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Harvesting techniques applied to microalgae: a review

Ana I. Barros, Ana L. Gonçalves, Manuel Simões, José C.M. Pires *

LEPABE, Departamento de Engenharia Química, Faculdade de Engenharia,
Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

*Corresponding author

Telephone: +351 22 508 2262

Fax: +351 22 508 1449

E-mail address: jcpires@fe.up.pt

Abstract

Research studies on microalgae have increased in the last decades due to the wide range of applications associated to these photosynthetic microorganisms. Microalgae are an important source of oils and other biomolecules that can be used in the production of biofuels and high-valued products. However, the use of microalgae in these green processes is still not economically viable. One of the main costs associated to microalgal production is related to the harvesting process, as it usually accounts for about 20-30% of total cost. Therefore, this review focuses on the main harvesting processes applied to microalgae, presenting the main advantages and disadvantages of each method, to allow the selection of an appropriate procedure to effectively separate microalgal biomass from the culture medium. To reduce the associated costs, it is common to harvest microalgae in a two-step separation: (i) thickening procedures, in which microalgal slurry is concentrated to about 2-7% of total suspended solids; and (ii) dewatering procedures, which result in the concentration of microalgal slurry to 15-25% of total suspended solids. Selection of the adequate harvesting methods depends on the characteristics of the target microorganism and also on the type and value of the end product.

Keywords: Bioenergy production; Economic viability; Green processes; Harvesting processes; Microalgae; Sustainable processes.

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1. Introduction

It is well known that microalgae have a huge potential in a wide variety of applications. Concerning environmental ones, microalgae can play an important role in bioremediation of wastewater and carbon dioxide sequestration [1-6]. Furthermore, these photosynthetic microorganisms have been considered as a potential renewable fuel source [7-9]: they can be used as raw material for the production of biodiesel, biomethane, bioethanol, biohydrogen and biobutanol. These biofuels are viewed as the most promising alternative to fossil fuels, being able to provide up to 25% of global required energy [9-11]. One of these energetic products is 3rd generation biodiesel obtained from the transesterification of microalgal lipids, which, in appropriate culture conditions, may represent a significant fraction of their biomass [5, 7, 9, 11-13]. Microalgal based biofuels present numerous economic and ecological advantages comparing to the terrestrial crop based ones: (i) continuous growth in waters with a wide range of salinities and chemical compositions; (ii) growth in any location (arable and non-arable land) without the need for herbicides or pesticides; and (iii) high specific production yields and photosynthetic efficiency [5, 8-11, 14]. Residual biomass, which is poor in lignin and very rich in proteins and other compounds of commercial interest, can then be used in animal feed production and in the synthesis of different high-valued compounds, such as nutritional supplements, cosmetics and pharmaceuticals in a biorefinery-based production [9, 11, 15-19].

Despite the wide range of applications, microalgal production is not economically viable yet. Several research pathways have been explored to improve the economics of the process [10, 20]. Firstly, microalgae may be cultivated using wastewater as culture medium. This approach aims to reduce both production costs and freshwater requirement. Microalgae have the ability to grow on these environments, assimilating nutrients and metals from the wastewater [9, 21]. Hence, microalgae would play an important remediation role during the tertiary wastewater treatment phase [9]. Secondly, a biorefinery-based production is a strategy that lowers the overall production costs by taking credit of all products of commercial interest that can be obtained from microalgae [7, 9]. Finally, a low cost harvesting process should be studied, as this production step represents 20-30% of the biomass production costs [9, 10, 22-24]. The main reasons for high process costs are the small size of microalgae and their growth in very dilute cultures (mass concentration less than 1 g L⁻¹) with densities close to that of the water [22, 23]. In addition, microalgal surface is negatively charged and the cells carry algogenic organic

matter (AOM), which keeps stable their dispersed state [25]. At this moment, there is no microalgal harvesting method that is both economically viable and efficient. Lowering harvesting costs is thus considered a key factor for the development of sustainable full-scale production of microalgal biomass [10]. Accordingly, this study aims to review the recent research concerning harvesting methods applied to microalgae, discussing their advantages and disadvantages in terms of applicability, environmental impacts and cost-effectiveness.

2. Microalgal harvesting methods

An ideal harvesting process should be effective for the majority of microalgal strains and should allow the achievement of high biomass concentrations, while requiring moderate costs of operation, energy and maintenance [22]. The initial harvesting stage is generally costly and determines the following downstream processing. One of two methodologies is generally applied: (i) a two-step concentration where the suspension is primarily thickened to a slurry of about 2-7% of total suspended solids, TSS, (concentration factor of 100-200) and further dewatered to a cake with 15 to 25% TSS (concentration factor of 2-10); or (ii) a single-step concentration process [26, 27]. Selection of an appropriate harvesting method depends on the end product, namely its value and properties [23]. Therefore, one must consider the acceptable level of moisture, salt concentrations, cell damage and strain features, such as their density and size. Furthermore, selection of an appropriate harvesting procedure must take into account that microalgal biomass must be further processed. Therefore, these procedures must not be toxic or contaminate microalgal biomass. It is also desirable that the selected harvesting method allows the recycling of the culture medium [27]. For almost applications, microalgal harvesting generally comprises the two-step concentration method: thickening and dewatering (see Figure 1). These stages are crucial to obtain thick algal slurry from the initial suspension and to enable further downstream processes [28, 29].

Microalgal harvesting currently involves mechanical, chemical, biological and, to a lesser extent, electrical based methods. It is very common to combine two or more of these methods to obtain a greater separation rate at lower costs. In fact, the combination of flocculation-sedimentation with centrifugation can significantly reduce process costs [30]. Biological approaches are emerging techniques that can lead to further reduction of operational costs. Mechanical methods are the most reliable and therefore the most

commonly used to harvest microalgal biomass [10, 23]. However, these methods are often preceded by a chemical or biological coagulation/flocculation thickening stage to improve effectiveness and to reduce operation and maintenance costs. Table 1 presents the main advantages and disadvantages of the most commonly used methods for microalgal harvesting that are described in the following sections.

2.1. Screening

Screening is an operation method applied as pre-processing of microalgal cultures. Its efficiency depends on the spacing between the screen openings and cell size [31]. Microstrainers and vibrating screens are the primary screening used devices. In microstraining, a rotary drum is covered with a straining fabric, stainless steel or polyester, and suffers frequent backwash [27]. The flow-through rate is the most determinant factor to overall production costs. Larger microalgae will be effectively processed with larger openings, thus resulting in faster flow rates and lower operational costs. Despite the simplicity of the method and low investment, given the wide character of the mesh that can be used, its efficiency in recovering bacterial-sized microalgae is very low and further processing is required [31]. Additionally, there is bacterial and microalgal biofilm formation on the fabric or mesh, which implies constant maintenance. Microstraining is largely influenced by initial microalgal concentration and incomplete solids removal can occur [27]. For instance, *Coelastrum proboscideum* harvested by this method resulted in 1.5% TSS consuming 0.2 kWh m⁻³, while the use of vibrating screens in continuous and batch mode allowed the recovery of 5-6% TSS and 7-8% TSS, respectively [32]. One can thereby conclude that greater algal solid content is achievable by vibrating screens, which are also more area efficient [31]. In fact, the harvesting of larger algae, such as *Spirulina* sp., resulted in 8-10% TSS with a flow rate of 20 m³ h⁻¹ [33]. This operation unit is currently used in the commercial production of multicellular filamentous blue-green algae [31].

2.2. Thickening

To increase solid concentration of the microalgal suspension and to reduce the volume to be processed, thickening methods must be applied, since volume reduction contributes to considerable savings along the downstream processes [31]. Typically, thickening

processes consist in coagulation/flocculation (both chemical and biologically based), gravity sedimentation, flotation or an electrical approach to these [27].

2.2.1. Chemical coagulation/flocculation

Chemical coagulation/flocculation is the main approach towards the economical optimization of microalgal harvesting processes. Application of these methods is required mostly due to the large volumes of microalgal cultures that must be processed and to the need for a universal method that can be applicable to a wide variety of species [27]. This harvesting step is used to concentrate the suspension 20-100 times [19]. It increases the effective particle size, prior to dewatering, thus significantly reducing its energy demand [23, 34]. For low cost harvesting of microalgae, coagulation/flocculation is generally followed by gravity sedimentation [35].

Whereas coagulation involves pH adjustment or electrolyte addition, flocculation is based on the addition of cationic polymers to the broth [16]. In this way, chemical coagulation/flocculation can be defined as the coalescence of finely divided particles in suspension onto larger aggregates followed by the agglomeration of these into larger flocs that settle to the bottom of the vessel, leaving a clear supernatant. Flocculation can be induced by different ways: (i) electrostatic patch (or patching), which occurs when a charged polymer binds to an opposite charged particle, locally reversing that charge and creating a patch that will connect with opposite charged patches; (ii) bridging, which occurs when polymers or colloids bind to the surface of two different particles forming a bridge between them; and (iii) sweep flocculation, which occurs when particles are entrapped in a massive mineral precipitation [19]. Cellular concentration and surface properties, namely net charge and hydrophobicity, concentration of the coagulant/flocculant species, as well as pH and ionic strength of the broth are key factors that influence this process [16]. Microalgal growth phase also influences flocculation, as pH, dissolved CO₂, zeta potential and particle size suffer significant variations throughout the cultivation time [36]. Therefore, it is advantageous to consider the stationary phase to harvest microalgal biomass. In this phase, microalgae have lower metabolic activity and cell mobility, presenting higher intercellular interactions, as the zeta potential is lower. This is also observed by the higher particle size, which may be due to cell agglomeration. Storage conditions before harvesting are preponderant as well. The presence of daylight enables photosynthesis and thus higher metabolic rate and unicellular mobility, resulting

in lower settling rates [22]. Ideally, chemical coagulation/flocculation should: (i) result in no biomass contamination; (ii) lead to subsequent high efficiency biomass settling; (iii) allow the reuse of the culture medium; (iv) consider environmental impact; and (v) be cheap and non-toxic when applied in large scale [23].

A wide variety of salts has been tested as coagulants for microalgal harvesting. Multivalent metal salts, such as FeCl_3 , $\text{Al}_2(\text{SO}_4)_3$ and $\text{Fe}_2(\text{SO}_4)_3$, have been effectively tested, as it is shown in Table 2. Dissociation of these salts in the culture medium lowers electrostatic repulsion between the negatively charged cell surfaces, enabling cell aggregates formation [9, 16]. The activity of an ion is largely determined by its electronegativity: the more electronegative the ion, the faster the coagulation (under 24 h) [16]. Solubility is also a key factor: salts with lower solubility are more effective. For example, chloride salts are more efficient than the equivalent sulphate ones, due to their lower solubility. Furthermore, the higher the charge density of an ion, the more efficient the charge neutralization between microalgal cell surfaces is expected to be. Finally, pH has to be low enough to form cationic hydrolysis products, which are crucial in coagulation, since this method functions by charge neutralization [27].

As to all harvesting methods, selection of the appropriate coagulant is determined by the target of the subsequent processes. The use of ferric salts has shown to lead to a brown-yellow coloration of the microalgae. However, no effect on the photosynthetic apparatus and cell viability was observed. In this way, if pigment extraction is intended, ferric salts cannot be applied. On the other hand, if the aim is to reduce the overall production cost, a cheaper and faster coagulant, such as an aluminium salt should be applied. When using aluminium salts, cell lysis was observed (10 to 25%), possibly due to rapid cell aggregation or cell membrane destabilization [16]. Despite being easily flocculated by metal coagulants, such as alum and iron chloride, large amounts of these salts are required, making it a very expensive option [34]. Furthermore, microalgal biomass treated with these chemical species can be contaminated by the used metals, hindering its application as biofuel or animal feedstock [37].

Although coagulation/flocculation followed by gravity sedimentation is a cheap approach for microalgal harvesting, coagulant costs represent a significant portion of the overall process (4 to 7%). Therefore, the use of naturally available coagulants/flocculants, such as phosphates, carbonates, calcium and magnesium ions, frequently found in wastewater, brackish water or seawater, has been considered [35]. However, phosphate based

coagulation is only feasible for phosphate rich wastewater, since when added to a free medium, microalgae hoard it in addition to their metabolic uptake. On the other hand, the use of magnesium ions as coagulants may be advantageous, as they present similar efficiencies to those achieved with Al^{3+} and Fe^{3+} ions and can be easily obtained from wastewaters. Additionally, the coagulation efficiency can also be enhanced through lime addition to the culture broth [30, 35]. The mechanism of coagulation by magnesium is thought to begin with charge neutralization at the cell surface that, with further hydroxide precipitation, leads to sweep flocculation [35]. Limestone or dolomites can be applied, as they bring not only magnesium ions but also other carbonates, hydroxides and oxides, which present pH related coagulating activity as well [30, 35].

The relationship between coagulant dose and microalgal cell concentration is not clear, as it has been reported as being linear [16, 38], as well as proportional to the cell concentration logarithm. Nonetheless, high density cultures require almost 10-fold less coagulant addition than expected. This might be due to the presence of less charged material on the surface of cell walls or to the shorter distance between cells that leads to higher collision rates [30]. Coagulant dose is also greatly influenced by AOM produced and excreted by microalgal cells. Excretion of AOM affects flocculation by alum, chitosan and cationic starch addition, as well as pH-induced flocculation. AOM is mainly constituted by extracellular polymeric substances (EPS), normally of polysaccharide or proteic nature. Proteins form complexes with the metal ions of most chemical coagulants and polysaccharides (with negatively charged carbonyl groups) interact with the positively charged coagulants, both making them unavailable microalgal flocculation. Nevertheless, the number and position of the carbonyl groups on the polysaccharides differ with microalgal species and cultivation conditions, showing different influence upon flocculation [34]. For instance, nutrient-limited cells (conditions that maximize lipid yield) generate more AOM than exponential growing ones. The ionic strength of the broth also influences the coagulant dose required. For example, the flocculation of marine microalgae demands five to ten times more coagulant dose than that of freshwater ones, since the chemical activity of the coagulant is reduced by the presence of marine salts [27].

Polyelectrolyte flocculants are natural or synthetic polymers of ionic or non-ionic species. The use of polymeric materials allows the reduction required dose by increasing their molecular weight [27, 39]. Nevertheless, the presence of some chemical substances and

pH of the medium are crucial to effective flocculation. These flocculants can either be cationic, anionic or non-ionic. However, due to the negative net charge of microalgal cells, anionic or non-ionic polymers have no effect on their flocculation [27]. Some cationic polymers, such as chitosan, cationic polyacrylamides, cellulose, surfactants and other man-made fibers proved to have successful flocculating activity towards microalgal cultures [31]. Cationic polymers reduce microalgal cell surface electronegativity and bridge them to one another [23]. These methods are effective in the flocculation of freshwater microalgae; however, the high salinity of the marine environment can be inhibitory, as the polymers shrink to smaller dimensions, failing in bridging the cells [23, 30]. This effectiveness reduction to flocculate marine microalgae is attributed to the ionic strength of sea and brackish waters [27]. Effective polyelectrolyte flocculation can be employed only when the salinity level is lower than 5 g L^{-1} . Polyelectrolytes, such as polyacrylamide, cannot be used to harvest microalgae, due to its toxicity. Chitosan has been effectively used in the harvesting of both fresh and seawater microalgae and does not contaminate microalgal biomass; however it is too expensive for large scale applications [30]. An alternative to chitosan is cationic starch, which is less pH-dependent, but requires higher dosage [39]. In order to improve its effectiveness, cationic starch must be designed to have more substitutions. Nonetheless, this would increase the polymer production cost.

2.2.2. Auto and bioflocculation

Despite being different phenomena, it is common to refer to auto and bioflocculation as being the same concept. Autoflocculation (flocculation merely by pH increase) is an attractive alternative, as it is low cost, low energy, non-toxic to microalgae and does not require the use of flocculants, enabling simple medium reuse [40]. This process may occur naturally in microalgal cultures exposed to sunlight (in warm and sunny days) with limited CO_2 supply [19, 41]: through photosynthesis, microalgae remove CO_2 dissolved in the culture medium, increasing its pH value. Magnesium and calcium hydroxide have been used to study autoflocculation (Table 3). This phenomenon can also be simulated by the addition of NaOH, which is a low cost product [19, 40]. Horiuchi et al. [40] flocculated *Dunaliella tertiolecta* by adding a NaOH solution. The settling time was very short (few minutes) with a pH value between 8.6 and 10.5, resulting in a biomass recovery efficiency above 90%. High pH-induced flocculation may have further advantages when applied to microalgal cultures in wastewaters, due to its negative effect on pathogenic

microorganisms [34]. Additionally, autoflocculation has greater influence in the recovery of marine microalgae rather than in the recovery of freshwater ones [42]: under high pH values, ions present in seawater react producing chemical formations that lead to changes on microalgal cell surfaces and consequent acceleration of their settling. Studies using ammonia to alter the pH of the culture broth have suggested the existence of a pH threshold above which the process becomes more efficient. pH-induced flocculation mechanisms were thought to be affected by the balance between electrostatic repulsion and van der Waals attraction (reducing or neutralizing microalgal surface cell charge in response to an increase in the ionic strength of the culture medium). However, some studies currently indicate that an increase in pH generates precipitation of magnesium hydroxide, calcium hydroxide [34], calcium phosphate and other insoluble particles that cover the cell surface. This, in combination with charge neutralization or adhesive attraction, results in heavier flocs and, therefore, more stable settling [42]. Despite their advantages, these methods are not preferred at industrial scale for pre-concentration of microalgae, as they are not reliable for controlled flocculation and cause changes in cell composition [24].

Bioflocculation relates to microalgal flocculation caused by secreted biopolymers, especially by EPS [10]. Flocculants produced by bacteria can be an important economical step towards sustainable microalgal based biofuel production. Bioflocculation eliminates the need for chemical flocculants, which represent an expensive, non-feasible and toxic alternative. However, co-culture of microalgae with bacteria, fungi or flocculating microalgae results in microbiological contamination, interfering with food or feed applications of microalgal biomass [19]. In the case of biofuel production, the added microorganisms may even contribute to the increase in lipid yields. [24, 43]. Resulting culture media from these methods can also be effectively reused, thus significantly reducing microalgal cultivation costs [44]. The success of microbial flocculation depends on the production of EPS by the bacteria in high concentrations and the ability of microalgae to attach to them to form flocs [37].

Microbial flocculants have been widely used for wastewater treatment, as the wastewater can provide the necessary carbon source for flocculating microorganisms [19]. In fact, a large amount of common soil bacteria excrete mucous material with potential flocculation activity. In the study performed by Oh et al. [43], *Paenibacillus* sp. showed the highest flocculant activity (Table 4). However, to be effective, the produced flocculant needed

the addition of chemical flocculants, such as CaCl_2 . Although this method reduces the amount of chemical coagulant needed for efficient flocculation, it is not ideal. Nonetheless, a bioflocculant produced by *Solibacillus silvestris* was reported to be effective in flocculating microalgae without the addition of chemical coagulants. This bioflocculant is a significant improvement from earlier ones, since it can be recycled losing only 3% of flocculation efficiency and it is innocuous to microalgal cells [45].

Microalgal bacterial flocs (MaB flocs) are aggregations of these microorganisms able to settle quicker than microalgae alone, due to their larger size [46]. Both microalgae and bacteria produce EPS that are indistinguishable from one another. Furthermore, these polymers are responsible for cell adhesion without cell stress or lysis over an extended period [47]. However, it appears that the presence of these microorganisms is required for a predictable flocculation method [47, 48]. In fact, some bacteria from the genera *Flavobacterium*, *Terrimonas* and *Sphingobacterium*, which are frequently associated to microalgal growth, have shown a collective role on harvesting the microalga *Chlorella vulgaris*. Flocs formed in these xenic cultures presented diameters of about 100 μm , which resulted in higher sedimentation and flocculation ability (Table 4) when compared to axenic *C. vulgaris* flocs (20 μm). Also, the addition of the bacterial broth to the microalgal culture in a later growth stage showed a greater flocculation efficiency than the axenic culture, proving that both bacterial cells and bacterial extracellular metabolites play an important role in the process [48]. Lower substrate concentration seemed to be related to the formation of denser flocs with a large number of microalgal cells embedded. However, the recovery efficiency remained similar [47]. Typical MaB flocs present a diameter of 148 to 305 μm and the carbon source has a clear effect on its settleability. In cultures using an inorganic carbon source, filamentous cyanobacteria, such as *Phormidium* sp., tend to dominate, resulting in poor settling flocs [49].

Flocculation of microalgae by adding bacteria may require the addition of extra organic substrate and energy source to enable their growth. This will potentiate the incidence of bacterial and fungal contaminations upon microalgal cultures. In this way, the harvesting of non-flocculating microalgae by adding a flocculating species appears as a promising alternative method. This method enables total broth reuse, as it does not require the addition of chemicals. Also, it does not require different cultivating conditions, resulting in further savings. Furthermore, the addition of flocculating microalgae does not interfere with later downstream processing of the lipids into biodiesel. The only reason why these

microalgae are not used for biodiesel production in the first place is their low lipid contents [24]. Studies suggest that the mechanism of this flocculation method is both bridging and patching: when a large network of cells is formed the mechanism involved is bridging, while if the cells seem more closely attached, the mechanism involved may be patching by the EPS excreted from flocculating microalgae. Recovery efficiencies, as well as the time needed for microalgal sedimentation, are in the same range as the ones observed for chemically induced flocculation (Tables 2 and 5). Considering a raceway pond cultivation system of 100 ha and a disk stack bowl centrifuge, the application of this pre-concentrating method, with a ratio between flocculating and non-flocculating microalgae of 0.25, generates an energy saving per biomass dry weight of approximately 12 MJ kg_{dry weight}⁻¹ [50].

Microalgal/fungi association occurs naturally in lichens. Symbiotically, microalgae fix CO₂ and produce organic compounds, promoting fungal growth, which, in return, entrap the microalgae by hyphae production [44, 51]. Fungal assisted microalgal flocculation is effective for both heterotrophic and autotrophic microalgal species. Some filamentous fungi, for instance, *Rhizopus oryzae*, *Penicillium expansum* and *Mucor circinelloides*, were reported to form pellets with 2 to 5 mm of diameter. The use of fungi for pelletization has already been successfully applied in wastewater treatment, where they are used to entrap the sludge solids [52]. Furthermore, some fungal species were reported to have lipid contents of over 30% of total biomass, making them suitable for biodiesel feedstock along with the microalgal biomass [51]. Additionally, as the previous one, this flocculation technique does not require different cultivation conditions and allows total medium reuse without additional treatment [44]. Xie et al. [53] studied mixed cultures with *C. vulgaris* (fungi-to-algae ratio of 1:2) and observed that 99% of the biomass was removed after two days of co-cultivation. Besides the reduction of harvesting costs when applying this technology, biomass and lipid yields can be significantly increased. The presence of an organic carbon source along with pH adjustment to 4.0 to 5.0 seem to favour the process (Table 4) [51]. The harvested pellets have been successfully used for municipal and animal wastewater treatment, presenting higher nutrient removal rates than free microalgal cells. Therefore, this is a suitable way of bioenergy and bioproducts production, while contributing to the effective treatment of wastewaters [44].

2.2.3. Gravity sedimentation

Despite the rudimental character of the process, sedimentation works for various types of microalgae and is highly energy efficient [9]. Thus, when the end product has extremely low value, such as biofuels, gravity sedimentation should be selected for microalgal harvesting [9, 23]. Since microalgal density is a key to ensure the process efficiency, the reliability of this method is low. Microalgal settling rates of 0.1-2.6 cm h⁻¹ result in a very slow sedimentation process that leads to the deterioration of most of the biomass during the settling time, limiting the application of this method for routine harvesting [10]. In this way, to fasten microalgal settling, it is common to apply a coagulation/flocculation step prior to gravity sedimentation [23, 54, 55].

The best results of microalgal harvesting using gravity sedimentation were achieved through lamella-type separators (recovery of 1.6% TSS) and sedimentation tanks (recovery of 3% TSS) attributable to microalgal autoflocculation [31]. The use of sedimentation tanks is viewed as a simple and inexpensive process, but the concentration achieved is very low without previous coagulation/flocculation. In the same way, microalgal concentration by lamella-type separators is low and unreliable, requiring further thickening.

2.2.4. Flotation

Flotation is often defined as “inverted” sedimentation where gas bubbles fed to the broth provide the lifting force needed for particle transport and separation. This process is commonly applied in wastewater treatment processes and is often preceded by coagulation/flocculation [56]. Given microalgal low density and self-float characteristics, flotation is more effective and beneficial in microalgal removal than in sedimentation [57]. Microalgae such as *Microcystis* sp., which are often associated to wastewater, have a very reduced size (3-7 µm), requiring a pre-coagulation/flocculation step in order to produce aggregates of at least 10 µm, feasible of flotation recovery [56]. The major advantage of flotation is that it has been proved at large scale although it generally requires the use of flocculants [57]. Further advantages of flotation are low space requirements, relatively short operation times and high flexibility with lower initial equipment costs [56].

The success of flotation can be described as a product of two probabilities: (i) bubble-particle collision; and (ii) bubble-particle adhesion after a collision has occurred. In this way, it depends on the instability of the suspended particle, lower instability will result in

higher air-particle contact, and on particle size, the smaller they are, the more likely they are to be levitated by the bubbles [27, 31]. Particles in suspension must be hydrophobic, in order to attach to gas bubbles [57]. Flotation has been successfully applied in the separation of freshwater microalgae, such as *C. vulgaris*, and it is a promising low cost large scale harvesting method [58]. On the other hand, flotation of marine microalgae may be compromised, as salinity is a key factor for bubble-cell adhesion [58, 59]. Under high ionic strength, gas bubbles were reported to be larger and with tendency to rupture more easily. At controlled pH, an increase in ionic strength of the medium results in a decrease of flotation efficiency from 90-92% to 32% [58]. In this process, coagulation can be applied, by suppression of the electrical double layer, leading to floc formation [57]. Microalgal removal depends on recycling rate, air tank pressure, hydraulic retention time and particle floating rate, while the concentration of the produced slurry depends on skimmer velocity and relative positions towards the surface of the water [27].

Presently, there are four main flotation techniques: (i) dissolved air flotation (DAF – bubble diameter <100 μm); (ii) dispersed air flotation (DiAF – bubble diameter 100-1000 μm); (iii) electrolytic flotation; and (iv) ozonation-dispersed flotation (ODF) [9, 31, 54, 55]. DAF is the most efficient and widely employed option: this process requires dissolving air in water at very high pressure, so that the solution becomes supersaturated, leading to nucleation of bubbles when the pressure is reduced in the nozzle. The bubbles then float to the surface carrying the suspended solids that later can be skimmed off. In this way, DAF is very energy intensive. It is convenient to recycle a portion of the clarified liquid and saturate it with air to provide bubble flux [27]. On the other hand, DiAF generates bubbles by passing air continuously through a porous material. This process consumes less energy, but requires more expensive equipment and demands higher pressure drop for bubble generation. Electro-flotation depends on the formation of fine hydrogen bubbles by electrolysis [31]. Finally, ODF is a method that creates charged bubbles: as microalgal cells are negatively charged, the mechanism consists in the interaction of the charged bubbles with them [9]. Harvesting of *C. vulgaris* by this method has shown an increase on its lipid content by 24% and caused cell lysis. Cell lysis can be beneficial, as it releases to the broth a number of biopolymers that can act as coagulants, thus enabling a more effective separation (Table 5). However, it is a very expensive process that poses contamination problems when applied at large scale.

Separation of flocs depends on bubble flux and size. Small bubbles have high surface area to volume ratio and low rise velocity, which leads to faster attachment and tender contact with particles. In this way, the use of micro-sized bubbles (microflotation) is a good alternative towards separation of fragile flocs. However, the efficiency of microflotation in microalgal separation strongly depends on pH and coagulant dosage. This technique must use preferably a fluidic oscillator, since neither DAF nor DiAF are capable of generating the right size bubbles in a sustainable way [57].

2.2.5. Electrical based processes

Electrical approaches to microalgal harvesting are not largely disseminated. Nonetheless, these methods are versatile, as they are applicable to a wide variety of microalgal species, while being environmentally friendly (they do not require the addition of chemicals) [27, 60]. As microalgal cells are negatively charged, when an electrical field is applied to the culture broth, the cells can be separated [27]. They can form precipitates on the electrodes (electrophoresis), as well as accumulate on the bottom of the vessel (electro-flocculation). On the other hand, electro-flotation mechanism only differs from DAF in the way of the bubbles are made. Hydrogen bubbles are formed through water electrolysis, which can be cheaper than conventional DAF [31]. The generation of these bubbles can be done at the anode and coupled with the electro-coagulation that occurs through the electrolytic oxidation that happens at the cathode [61]. This process is described as electro-coagulation-flotation (ECF) [61].

Two types of electrodes can be applied: sacrificial and non-sacrificial [27]. The use of sacrificial electrodes results in metal ions being released to the broth, depending on the amount of electricity that passes through the electrolytic solution [27, 60]. The dissolution of the released ions from the reactive anode results in coagulant formation that destabilizes the microalgal suspension. Consequently, microalgal cells aggregate by combining with the positively charged metal ions [27]. This electrode dissolution is not comparable, toxicity wise, with the addition of chemical coagulants [60]. In this way, the probability of contamination with metallic hydroxides is significantly lowered for both methods [27]. The use of a non-sacrificial electrode is based only on the movement of the negatively charged microalgae towards the anode. Upon reaching the anode, cells lose their charge forming aggregates. Thus, this type of equipment is very prone to fouling when compared to chemical methods. Electrical based methods are largely influenced by

the material by which the electrodes are made. Aluminium and iron are the most commonly used metals in this type of process. Considering an harvesting period of 45 min, while an aluminium electrode performed a total removal efficiency (100%), an iron one only achieved a removal of 78.9%, mainly because these electrodes have less current efficiency and dissociate less than the aluminium ones. Hence, ferric electrodes are not convenient, as they consume more energy and result in brown coloured slurry, due to ferric oxide formation, the opposite from aluminium electrodes.

Current density deeply affects the reaction kinetics, showing a great influence in the harvesting efficiency, as well as in the energy consumption of the operation. The increase in current density results in shorter harvesting periods [61]. This can be attributed to the fact that the formation of aluminium ions by oxidation at the anode increases with current density and electrolysis time. High ion concentration increases coagulant surface area and active binding sites, promoting microalgal floc formation. However, while microalgal harvesting using a current density of 0.5 mA cm^{-2} required an energy consumption of 0.2 kWh m^{-3} , when a current density of 5.0 mA cm^{-2} was applied, energy consumption increased to 2.28 kWh m^{-3} [61]. Thus, despite the achievement of higher harvesting efficiencies with higher current densities, a balance must be set between the harvesting period and the energy consumption. Further optimization can be done by stirring the mixture in order to increase the probability of particle contact and therefore overcome the electrical double layer that prevents their aggregation. Electro-flocculation followed by mixing and settling lowers the energy requirements for microalgal harvesting. It requires higher operation times and results in a final slurry with a low concentration. On the other hand, electro-flocculation followed by flotation results in higher concentrations and energy requirements with lower processing times [62]. Particle collision and transport rate can also be increased by increasing process temperature [61]. Considering the ECF process time of 15 min, an increase in temperature from 18 to 36 °C resulted in an increase from 46 to 98% in the process recovery. Electrolysis time can also be shortened by increasing process temperature, as it improves the dissolution rate of aluminium. Therefore energy consumption of the electrolysis process can be lowered from 0.36 to 0.16 kWh m^{-3} , considering the same temperature range.

Broth properties, such as pH and initial cell concentration, can offer resistance to cell motion towards the electrodes [31, 61]. It is known that seawater microalgae are more easily separated and require approximately half the energy consumption than the

harvesting of freshwater species, as the low ionic strength of the culture medium hinders the separation process [60]. In the same way, the initial pH of the medium influences microalgal cell recovery, as it determines the type of aluminium ions formed. In a pH range of 4 to 7, the formed species are aluminium hydroxide, monomeric hydroxoaluminum cations and polymeric species such as $Al_{13}O_4(OH)_{24}^{7+}$. Hence the negatively charged microalgal cells are easily absorbed onto these positively charged precipitates, facilitating the harvesting process. On the other hand, in alkaline conditions, monomeric hydroxoaluminum anions dominate the solution, reducing the adsorption capacity of microalgal cells. In these conditions, efficient harvesting can only be achieved by increasing the electrolysis time, resulting in microalgal flocculation by sweeping and enmeshment mechanisms. Initial cell density does not strongly affect the energy consumption for complete microalgal removal. Nevertheless, microalgal recovery significantly decreases with the increase of initial cell density, as no sufficient aluminium is available for the removal of the excessive cells with short electrolysis time. While total recovery was achieved in only 25 min for an initial cell concentration of 0.55×10^9 cells L^{-1} , the same was only possible in 75 min for an initial cell concentration of 2.10×10^9 cells L^{-1} [61].

Despite these electrical methods present several advantages, energy requirements and equipment costs remain too high for sustainable large scale application at the time [10]. One must consider the scaling up of the electrodes and its effect on the optimization of operation parameters, such as pH, temperature, stirring, distance between the electrodes and their surface area.

2.3. Dewatering

Dewatering of the microalgal slurry is achieved by various mechanical means, including various types of filtration and centrifugation. After harvesting, the dewatered cake is usually dried to improve the efficiency of the downstream processes (e.g., lipid extraction).

2.3.1. Filtration

Filtration is mainly a dewatering means and it is normally applied following coagulation/flocculation to improve harvesting efficiency. Its application requires the maintenance of a pressure drop across the system to force fluid flow through a membrane.

In this process, microalgal deposits on the filtration membrane tend to grow thicker throughout the process, increasing resistance and decreasing filtration flux upon a constant pressure drop [31]. This phenomenon (called fouling/clogging) represents the main drawback associated to filtration methods, increasing their operational costs [10]. Critical flux is defined as the lowest flux that creates irreversible deposit on the membrane. However, limiting flux represents the maximum stationary permeation flux that can be reached, for a given tangential velocity, by increasing transmembrane pressure. Therefore, with the purpose of optimizing performance and minimize cleaning steps, it is necessary to work in the sub-critical zone. Nevertheless, even working at these conditions, gradual minor fouling can occur followed by a drastic increase that requires chemical cleaning. This phenomenon is further affected by the production of EPS, commonly secreted by microalgae when in stress conditions. These substances cause a gel-like layer in the filtration cake, increasing the resistance to flow, also requiring chemical cleaning to be eliminated [63]. Membranes must then be regularly cleaned to ensure sanitization and reusability.

Filtration is only sustainable for harvesting long length microalgae or those forming large colonies [51]. Despite microalgal cells of very low densities can be harvested by this method (a major advantage), membrane filtration is not commonly applied in large scale processes [23]. Both tangential flow and dead-end filtration modes can be applied. Also, different methods can be applied upon the required pressure drop, namely gravity, pressure, vacuum, or magnetic filtration [31, 64]. Dead-end filtration is effective in the recovery of large microalgal cells (diameter over 70 μm) [9, 23]. Tangential flow filtration (TFF) is considered more appropriate for the harvesting of smaller suspended algae due to minor fouling problems (Table 6) [10, 25]. Furthermore, this method allows the separation of shear sensitive suspensions [27]. As the medium flows tangentially across a membrane and the retentate is recirculated, the cells are kept in suspension, thus reducing membrane fouling. TFF has a better anti-fouling performance, given the deposit removal effect caused by the high fluid velocity tangential to the membrane [65]. Additionally, higher filtration rates can be achieved, enabling complete removal of microalgal cells and debris. Micro- or ultrafiltration membranes tend to be costly, energy intensive and need frequent membrane replacements [9]. Microfiltration (pore size from 0.1 to 10 μm) is suitable for harvesting fragile smaller cells. These membranes allow higher initial fluxes, but clog more easily [27]. On the other hand, ultrafiltration (pore

diameter: 1 to 100 nm) is appropriate for separation of solutes within 1 to 500 kDa of molecular weight. The performances of both methods rely on hydrodynamic conditions, concentration and culture properties. However, through ultrafiltration and for a long-term operation, low pressure and tangential velocity fluxes similar to other industrial installations for biotechnological applications are achieved. Ultrafiltration also seems to retain all cells and debris, which is convenient for these applications [66]. High gradient magnetic filtration can also be efficiently applied. Biosorption of submicron-sized magnetic particles has great potential on microalgal harvesting. Efficiencies of over 95% have been reported [67]. These particles must be low cost, reusable, chemically stable and have high adsorption capacity.

Filtration major costs are related with membrane replacement and pumping; thus, it is cost-effective only for small volumes [27]. In fact, microfiltration can be more cost-effective than centrifugation when the volume to be processed is less than $2 \text{ m}^3 \text{ d}^{-1}$. For volumes greater than $20 \text{ m}^3 \text{ d}^{-1}$, centrifugation may be more economic [23, 66]. The recommended design for pressure filtration, with respect to energy savings, reliability and concentration capability, are chamber filter press, cylindrical sieve and filter basket [31].

2.3.2. Centrifugation

Centrifugation is the fastest harvesting method, but also the most expensive due to its high energy consumption, which limits its application to high-valued products, such as highly unsaturated fatty acids, pharmaceuticals and other commodities [9, 10, 23, 51]. Centrifuges are able to harvest the great majority of microalgae [9]. Some are even efficient as one-step separation process, while others require a pre-concentrated algal slurry [31]. However, there are evidences that the exposure of microalgal cells to high gravitational and shear forces results in cell structure damage [28].

Normally, centrifuges are set to maximize capture efficiency. However, cost-effective microalgal harvesting may not coincide with the maximum capture efficiency [20]. To achieve high harvesting efficiencies, longer retention times in the bowl are needed to enable their sedimentation, due to the small size of these cells. High cell removal efficiency (94%) and low flow rate (0.94 L min^{-1}) implied a 20 kWh m^{-3} of culture water energy input, whereas only 0.80 kWh m^{-3} was needed for a 17% recovery at a flow rate of 23 L min^{-1} . In this way, while high capture efficiency (slower flow rates) required more energy per volume of culture, lower recoveries were offset by the increase in the

processed volume. This low energy conditions result in a decrease in overall cost per liter of produced oil (Table 7) [20]. If coagulation/flocculation is applied prior to centrifugation, its high energy consumption might be lowered, as it reduces the volume to be processed in 65%. Additionally, this combination resulted in a 3.8 fold increase in final algal concentration [30].

3. Research needs

The optimization of a pre-concentration step before the dewatering process is the most promising approach towards lowering microalgal harvesting costs. At the same time, environmental sustainability must be taken into account. Regarding biologically based harvesting methods, better understanding and control of auto and bioflocculation processes could improve their performance and reduce operational costs. Biological production of flocculants shows great potential, given the absence of microbial and chemical contamination. Nevertheless, little is known about which molecules have these properties, which microorganisms produce them and how they do it. On the other hand, the effect of co-cultivation of flocculating microorganisms and oil producing microalgae on their growth kinetics is yet to be accessed. If a favourable relationship exists, this method should also be further explored, given its cheapness and convenience of application. Manipulating microalgal characteristics through genetic engineering is also of importance. Clear identification of the mechanisms that allow some microalgal species to autoflocculate in a predictable way is imperative. These could then be genetically engineered onto high oil bearing species allowing the development of a reliable bioflocculation method. Other alternative may be the incorporation of the microalgal lipid overproduction apparatus onto microorganisms that are more commonly used in large scale operations, such as *Escherichia coli* and *Saccharomyces cerevisiae*, easing the scale up of all the processes related to biodiesel production, including biomass harvesting. Electrical approaches also present a high potential towards energetic optimization of microalgal harvesting, but the effect of the scale up of these methods should be determined. A low cost reliable method for pre-concentrate microalgal cultures is yet to be developed.

4. Conclusions

The harvesting methods described in this review constitute efficient ways to recover microalgal biomass from the culture medium. However, there is not a universal method that can be applied to harvest all microalgal strains with the same efficiency. An efficient method should be designed basing on microalgal properties, such as cell morphology and size and cell surface properties, on the properties of the culture medium and on the quality and value of the end product. Additionally, to improve harvesting efficiencies and costs, it is common to apply a combination of processes. Normally, a two-stage separation, composed of thickening and dewatering processes, is required. Concerning biofuel applications, bioflocculation followed by gravity sedimentation seems to be a cost-effective way of microalgal biomass recovery. However, the possibility of microbiological contaminations may limit the application of bioflocculation when the aim of the separation is animal feed or the production of high-valued compounds. Therefore, to avoid the toxicity of chemical coagulants/flocculants and microbiological contaminations from bioflocculation processes, electrical based methods may be the alternative.

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Figure captions

Figure 1. Diagram of microalgal harvesting and drying techniques.

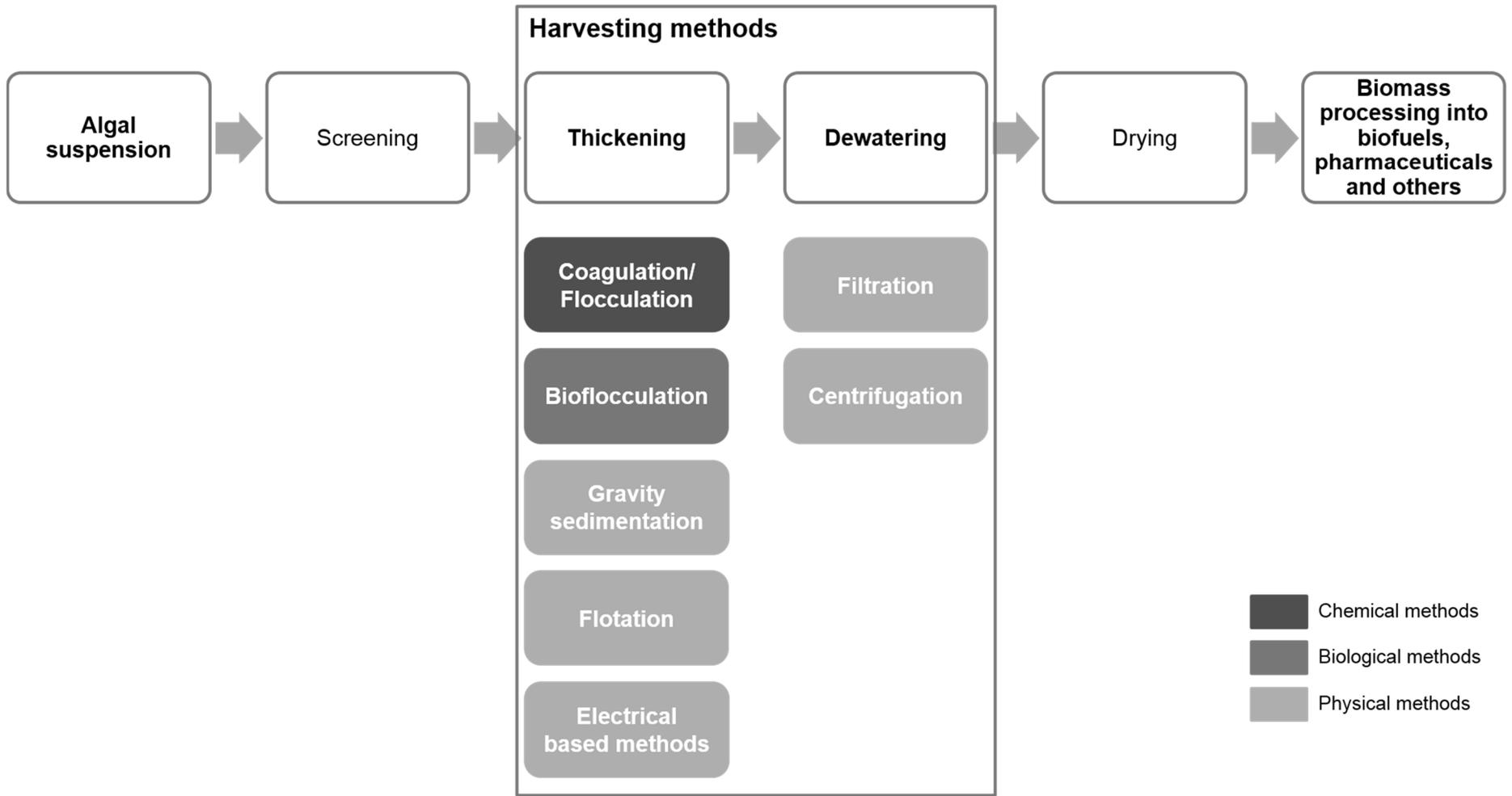


Figure 1.

Table 1. Advantages and disadvantages of different harvesting methods applied to microalgal biomass

Harvesting method	Advantages	Disadvantages
Chemical coagulation/flocculation	<ul style="list-style-type: none">• Simple and fast method;• No energy requirements.	<ul style="list-style-type: none">• Chemical flocculants may be expensive and toxic to microalgal biomass;• Recycling of culture medium is limited.
Auto and bioflocculation	<ul style="list-style-type: none">• Inexpensive method;• Allows culture medium recycling;• Non-toxic to microalgal biomass.	<ul style="list-style-type: none">• Changes in cellular composition;• Possibility of microbiological contamination.
Gravity sedimentation	<ul style="list-style-type: none">• Simple and inexpensive method.	<ul style="list-style-type: none">• Time-consuming;• Possibility of biomass deterioration;• Low concentration of the algal cake.
Flotation	<ul style="list-style-type: none">• Feasible for large scale applications;• Low cost method;• Low space requirements;• Short operation times.	<ul style="list-style-type: none">• Generally requires the use of chemical flocculants;• Unfeasible for marine microalgae harvesting.
Electrical based processes	<ul style="list-style-type: none">• Applicable to a wide variety of microalgal species;• Do not require the addition of chemical flocculants.	<ul style="list-style-type: none">• Poorly disseminated;• High energetic and equipment costs.
Filtration	<ul style="list-style-type: none">• High recovery efficiencies;• Allows the separation of shear sensitive species.	<ul style="list-style-type: none">• The possibility of fouling/clogging increases operational costs;• Membranes should be regularly cleaned;• Membrane replacement and pumping represent the major associated costs.
Centrifugation	<ul style="list-style-type: none">• Fast method;• High recovery efficiencies;• Suitable for almost all microalgal species.	<ul style="list-style-type: none">• Expensive method;• High energy requirements;• Suitable only for the recovery of high-valued products;• Possibility of cell damage due to high shear forces.

Table 2. Microalgal biomass recovery by coagulation/flocculation followed by sedimentation.

Microalgae	Coagulant	Experimental set-up	R (%)	Ref.
<i>Chlorella vulgaris</i>	Nano-aminoclays (Mg-APTES)	CoC: CM - F/2 medium; S = 30 g L ⁻¹ ; T = 30 °C; V = 500 mL; BC = 1.0 g _{dry weight} L ⁻¹ ; CD = 1.0 g L ⁻¹ ; pH 5.0-12.0; HP = 30 min; HM - batch.	99	[68]
<i>Chlorella vulgaris</i>	Chitosan	CoC: CM - BG-11; V = 250 mL; T = 25 °C; pH 6.0 (adjusted) CD = 30 mg L ⁻¹ ; HP = 10 min; HM - batch.	92	[69]
<i>Chlorella sorokiniana</i>	Chitosan	CoC: CM - modified Tris acetate phosphate medium with 10% NH ₄ Cl; V = 1 L; BC = 0.2-1 g _{dry weight} L ⁻¹ ; CD = 10 mg g ⁻¹ microalgal biomass; pH 6; FC = 200 USD ton ⁻¹ microalgal biomass; HM - batch.	99	[70]
<i>Chlorella minutissima</i>	Fe ₂ (SO ₄) ₃	CoC: CM - f/2 medium; V = 20 mL; BC = 220 ×10 ⁶ cells mL ⁻¹ ; CC = 0.75 g L ⁻¹ ; HP = 4 h; HM - batch.	80	[16]
<i>Chlorella minutissima</i>	AlCl ₃	CoC: CM - f/2 medium; V = 20 mL; BC = 220 ×10 ⁶ cells mL ⁻¹ ; CC = 0.5 g L ⁻¹ ; HP = 1 h; HM - batch.	80	[16]
<i>Phaeodactylum tricorutum</i>	PAC (polyaluminium chloride)	CoC: CM - filtered and sterilized seawater with commercial fertilizer, B group vitamins and sodium silicate; V = 250 mL; BC = 104.62 mg _{dry weight} L ⁻¹ ; CD = 0.27 kg kg ⁻¹ microalgal biomass; pH 7.5; FC = 0.429-1,429 USD kg ⁻¹ coagulant; HM - batch.	67	[41]
<i>Phaeodactylum tricorutum</i>	Al ₂ (SO ₄) ₃	CoC: CM - filtered and sterilized seawater with commercial fertilizer, B group vitamins and sodium silicate; V = 250 mL; BC = 104.62 mg _{dry weight} L ⁻¹ ; CD = 0.27 kg kg ⁻¹ microalgal biomass; pH 5.9; FC = 0.976-2.073 USD kg ⁻¹ coagulant; HM - batch.	83	[41]
<i>Phaeodactylum tricorutum</i>	Chitosan	CoC: CM - filtered and sterilized seawater with commercial fertilizer, B group vitamins and sodium silicate; V = 250 mL; BC = 104.62 mg _{dry weight} L ⁻¹ ; CD = 0.18 kg kg ⁻¹ microalgal biomass; pH 9.9 (adjusted); FC = 2-100 USD kg ⁻¹ coagulant; HM - batch.	91,8	[41]
<i>Dunaliella salina</i>	Electrolytic flocculation;	CoC: n/a; CD = 50 A m ⁻² ; HP = 7 min; EC = 0.23 kWh m ⁻³ .	98.9	[60]

<i>Coelosphaerium</i> sp., <i>Aphanizomenon</i> sp., <i>Closterium</i> sp., <i>Pediastrum</i> sp., <i>Cryptomonas</i> sp., <i>Staurastrum</i> sp., <i>Asterionella</i> sp., <i>Cyclotella</i> sp. and <i>Melosira</i> sp.	Electrolytic flocculation	6 cathodes and 3 anodes 26.3 cm apart; Voltage = 26.5 V; CI = 1.0 A; BC = 1 - 50 mg _{dry weight} L ⁻¹ ; HP = 75 min; HM - batch; EC = 0.331 kWh m ⁻³ .	96.3	[71]
<i>Tetraselmins</i> sp.	Electro-flocculation	CoC: CM - f/2 medium; T= 22 °C; S = 1 M; pH 8.4 (adjusted); C _i = 0.25 kg _{dry} weight m ⁻³ ; V = 150 mL; CI = 1.33 A; CD = 100 A m ⁻² ; HP = 5.75 min; EC = 0.559 MJ m ⁻³	87	[62]
<i>Tetraselmins</i> sp.	Electro-flocculation followed by gravity sedimentation	CoC: CM - f/2 medium; T= 22 °C; S = 1 M; pH 8.4 (adjusted); C _i = 0.25 kg _{dry} weight m ⁻³ ; V = 150 mL; Voltage = 10 V, CI = 5 A; SS = 7.5 rad s ⁻¹ ; HP = 45.5 min; EC = 0.328 MJ m ⁻³ .	91	[62]

BC – initial biomass concentration, CC – coagulant concentration, CD – coagulant dose, CI – current intensity, CM – culture medium, CoC – conditions of culturing, EC – energy consumption, FC – Flocculation cost, HM – harvesting mode, HP – harvesting period, R – recovery efficiency, $R = C_i - C_f / C_i \times 100\%$, where C_i and C_f are the initial and final microalgal concentrations, respectively, S – salinity, SS – stirring speed, T – temperature, V – volume.

Table 3. Microalgal biomass recovery by autoflocculation followed by sedimentation

Microalgae	Procedure	Experimental set-up	R (%)	Ref.
<i>Chlorella vulgaris</i>	pH adjustment, NaOH	CoC: CM - dechlorinated tap water enriched with inorganic nutrients, with aeration; V = 30 L; BC = 0.5 g _{dry weight} L ⁻¹ ; CD = 9 mg g ⁻¹ microalgal biomass; pH 10.8; HM - batch.	98	[34]
<i>Chlorella vulgaris</i>	pH adjustment, KOH	CoC: CM - dechlorinated tap water enriched with inorganic nutrients, with aeration; V = 30 L; BC = 0.5 g _{dry weight} L ⁻¹ ; CD = 12 mg g ⁻¹ microalgal biomass; pH 10.8; HM - batch.	98	[34]
<i>Chlorella vulgaris</i>	pH adjustment, Ca(OH) ₂	CoC: CM - dechlorinated tap water enriched with inorganic nutrients, with aeration; V = 30 L; BC = 0.5 g _{dry weight} L ⁻¹ ; CD = 18 mg g ⁻¹ microalgal biomass; pH 10.8; HM - batch.	98	[34]
<i>Chlorella vulgaris</i>	pH adjustment, Mg(OH) ₂	CoC: CM - dechlorinated tap water enriched with inorganic nutrients, with aeration; V = 30 L; BC = 0.5 g _{dry weight} L ⁻¹ ; CD = 27 mg g ⁻¹ microalgal biomass; pH 9.7; FC = 18 USD ton ⁻¹ microalgal biomass; HM - batch.	98	[34]
<i>Chlorella vulgaris</i>	None	CoC: CM - marine medium from Salim et al [24]; SS = 100 rpm; 2% CO ₂ enriched airflow; light:dark cycle of 16:8 h; LI = 50 μmol m ⁻² s ⁻¹ ; V = 100 mL; T = 25 °C; HP = 3 h; HM - batch.	25	[50]
<i>Neochloris oleoabundans</i>	None	CoC: CM - marine medium from Salim et al. [24]; SS = 100 rpm; 2% CO ₂ enriched airflow; light:dark cycle of 16:8 h; LI = 50 μmol m ⁻² s ⁻¹ ; V = 100 mL; T = 25 °C; HP = 3 h; HM - batch.	40	[50]

BC – initial biomass concentration, CC – coagulant concentration, CD – coagulant dose, CI – current intensity, CM – culture medium, CoC – conditions of culturing, HM – harvesting mode, HP – harvesting period, LI – light intensity, R – recovery efficiency, $R = C_i - C_f / C_i \times 100\%$, where C_i and C_f are the initial and final microalgal concentrations, respectively, SS – stirring speed, T – temperature, V – volume.

Table 4. Microalgal biomass recovery by bioflocculation followed by sedimentation

Microalgae	Microorganism	Experimental set-up	R (%)	Ref.
<i>Chlorella vulgaris</i>	<i>Paenibacillus</i> sp.	CoC: CM - modified Chu 13 medium; V = 50 mL. BC - use of diluted <i>Paenibacillus</i> sp. broth as flocculating agent; pH 5-11 (adjusted); CC(CaCl ₂) = 6.8 mM; HM - batch.	93	[43]
<i>Botryococcus braunii</i> , <i>Scenedesmus quadricauda</i> and <i>Selenastrum capricornutum</i>	<i>Paenibacillus</i> sp.	CoC: CM - modified Chu 13 medium; V = 50 mL; BC - use of diluted <i>Paenibacillus</i> sp. broth as flocculating agent; pH 5-11 (adjusted); CC(CaCl ₂) = 6.8 mM; HM - batch.	91-95	[43]
<i>Anabaena</i> <i>flos-aquae</i> and <i>Microcystis</i> <i>aeruginosa</i>	<i>Paenibacillus</i> sp.	CoC: CM - modified Chu 13 medium; V = 50 mL; BC - use of diluted <i>Paenibacillus</i> sp. broth as flocculating agent; pH 5-11 (adjusted); CC(CaCl ₂) = 6.8 mM; HM - batch.	38-49	[43]
<i>Nannochloropsis oceanica</i>	<i>Solibacillus silvestris</i>	CoC: CM - f/2 medium; V = 200 mL; Purified bioflocculant from <i>Solibacillus silvestris</i> growth media was used at the concentration of 0.1% (w/v); pH 8.7; T = 25 °C; HM - batch.	88	[45]
<i>Pleurochrysis carterae</i>	Tap water bacterial inoculum	CoC: CM - modified BG-11 medium, 0.5 g L ⁻¹ substrate (acetate, glycerine or glucose) with mixing; V = 100 mL; no illumination; 0.09% (v/v) of the microbial culture is used as flocculating agent; HP = 30 min; HM - batch.	90-93 (CF = 100 -131)	[47]
<i>Chlorella</i> sp., <i>Pediastrum</i> sp., <i>Phormidium</i> sp. and <i>Scenedesmus</i> sp. consortium	Aerobic activated sludge bacterial inoculum from a municipal wastewater treatment plant	CoC: co-cultivation in synthetic wastewater; light:dark cycle of 15:9 h; LI = 100 μmol m ⁻² s ⁻¹ ; V = 1.5 L; T = 23-29 °C; HP = 1.5 h; HM - batch.	98 (23.2 g TSS L ⁻¹)	[46]
<i>Chlorella vulgaris</i>	<i>Ettlia texensis</i>	CoC: co-cultivation; CM - marine medium from Salim et al. [24]; SS = 100 rpm; 2% CO ₂ enriched airflow; light:dark cycle of 16:8 h; LI = 50 μmol m ⁻² s ⁻¹ ; V = 100 mL; T = 25 °C; HP = 3 h; R _{inf} = 1; HM - batch.	55	[50]
<i>Chlorella vulgaris</i>	<i>Scenedesmus obliquus</i>	CoC: co-cultivation; CM - marine medium from Salim et al. [24]; SS = 100 rpm; 2% CO ₂ enriched airflow; light:dark cycle of 16:8 h; LI = 50 μmol m ⁻² s ⁻¹ ; V = 100 mL; T = 25 °C; HP = 3 h; R _{inf} = 1; HM - batch.	34	[50]

<i>Chlorella vulgaris</i>	<i>Ankistrodesmus falcatus</i>	CoC: co-cultivation; CM - marine medium from Salim et al. [24]; SS = 100 rpm; 2% CO ₂ enriched airflow; light:dark cycle of 16:8 h; LI = 50 μmol m ⁻² s ⁻¹ ; V = 100 mL; T = 25 °C; HP = 3 h; R _{mf} = 1; HM - batch.	50	[50]
<i>Neochloris oleoabundans</i>	<i>Tetraselmis suecica</i>	CoC: co-cultivation, CM - marine medium from Salim et al. [24]; SS = 100 rpm, 2% CO ₂ enriched airflow, light:dark cycle of 16:8 h, LI = 50 μmol m ⁻² s ⁻¹ , V = 100 mL, T = 25 °C; HP = 3 h, R _{mf} = 0.74; HM: batch.	72	[50]
<i>Chlorella vulgaris</i>	<i>Aspergillus oryzae</i>	CoC: co-cultivation, heterotrophic; CM - supplemented BG-11; CS = 10 g L ⁻¹ ; glucose; under dark; T = 25 °C; SC = 1.1×10 ⁴ spores mL ⁻¹ ; pH 4.5-7.0 (adjusted); HP = 3 d; HM - batch.	97	[51]
<i>Chlorella vulgaris</i>	<i>Aspergillus oryzae</i>	CoC: co-cultivation, autotrophic; CM - supplemented BG-11; light:dark cycle of 24:0 h; LI = 120 μmol m ⁻² s ⁻¹ ; T = 25 °C; SC = 1.1×10 ⁴ spores mL ⁻¹ ; pH 4.0-5.0 (adjusted); CS = 10 g L ⁻¹ glucose (later added); HP = 2 d; HM - batch.	93	[51]
<i>Chlorella vulgaris</i>	<i>Aspergillus oryzae</i>	CoC: co-cultivation, autotrophic; CM - supplemented BG-11; light:dark cycle of 24:0 h; LI = 120 μmol m ⁻² s ⁻¹ ; T = 25 °C; SC = 1.1×10 ⁴ spores mL ⁻¹ ; pH 4.0-5.0 (adjusted); HP = 3 d; HM - batch.	63	[51]
<i>Chlorella vulgaris</i>	<i>Cunninghamella echinulata</i>	CoC: co-cultivation; T = 25 °C; SS = 120 rpm; light:dark cycle of 24:0 h; R _{fm} = 1:2; SC = 3.44×10 ⁷ spores L ⁻¹ .	99	[53]

BC – initial biomass concentration, CC – coagulant concentration, CoC – conditions of culturing, CS – carbon source, CF – concentration factor, HP – harvesting period, LI – light intensity, R – recovery efficiency, $R = C_i - C_f / C_i \times 100\%$, where C_i and C_f are the initial and final microalgal concentrations, respectively, R_{mf} – ratio of flocculating/non-flocculating microalgae, SC – spore concentration, SS – stirring speed, T – temperature, TSS – total suspended solids, V – volume.

Table 5. Microalgal biomass recovery by flotation

Microalgae	Process	Experimental set-up	R (%)	Ref.
<i>Chlorella</i> sp.	Flotation	CoC: NC medium; T = 25 °C; SS = 75 rpm; V = 200 mL; pH 8; AFR = 114 mL min ⁻¹ ; BC = 6.8 × 10 ⁵ cells mL ⁻¹ ; addition of chitosan (10 mg L ⁻¹) and SDS (20 mg L ⁻¹).	90	[58]
<i>Dunaliella salina</i>	Microflotation preceded by flocculation	CoC: <i>D. salina</i> growth medium; V = 2 L; T = 20 °C; microflotation preceded by flocculation with 150 mg L ⁻¹ FeCl ₃ ; pH 5, DD = 26 cm.	98.2	[57]
<i>Microcystis aeruginosa</i>	Electro-coagulation-flotation	CoC: n/a; T = room temperature; pH 7; V = 1 L; SS = 200 rpm; C _i = 1.2×10 ⁹ - 1.4×10 ⁹ cells L ⁻¹ ; HP = 45 min; aluminum electrode.	100	[61]
<i>Microcystis aeruginosa</i>	Electro-coagulation-flotation	T = room temperature; pH 7; V = 1 L; SS = 200 rpm; C _i = 1.2×10 ⁹ - 1.4×10 ⁹ cells L ⁻¹ ; CD = 1 mA cm ⁻² ; HP = 45 min; iron electrode.	78.9	[61]
<i>Microcystis aeruginosa</i>	Electro-coagulation-flotation	T = room temperature; pH 7; V = 1 L; SS = 200 rpm; C _i = 1.2×10 ⁹ - 1.4×10 ⁹ cells L ⁻¹ ; CD = 0.5 mA cm ⁻² ; HP = 25 min aluminum electrode; EC = 0.20 kWh m ⁻³ .	45	[61]
<i>Microcystis aeruginosa</i>	Electro-coagulation-flotation	T = room temperature; pH 7; V = 1 L; SS = 200 rpm; C _i = 1.2×10 ⁹ - 1.4×10 ⁹ cells L ⁻¹ ; CD = 5.0 mA cm ⁻² ; HP = 25 min aluminum electrode; EC = 2.28 kWh m ⁻³ .	100	[61]

AFR – air flow rate, BC – initial biomass concentration, CD – current density, CoC – conditions of culturing, DAF – Dissolved air flotation, DD – distance from the diffuser, EC – energy consumption, HP – harvesting period, R – recovery efficiency, $R = C_i - C_f / C_i \times 100\%$, where C_i and C_f are the initial and final microalgal concentrations, respectively, SS – stirring speed, T – temperature, V – volume.

Table 6. Microalgal biomass recovery by filtration

Microalgae	Process	Experimental set-up	Resulting TSS (%)	Ref.
<i>Coelastrum proboscideum</i>	Pressure filtration; Netzsch chamber filter	HM - batch; one step; EC = 0.88 kWh m ⁻³ .	22-27 (CF = 245)	[23]
<i>Coelastrum proboscideum</i>	Pressure filtration; Cylindrical sieve rotator; Englesmann	HM - continuous; one step; EC = 0.3 kWh m ⁻³ .	7.5 (CF = 75)	[23]
<i>Coelastrum proboscideum</i>	Vacuum filtration; Non-precoat vacuum drum filter	HM - continuous; one step; EC = 5.9 kWh m ⁻³ .	18 (CF = 180)	[23]
<i>Coelastrum proboscideum</i>	Vacuum filtration; Suction filter	HM - batch; one step; EC = 0.1 kWh m ⁻³ .	8 (CF = 80)	[23]
<i>Chlorella vulgaris</i>	Magnetic filtration	CoC: CM - modified TAP-medium (no TRIS, no acetate); C _{salt,0} = 0.7-3.1 g L ⁻¹ ; V = 5 mL; pH 8 (adjusted); MagSilica seeding m _{mp} /m _{X,0} = 1; HP = 30 min; HM - continuous.	90	[72]
<i>Chlorella vulgaris</i>	Submerged microfiltration; Membrane PVDF-9	CoC: CM - WC medium; pH 8.5 (adjusted); BC = 0.41 g _{dry weight} L ⁻¹ ; EC = 0.27 kWh m ⁻³ .	98	[73]
<i>Phaeodactylum tricornutum</i>	Submerged microfiltration; membrane PVDF-9	CoC: CM: WC medium supplemented with synthetic seawater; pH 8.5 (adjusted); BC = 0.23 g _{dry weight} L ⁻¹ ; EC = 0.25 kW h m ⁻³ .	70	[73]
<i>Phaeodactylum tricornutum</i>	Ultrafiltration	CoC: CM - f/2 medium; BC = 3.7 x 10 ⁶ cells L ⁻¹ ; V = 900 L; HP = 1 h; EC = 0.75 USD kg ⁻¹ _{biomass} .	CF = 10	[74]

BC – initial biomass concentration, CF – concentration factor, CM – culture medium CoC – Conditions of culturing, C_{salt,0} – initial salt concentration, EC – energy consumption/cost, HM – harvesting mode, HP = harvesting period, m_{mp}/m_{X,0} – ratio between mass of magnetic particles and biomass at initial time, V – volume, TSS – total suspended solids.

Table 7. Microalgal biomass recovery by centrifugation

Microalgae	Equipment	Experimental set-up	Resulting TSS (%)	Ref.
<i>Scenedesmus</i> sp. and <i>Coelastrum proboscideum</i>	Self-cleaning, disk-stack centrifuge; Westfalia	CoC: n/a; HM: continuous; one-step; EC = 1 kWh m ⁻³ .	12 (CF = 120)	[23]
<i>Scenedesmus</i> sp. and <i>Coelastrum proboscideum</i>	Nozzle discharge centrifuge; Westalia	CoC: n/a; HM: continuous; EC = 0.72 kWh m ⁻³ .	2-15 (CF = 20-150)	[23]
<i>Nannochloropsis</i> sp.	1.5-HP (1.12 kW) continuous-flow centrifuge; US Filter-Maxx	CoC: CM - f/2 medium; BC = 100 mg _{dry weight} L ⁻¹ ; HM - continuous; EC = 20 kWh m ⁻³ .	96	[20]
<i>Nannochloropsis</i> sp.	1.5-HP (1.12 kW) continuous-flow centrifuge; US Filter-Maxx	CoC: CM - f/2 medium; BC = 100 mg _{dry weight} L ⁻¹ ; HM - continuous; EC = 0.8 kWh m ⁻³ .	17	[20]

BC – initial biomass concentration, CF – concentration factor, CM – culture medium, CoC – conditions of culturing, EC – energy consumption, HM – harvesting mode, TSS – total suspended solids.