l dry matter 20–100 times to a slurry of 10–50 g/l. Further dewatering using a mechanical method such as centrifugation is then required to obtain an algal paste with a 25% dry matter content [20]. The energy requirements for this final mechanical dewatering step are acceptable because the particles are relatively large and the volumes of water to be processed small [17].

Flocculation is a widely used technology in different industries ranging from brewing to water treatment and mining. In these industries, flocculation is generally used to separate a small amount of impurities from a large volume of liquid and the liquid is the end product. On

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**Box 1. Production of microalgae**

Microalgae and cyanobacteria are unicellular microorganisms that live in water and produce biomass through photosynthesis. They evolved billions of years ago in the oceans, long before plants colonized the land. Many microalgae belong to different evolutionary lineages than terrestrial plants and are therefore capable of producing unique products such as polyunsaturated fatty acids [e.g., eicosapentaenoic or docosahexaenoic acid) or natural pigments (e.g., phycocyanin or astaxanthin) that cannot be found in terrestrial plants.

Microalgae occur in lakes, rivers, and oceans but biomass concentrations in natural ecosystems are generally too low to be harvested commercially (except for certain lakes dominated by *Arthrospira*). Microalgae are produced commercially in special reactors designed to maximize photosynthesis: open 'raceway ponds' or closed photobioreactors. Raceway ponds consist of shallow basins, whereas photobioreactors are transparent tubular or flat panel reactors (Figure I). Productivity of microalgae is generally limited by self-shading of the cells in the culture, therefore, photobioreactors have a higher productivity than raceway ponds because light supply to the cells is more efficient. The main disadvantage of photobioreactors is their cost, although several potentially low-cost designs have been developed by the microalgae industry in the past years (e.g., Proviron and Solix; Figure I).

Microalgae have been produced commercially for several decades but total production is still low (1000 tones/year) and aimed mainly at high-value products such as nutritional supplements, natural pigments, or aquaculture feed. Only a few species are being produced on a scale of hundreds to thousands of tonnes (*Arthrospira*, *Chlorella*, *Dunaliella*, and *Haematococcus*), whereas about 10 additional species are produced on a smaller scale [70]. The majority of the commercial production today is done in open raceway ponds, but closed photobioreactors appear to be favored by most new companies.

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**Figure I**. Example of open 'raceway pond' reactors (Ingrepro, The Netherlands) and a novel design of a low-cost photobioreactor (Proviron, Belgium).

the contrary, when flocculation is used for harvesting microalgae the harvested biomass is the end product. As a result, the economics are very different when flocculation is used for harvesting microalgal biomass than when it is used for removing impurities from a liquid. Also, contamination is a major issue because any chemicals added to induce flocculation end up in the harvested biomass. These chemicals can interfere with the final applications of the biomass (e.g., food or feed) or with further processing of the biomass (e.g., lipid extraction) [15].

## Approaches for microalgae flocculation

Flocculation can be achieved in several ways (Box 3) and a wide range of approaches for flocculating microalgae have been explored in recent years. These approaches range from traditional flocculation methods that are widely used in other fields of industry (e.g., chemical flocculation) to novel ideas based on the biology of microalgae (e.g., bioflocculation) and the use of emerging technologies (e.g., use of magnetic nanoparticles). Here, we give a concise overview of these technologies with their advantages and disadvantages.

### *Chemical flocculation*

Metal salts such as alum and ferric chloride are widely used for flocculation in industries such as water treatment and mining. Although metal salts are being applied for harvesting microalgae (e.g., *Dunaliella*; [21]), their use results in high concentrations of metals in the harvested biomass. These metals remain in the biomass residue after extraction of lipids or carotenoids [22]. The metals in the biomass residue may however interfere with the use of the protein fraction in this residue as animal feed. The valorization of the protein fraction as animal feed is said to be important for making microalgal biofuels economically viable [23]. Despite this shortcoming, metal coagulants provide a good model system to study the interaction between flocculants and microalgal cells because their properties are well understood [24,25].

Other commonly used chemical flocculants in other industries are synthetic polyacrylamide polymers. These may however contain traces of toxic acrylamide and thus also contaminate the microalgal biomass [26]. Flocculants based on natural biopolymers are therefore a safer

**Box 2. Stabilization of microalgal suspensions**

Particles suspended in water usually carry a positive or negative surface charge. To maintain electrical neutrality, such charged particles will attract ions with an opposite charge from the solution (counter ions). The system of the particle surface charge and associated counter ions in the surrounding solution is called the electrical double layer Figure I. Close to the particle surface, the counter ions form a dense layer of ions that is inaccessible to other counter ions, the Stern layer. Further away from the particle surface, the counter ions form a diffuse layer as a result of a balance between electrostatic attraction and thermal diffusion. As a result, the potential difference between the particle surface and the bulk solution decreases more or less exponentially with distance from the particle surface.

The cloud of counter ions surrounding charged particles in a suspension results in an electrical repulsion between the particles. The $\zeta$ potential is the potential difference between the bulk fluid and the layer of counter ions that remains associated with the charged particle when the particle is moving through the solution (the slipping plane). The $\zeta$ potential can relatively easily be estimated from the mobility of the charged particles in an electric field, therefore, it is a useful indicator of the degree of repulsion between charged particles in a suspension. When the $\zeta$ potential is high ($>$25 mV, positive or negative), electrical repulsion between particles is strong and the suspension is said to be stable. When the $\zeta$ potential is close to zero, particles can approach each other to a point where they will be attracted by Van der Waals forces. When that happens, particles will aggregate and flocculation or coagulation will occur.

Just as in other stable suspensions of particles, microalgal cell suspensions are stabilized by the surface charge of the cells. This surface charge originates predominantly from the presence of carboxylic (-COOH) and amine (-$NH_2$) groups on the cell surface. The carboxylic groups dissociate and are negatively charged above pH 4–5, whereas the amine groups are uncharged at this pH. This results in a net negative surface charge above pH 4–5.

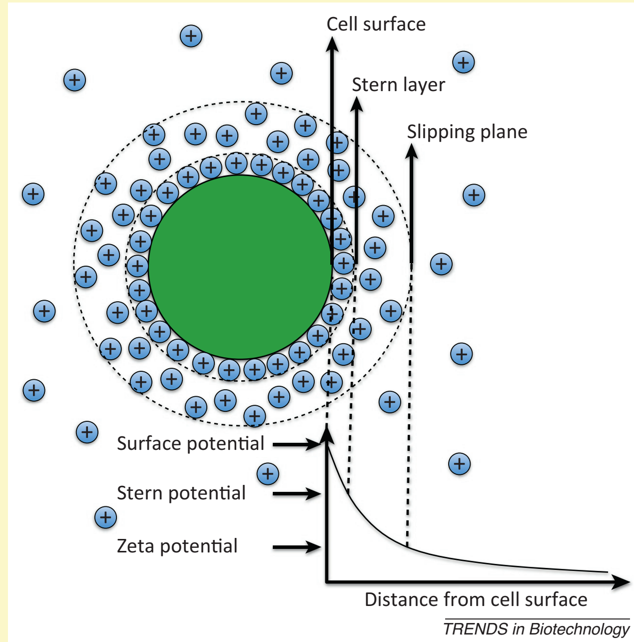


**Figure I**. Structure of the electrical double layer of charged ions in solution surrounding a negatively charged microalgal cell and the potential difference between the particle and the bulk fluid as a function of the distance from the particle surface.

alternative. To be able to interact with the negative surface charge of microalgal cells, these biopolymers should be positively charged, which is rare in nature. A well-known positively charged biopolymer is chitosan, which is derived from chitin, a waste product from shellfish production. Chitosan is a very efficient flocculant but it works only at low pH, but pH in microalgal cultures is relatively high [27]. An alternative to chitosan is cationic starch, which is prepared from starch by addition of quaternary ammonium groups. The charge of those quaternary ammonium groups is independent of pH and therefore cationic starch works over a broader pH range than chitosan [28]. Other examples of biopolymers than can be used to flocculate microalgae are poly-$\gamma$ glutamic acid (an extracellular polymer produced by *Bacillus subtilis*) [29] or polymers present in flour from *Moringa oleifera* seeds [30]. A general problem of polymer flocculants is that they undergo coiling at high ionic strengths and become ineffective

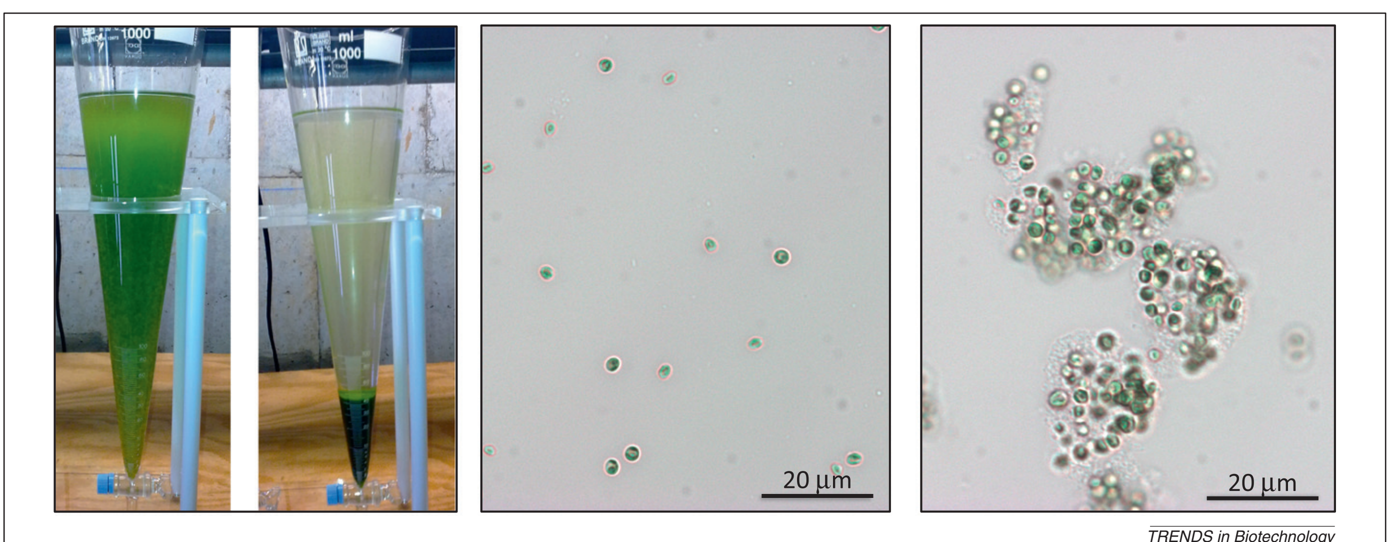


**Figure 1**. Macroscopic and microscopic view of flocculation of a culture of *Chlorella vulgaris* using autoflocculation. The macroscopic view (left) shows a culture of *C. vulgaris* in an Imhoff cone before and after flocculation. The microscopic view shows *C. vulgaris* cells before (middle) and after (right) flocculation.

**Box 3. Approaches to induce flocculation**

Flocculation or coagulation is commonly used in wastewater treatment, drinking water production, mining, and brewing to remove impurities from water. Flocculation of suspensions of particles can often be attributed to three common mechanisms, which can act alone or in combination.

- *Charge neutralization:* the phenomenon when positively charged ions, polymers or colloids strongly absorb on the negative surface charge of a particle, ultimately canceling the negative surface charge. As a result, the electrostatic repulsion between the particles disappears and particles will coagulate or flocculate.
- *Electrostatic patch mechanism:* the phenomenon where a charged polymer binds to a particle with opposite charge. The polymer locally reverse the charge of the particle surface, resulting in patches of opposite charge on the particle surface. Particles connect with each other through patches of opposite charge, causing flocculation of the suspension.
- *Bridging*: polymers or charged colloids simultaneously bind to the surface of two different particles to form a bridge between these particles. This bridge brings the particles together and causes flocculation.
- *Sweeping flocculation*: particles are entrapped in a massive precipitation of a mineral. This causes flocculation of these particles.

Flocculation can be induced by several approaches. Metal salts such as alum and ferric chloride are commonly used flocculants or coagulants. These metal salts dissociate in water and the metal ions can cause flocculation through charge neutralization. Metal ions readily hydrolyze in water to form metal hydroxides. Metal hydroxides can precipitate even at low metal concentrations. These metal hydroxide precipitates are often positively charged and can cause flocculation through charge neutralization, bridging, or sweeping flocculation. Other precipitates such as calcium phosphates or magnesium hydroxides that can be formed at high pH can cause flocculation through the same mechanisms as metal hydroxide precipitates. Another important class of flocculants is polymers. Charged polymers can bind to the surface of different particles and can cause flocculation through bridging. Charged polymers can also neutralize or even reverse the surface charge of particles to cause flocculation through charge neutralization or electrostatic patch aggregation.

[15]. Therefore, they are less suitable for harvesting microalgae cultivated in seawater.

### *Autoflocculation*

Flocculation often occurs spontaneously in microalgal cultures when pH increases above 9 [31]. This type of flocculation is often referred to as autoflocculation because it occurs spontaneously in microalgal cultures as a result of a pH increase due to photosynthetic $CO_2$ depletion (Figure 1). Autoflocculation is associated with the formation of calcium or magnesium precipitates. Depending on the conditions, these precipitates carry positive surface charges and can induce flocculation through charge neutralization and/or sweeping flocculation.

Calcium phosphate precipitates are positively charged when calcium ions are in excess of phosphate ions and interact with the negative surface charge of microalgal cells [8,17]. High phosphate concentrations are required for this type of flocculation to occur. As a result of the declining phosphate reserves and increasing prices of phosphate, flocculation by calcium phosphate precipitation is unsustainable, except perhaps in applications where microalgae are used for wastewater treatment and excess phosphate needs to be removed [32]. Magnesium hydroxide or brucite also precipitates at high pH. These precipitates are positively charged up to pH 12 and can therefore also interact with the microalgal cell surface to cause flocculation [33,34]. Most waters contain sufficiently high background concentrations of magnesium for this process to occur. Calcium carbonate or calcite also precipitates at high pH but whether it can induce flocculation of microalgae remains to be demonstrated. Flocculation at high pH is caused by formation of inorganic precipitates and not by pH as such, therefore, the harvested biomass contains high concentrations of minerals [35]. Although these have a low toxicity, it is nevertheless preferable to remove them from the biomass.

### *Physical flocculation methods*

Contamination of the biomass would be avoided if it were possible to induce flocculation by applying only physial forces. For instance, flocculation of microalgae can be accomplished by applying a field of standing ultrasound waves. Although this method works well in the laboratory, it is difficult to apply on larger scales [36]. In electrocoagulation flocculation, flocculation is induced through electrolytic release of metal ions from a sacrificial anode [37]. The efficiency of this method might be improved by changing the polarity of the electrodes [38]. Similar to flocculation by metal salts, electrocoagulation flocculation results in contamination of the biomass with metals, albeit to a lesser extent than when metal coagulants are directly used. OriginOil claims to have developed a solution for this problem. Its method uses only electromagnetic pulses to neutralize the surface charge of microalgal cells and induce flocculation [39].

Recently, several studies have explored the use of magnetic nanoparticles to harvest microalgae. Magnetite ($Fe_2O_3$) nanoparticles may adsorb directly on the microalgal cells, upon which the cells can be separated from the medium by applying a magnetic field. This method thus combines flocculation and separation in a single process step [40]. Magnetite nanoparticles seem to adsorb more easily on some microalgal species than on others [41]. Adsorption can be improved by coating the nanoparticles with cationic polymers [42,43]. An advantage of using magnetite nanoparticles for harvesting microalgae is that the nanoparticles can be recovered after harvesting and subsequently reused [40].

### *Bioflocculation*

In natural blooms of microalgae occurring in lakes or rivers, flocculation sometimes occurs spontaneously. This spontaneous flocculation is assumed to be caused by extracellular polymer substances in the medium and is called bioflocculation [10]. Bioflocculation is often successfully used for harvesting microalgae in facilities where microalgae are used in wastewater treatment [44]. The underlying mechanism, however, is poorly understood and deserves further research because it may lead to a chemical-free method for flocculating microalgae. Some microalgal species flocculate more readily than others and such naturally bioflocculating microalgae can be mixed with other species to induce flocculation [45,46]. There are indications that bioflocculation may be initiated by infochemicals [47]. Recently, an infochemical isolated from a senescent and flocculating culture of a *Skeletonema* species was found to be capable of inducing flocculation in a culture of another species of microalgae [48].

Bacteria or fungi can also induce bioflocculation of microalgae. Some fungi, for instance, have positively charged hyphae that can interact with the negatively charged microalgal cell surface and cause flocculation [49,50]. Specific consortia of bacteria can also induce flocculation of microalgae [51,52]. These flocculating fungi or bacteria can be cultivated separately or in combination with the microalgae. Cultivating bacteria or fungi in combination with microalgae requires a carbon source in the medium. In wastewater, a carbon source is usually present and this allows cocultivation of microalgae and bacteria. This results in a culture of mixed algal–bacterial flocs that can be easily harvested [53,54]. The use of bacteria or fungi as a flocculating agent avoids chemical contamination of the biomass but results in microbiological contamination, which may also interfere with food or feed applications of the microalgal biomass.

### *Genetic modification*

Many research efforts are currently directed towards genetic modification of microalgae. Most recently published studies and granted patents in this field are aimed at increasing biomass productivity or increasing production of specific metabolites, most often lipids [10,11]. However, genetic modification may also be a promising way to harvest microalgae [8,11]. Here, achievements in genetic modification of yeast may be used as an example. In yeast, genetically modified strains have been developed that express flocculin proteins in their cell walls, causing the cells to aggregate [55]. The expression of these proteins can be induced by an environmental trigger or during a specific growth stage. Sapphire Energy has described a method for flocculating microalgae in which ligand–receptor pairs can be expressed in different strains that are mixed to induce flocculation, or that are expressed sequentially in the same strain [56]. Genetic modification or selection may also be aimed at facilitating flocculation by other methods. For instance, a cell wall-deficient mutant of *Chlamydomonas* has been found to flocculate much more easily under alkaline conditions than the wild type strain [57]. This indicates that minor genetic modifications may greatly facilitate flocculation.

## Properties of microalgae that influence flocculation

Most studies on flocculation of microalgae carried out so far have focused on a single species cultured under one particular condition. However, flocculation depends on the properties of microalgal cell surfaces, and these properties differ between species and vary within a species depending on culture conditions. The cell surface to biomass ratio increases with decreasing cell size, therefore, smaller species will require a higher flocculant dose to harvest the same amount of biomass than larger species [45]. The biochemical composition of the cell surface differs between species and these differences influence flocculation [58]. Cell surface properties may even vary between different strains of the same species and cause differences in flocculation behavior between different strains [59]. These properties are also variable within a species, resulting in different flocculation behavior in, for example, exponential versus stationary phase cultures [58,60].

The composition of the culture medium will also affect flocculation of microalgae. pH influences the charge of not only the microalgal cell surface but often also of chemical flocculants, and is therefore an important parameter to consider. Furthermore, microalgae often excrete significant quantities of organic matter into the growth medium [61]. This algal organic matter consists of polysaccharides and proteins that can compete with the algal cell surface for flocculants and thus interfere with flocculation [62,63]. It appears that the flocculant demand is determined to a larger degree by the quantity and composition of the algal organic matter than by the biomass and the properties of the microalgal cells themselves [64,65]. Excretion of this algal organic matter is generally higher under nutrient stress [45], which is actually important to induce lipid accumulation in microalgae [47]. Higher lipid productivity in microalgae may thus be associated with a higher chemical demand for flocculation.

## Factors influencing the cost of flocculation

Production of microalgae today is still very expensive compared to agricultural biomass production, therefore, cost is an important factor to consider when evaluating flocculation technologies for harvesting microalgae. Harvesting microalgae using metal salts or chitosan is only marginally less expensive than centrifugation, which is currently the most commonly used method for harvesting microalgae [8]. Flocculation using standing ultrasound waves costs even more than centrifugation. Magnetic nanoparticles are prohibitively expensive today but their cost may go down in the future if new methods for producing such nanoparticles become available [66]. Cationic starch or other biopolymers are slightly cheaper than, for instance, chitosan but probably still too expensive for applications such as biofuel production.

Electrocoagulation flocculation has a low electricity demand when the method is used in seawater and may be a promising low-cost method for harvesting marine microalgae [37,38]. Other promising low-cost flocculation methods that are available today are autoflocculation at high pH and bioflocculation. The cost of autoflocculation is very low even if a base is required to increase pH [17,33]. Bioflocculation by addition of flocculating microalgae, fungi or bacteria requires cultivating these microorganisms, but the cost for doing this is substantially lower than the cost of centrifugation [46]. This cost can be avoided altogether if flocculating microalgae are cocultivated with flocculating bacteria, which is possible if the medium contains a carbon source for the bacteria, as is often the case in wastewater. Controlled flocculation of microalgae through infochemicals or genetic modification is a promising technology but requires further basic research before it can be applied. The use of both infochemicals and genetic modification is likely to be highly species specific. So far, virtually no information is available on the identity of infochemicals that induce bioflocculation. For most species of microalgae, a toolbox for genetic modification is not yet available. Therefore, upfront research and development costs for these flocculation methods are likely to be high.

The use of flocculation not only incurs direct costs for flocculation itself, but also indirect costs through its impact on other operations in the production process. For instance, many flocculation technologies cause contamination of the biomass with chemicals, minerals, or microorganisms. This is the case for chemical flocculation, autoflocculation, or bioflocculation by bacteria or fungi. These contaminants may limit the use of the biomass (e.g., for food or feed), or interfere with processing of the biomass. Some flocculation technologies also result in chemical contamination of the culture medium (e.g., metal salts or electrocoagulation flocculation) or cause large changes in pH (e.g., autoflocculation). This may limit recycling of the culture medium and result in significant indirect costs that should be accounted for.

Flocculation is part of a two-step harvesting process in which flocculation is used to preconcentrate the biomass before a physical method is used for the final dewatering. The more water can be removed during the first flocculation step, the lower the cost will be for the second mechanical dewatering step [67]. To minimize the cost for mechanical dewatering, it is important that flocculation results in a low algal sludge volume [68]. Not only the volume of water that can be removed during flocculation is important, but also the rate at which this can be done. Therefore, microalgal flocs should have a high sedimentation rate. A flocculation technology that results in rapidly settling flocs requires a smaller harvesting unit and thus incurs lower investment costs. So far, few studies on flocculation of microalgae have taken parameters such as the sludge volume or the sedimentation rate into account [69].

## Concluding remarks

Development of an efficient flocculation technology for microalgae may yield major cost and energy savings in large-scale production of microalgal biomass. As a result of this, numerous studies have started to explore various approaches for flocculating microalgae. Chemical flocculation has the disadvantage that it results in contamination of the biomass, although the use of natural polymers may minimize this problem. Alkaline flocculation promises to be a low-cost flocculation method but also results in contamination of the biomass, albeit with mineral precipitates with low toxicity. Biological flocculation using fungi or bacteria holds a lot of potential when microalgae production is combined with wastewater treatment, because wastewater can provide the necessary carbon source for the flocculating microorganisms. Physical flocculation methods have the advantage that they may avoid contamination of the biomass with chemicals or microorganisms. Fundamental research into infochemicals that induce flocculation in microalgae is urgently needed, because this may lead to a highly controllable method for inducing flocculation that avoids contamination. The same holds true for approaches to induce flocculation through genetic modification. Future studies should not only look at the efficiency of flocculation under specific conditions, but should also investigate how flocculation is influenced by properties of the microalgal cells or by culture conditions, particularly interference by organic matter in the culture medium. Cost is an important factor to consider when evaluating new flocculation methods for microalgae. Cost evaluation should not only take the cost of flocculation step itself into account, but also the influence on the entire production process.

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