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# A comparative analysis of algal biomass densification methods aimed at the biofuel production

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#### Abstract

The reduced size of the cells (< 30  $\mu$ m in diameter) and the low cell concentration of microalgae and cyanobacteria cultures (0.5 g/L on average) greatly hinder the densification of the biomass, a crucial step to becoming biofuel production from algal biomass economically viable. To identify the best densification method, about 90 studies applied to 60 different microalgae and cyanobacteria were compiled through an extensive literature survey, classified into eight methods of algal biomass densification, and analyzed regarding the harvesting efficiency, energy consumption, and costs. Low-cost methods (spontaneous settling, autoflocculation, and bioflocculation) can achieve high densification only with specific species and conditions. On the contrary, many species only achieve high densification using high-cost methods (centrifugation, membrane filtration, electrocoagulation). Based on the analyzed data, researchers should focus on selecting species that combine the desired characteristics for the downstream processes with their potential for biofuel production.

Keywords: Algal biomass; Biomass densification; Harvesting efficiency; Energy consumption; Biofuel

#### **1. Introduction**

To the detriment of traditional fossil fuels, using renewable energy sources, such as biofuels, presents itself as an alternative to guarantee the well-being of humanity and ecosystems. Third-generation biofuels, derived from microalgae and cyanobacteria, can be a potential energy source to solve disadvantages encountered in producing first-and second-generation biofuels [1]. Thus, several studies have investigated the potential of microalgae and cyanobacteria to capture  $CO_2$  and produce biofuels.

However, 3rd generation biofuel production depends directly on biomass concentration, low in microalgae and cyanobacteria cultures (final concentration below 0.5 g/L, as dry weight). Furthermore, the small cell size significantly complicates the recovery process being necessary in some cases to use one or more solid-liquid separation steps for efficient recovery [2]. An efficient and low-cost harvesting method is essential to produce biofuels from microalgae and cyanobacterial biomass. In addition to efficiency and low cost, for the use of the resulting concentrate in biogas production, the chemicals used in biomass harvesting must not have toxic or inhibitory effects on the microbial population involved in anaerobic digestion [3].

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The harvesting technologies are classified as mechanical (settling, dissolved air flotation, centrifugation, and filtration), electrical (electrocoagulation, electroflotation), chemical (chemical flocculation), and biological (bioflocculation, autoflocculation) methods [4].

Electrochemical methods, such as electroflotation and electro-coagulation-flotation, are based on the electrophoresis of the microalgae cells and the negative surface charge of microalgal cells. They have low cost and are energy-effective but have very low harvesting efficiency and the problem of residual chemicals that degrade biomass quality [5]. Flotation involves bubbling air through a suspension to create interactions with microalgal cells, resulting in froth or scum formation on the liquid surface. However, it needs to be combined with flocculation to achieve good results, and it is not suitable for a full-scale process [4].

Most researchers point out that improvements in the microalgae production step are essential to reducing biofuel production's downstream cost. However, this cost reduction can occur in itself harvesting/concentration step. It is possible to develop densification methods of algal biomass with high efficiency and low cost that does not impair biofuel production. This study systematically compared eight technologies (spontaneous settling, chemical flocculation, autoflocculation, bioflocculation, centrifugation, membrane filtration, flotation, and electrocoagulation) for their performance in harvesting microalgal biomass.

# 2. Densification methods of algal biomass

## 2.1. Spontaneous settling (or self-settling)

Settling occurs when microalgae cells form flocs and sediment spontaneously, mainly due to the extracellular polymeric substances (EPS) excretion in the culture medium. The EPSs are primarily composed of proteins and carbohydrates, available in microalgae suspension under stress conditions caused by the absence or excess of nutrients in the medium and by variations in lighting and temperature [6-9].

Few species of microalgae and cyanobacteria show high efficiencies of spontaneous settling, such as *Arthrospira platensis, Ettlia texensis, Scenedesmus quadricauda*, and *Desmodesmus* sp. [8,10-12]. Depraetere et al. [11] evaluated the behavior of the filamentous cyanobacterium *Arthrospira platensis* in cultures with low nitrogen concentrations. After the ninth day of cultivation under nitrogen stress, the cells accumulated large amounts of glycogen, increasing the specific density of the filaments and providing spontaneous settling at a maximum speed of 0.64 m/h.

Cui et al. [13] evaluated the gravitational sedimentation of *Heveochlorella* sp. and Lv et al. [14] evaluated the gravitational sedimentation of the microalgae *Chlorococcum* sp. Both authors related the sedimentation ability to the hydrophobic EPSs. Other examples of spontaneous settling are in Table 1, which shows harvesting efficiencies above 90% with sedimentation times from 1 to 24 h, depending on microalgae and cyanobacterial species and cultivation conditions. Table 1 also indicates studies with non-flocculating cells and cells washed to remove EPSs, verifying efficiencies below 30% in these cases. However, the reason for the poor flocculation of some species remains unclear.

Although the mechanisms involved in spontaneous settling are not yet fully understood, species that present high sedimentation efficiencies in reduced times may be promising because, in this method, the algal cells can be concentrated without the addition of chemicals and with low energy consumption equipment. The elucidation of the mechanisms of spontaneous settling of flocculating microalgae, as well as the study of the flocculating agents produced by microalgae, may benefit genetic modifications of non-flocculating strains with the flocculation phenotype for the concentration of biomass by sedimentation [15].

#### 2.2. Flotation

Flotation is a solid-liquid separation method commonly used in wastewater treatment and microalgae biomass concentration. This concentration method inserts air through a diffuser (dispersed air flotation) or pressurization (dissolved air flotation) into a column containing the biomass suspension. In this system, surfactants and flocculants can be used to destabilize the electrostatic forces in the microalgae suspension and for the formation of bubbles. In this way, the microalgae cells adhere to the surface of the bubbles and float to the top [16-18].

Flotation is an efficient method with low-cost implantation and operation and low energy cost. Because of that, freshwater and marine microalgae of the most diverse species are concentrated by flotation, as seen in Table 1. Flotation studies focus on the adequacy of parameters, such as bubble size, pH, salinity, column design, air flow, and feed flow

rate, as well as the dosage of surfactants and flocculants, to improve biomass recovery and increase the concentration factor [19,20].

Some modifications of the flotation method stand out as innovations in improving biomass concentration, such as microflotation, in which microbubbles (50  $\mu$ m) are generated through fluidic oscillation in a dispersed air flotation column [21]. Ballasted flotation is also an example in which microspheres of sodium borosilicate glass are used to aggregate microalgae cells [22]. The main obstacles in this method are related to the scale-up. Most studies are on a laboratory scale, and parameters are adjusted not to be energy intensive. Another obstacle would be the dosage of coagulants and surfactants. The process becomes expensive if a large volume of these chemicals is needed. In addition, the chemicals inserted into the microalgae suspension cannot cause contamination to the biomass [23].

## 2.3. Membrane filtration

Filtration in micro and ultrafiltration membranes aims to separate algal cells from the liquid fraction of the culture medium due to the reduced size of the cells ( $\leq$  30 µm). Compared to traditional separation methods, membrane filtration has the advantage of not having to add chemical compounds and, consequently, contamination of the biomass. The technique provides the separation of cells from the culture medium with high efficiency of biomass concentration without damaging the cells [24]. The concentration efficiency can be greater than 85% for cultures with different initial densities, as shown in Table 1. However, one of the main disadvantages of the method is membrane clogging, which reduces the permeate flux and, consequently, the concentration of the microalgae suspension. Therefore, many studies on microalgae filtration focus mainly on evaluating better operating conditions to reduce membrane clogging [24-26].

Researchers also study membrane surface modifications to reduce fouling and improve membrane performance. Some examples are surface-coating with a functional coating material like hydrophilic polyvinyl alcohol (PVA) polymer [27] or the use of membranes with a wave pattern on their surface [28]. Another obstacle in using membrane filtration is the amount of energy required when pumping is necessary. Das et al. [29] evaluated different densification methods of a suspension of *Tetraselmis* sp., verifying that the membrane filtration resulted in higher efficiency and energy consumption. The authors concluded that decision-makers should prefer membrane filtration when biomass presents many high-added value metabolites.

#### 2.4. Centrifugation

Centrifugation is a physical separation method based on centrifugal force in a rotational acceleration movement, which acts on the difference in density between the particles, separating the microalgae cells from the culture medium. The main centrifuges are disc-stacks, perforated baskets, and non-perforated baskets. Hydrocyclones are also used as a centrifugation method. Among the various microalgae densification techniques, centrifugation is one of the most used methods due to the speed of the separation process and its ability to process large volumes with high concentration efficiencies [30].

However, full-scale systems are potentially more expensive due to operating and equipment maintenance costs [25]. [30]. Although centrifugation provides high microalgae cell concentration rates (as presented in Table 1), the product must have high added value to make the microalgae densification method economically viable [31].

**Table 1** Harvesting of microalgae and cyanobacteria by mechanical methods

Specie (Initial conc. g/L <sup>a</sup> ) Conditions		Efficiency (%)	Reference		
Spontaneous settling (self-settling or gravity sedimentation)					
Arthrospira platensis (na)	N stressed cultures, settling time 1 h Control cultures, settling time 1 h	94.0 no settling	[11]		
Chlorella vulgaris (0.62-0.82 <sup>b</sup> )	Settling time 1 h	60.0	[32]		
Chlorella vulgaris (0.891 <sup>b</sup> ) Chlorella vulgaris (0.880 <sup>b</sup> )	Self-flocculating, settling time 0.5 h Non-flocculating, settling time 0.5 h	76.3 25.6	[15]		
Chlorococcum sp. (0.08) Parachlorella kessleri (0.08)	Self-flocculating, settling time 3 h Non-flocculating, settling time 3 h	84.4 16.2	[14]		
Chroococcidiopsis sp. (1.14)	Self-flocculating, settling time 1 h	97.0	[33]		
Desmodesmus sp. (0.81)	Stationary phase, settling time 0.5 / 2.5 h	12.4 / 90.0	[8]		
Ettlia texensis (0.89-1.19)	Stationary phase, settling time 1 h	90.0	[10]		
<i>Heveochlorella</i> sp. (4.5×10 <sup>7</sup> °)	Stationary phase, settling time 2 h	85.2	[13]		
Golenkinia sp. (1.90-2.05)	Self-settling, settling time 0.67 h	89.8	[34]		
Monoraphidium sp. (1.0) Monoraphidium sp. (0.218)	Heterotrophic growth, settling time 24 h Autotrophic growth, settling time 24 h	97.9 85.0	[35]		
Neocystis mucosa (na)	Self-flocculating, settling time 3 h	93.6	[36]		
Scenedesmus quadricauda (na)Self-flocculating with addition of ZnCl2, settling time 0.5 hFree of EPS (washed cells), settling time 0.5 h		86.7 26.5	[12]		
Scenedesmus sp. (0.25)	Self-flocculating, settling time 12 h	92.3	[37]		
Flotation	·				
Chlorella vulgaris (na) Scenedesmus obliquus (na)	Biosurfactant (saponin 20 mg/L) and flocculant (chitosan 5 mg/L)-aided IAF, column height 47 cm (20 min), $N_2$ flow rate 80 mL/min	> 93	[38]		
Chlorella zofingiensis (2.0)	Coagulation-aided DAF, 550 kPa, recycle ratio 20%, 10 min, pH 6.2-8.0, Chitosan 70 mg/g	81	[39]		

	CTAB 500 mg/g	86	
	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 180 mg/g	87	
	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 250 mg/g	91	
Chlorella vulgaris (na)	Surfactant (CTAB 35 mg/L)-aided IAF, column height 146 cm, air flow rate	95	[40]
Isochrysis galbana (na)	1 L/min, feed flow rate 0.1 L/min	93	
Tetraselmis suecica (na)		89	
Nannochloropsis sp. (na)	Flocculant (MPOE 50 mL/L)-aided (300 rpm/4 min) flotation (2h), pH 8	86.5	[41]
<i>Chlorella vulgaris</i> (na)	Surfactant (BCBD 10 mg/L)-aided IAF, air flow 6 L/min, flotation time 10 min	97.1	[42]
Chlorella sorokiniana (0.6)	Coagulation-aided DAF, tap water saturated with compressed air for 20		[17]
	min/500 kPa and injected in the column (750 mm), $V_F 8$ cm/min (150 s), pH 7, average bubble diameter 40 um. Zetag 8185 10 mg/L	98.4	
	Tanfloc SG 75 mg/L	94.5	
	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 500 mg/L	95.4	
	FeCl <sub>3</sub> 1000 mg/L	96.7	
Chlorella vulgaris (0.2)	Surfactant (cooking oil 0.33% (v/v) and 6.7 mg/L CTAB emulsion)-aided (700 rpm/1 min, 100 rpm/1 min) flotation (5 min), pH 10		[43]
Membrane filtration			
Chlorella saccharophila (0.07)	Surfactant (CTAB 100 mg/L)-aided IAF, column height 795 mm, pH 10, flow rate 57.9 mL/min	94.5	[18]
Arthrospira maxima (40)	Disc-type submerged U, PVDF 10-40 nm, 1 bar, EFA 417 cm <sup>2</sup> , flux 24-143 L/m <sup>2</sup> h	100.0	[44]
Aurantiochytrium sp. (25)	M and U with rotating disk, PVDF 0.2 $\mu$ m, 240 min	97.3	[45]
	PES 150 kDa, 1600 rpm 180 min	99.8	
	PVDF 1.5 bar, 240 min	99.9	
<i>Chlorella</i> sp. (4.86×10 <sup>9</sup> <sup>c</sup> )	M, cellulose acetate 1.2 $\mu\text{m}$ , TMP 1.5 bar, CFV 0.4 m/s	90.0	[25]
Chlorella sp. (1.2-1.4)	PET 4 $\mu m$ and PVDF 0.2-0.45 $\mu m$ , TMP 2 bar, CFV 1 m/s, EFA 14 cm², 1%PVA-PVDF/5%PVA-PET	99.0 / 97.0	[27]
Chlorella sp. (0.69 <sup>d</sup> )	U 500 kDa, 1.4 bar, EFA 1 m², permeate flux 11.3 L/m²h	98.7	[46]

Desmodesmus sp. (0.78)	18%PSF-22%PEG, TMP 2 bar, CFV 0.015 m/s, EFA 4×10 cm <sup>2</sup>	> 99.0	[28]
Euglena sp. (0.6)	PVDF 0.42 μm -1% PEG, 0.1 bar, EFA 140 cm², flux 72 L/m²h	na	[47]
Phaeodactylum tricornutum (3.7×10 <sup>6</sup> °) Nannochloropsis gaditana (1.5×10 <sup>6</sup> °) Chaetoceros calcitrans (9.9×10 <sup>6</sup> °)	Pilot-scale dynamic M, ceramic membrane 2 μm, TMP 1 bar, 1110 rpm, 250 L/h	95.0 98.1 97.6	[48]
Tetraselmis sp. (0.68)	Pilot-scale crossflow filtration, 3 bar, EFA 25 $m^2,$ permeate flux 40-120 $L/m^2h$	100.0	[29]
Centrifugation			
Scenedesmus sp. (0.4)	Laboratory centrifuge of tubes, 2000 rpm/ 15 min Industrial nozzle disc (continuous) centrifuge, 5500 rpm/ 14 L/min	96.0 82.1	[49]
Nannochloris sp. (0.1)	Continuous-flow centrifuge, 3000g, 0.94 L/min / 23 L/min	94.0 / 17.0	[50]
<i>Chlorella</i> sp. (4.86×10 <sup>9</sup> °)	Laboratory centrifuge, 4000 rpm/ 10 min	100.0	[25]
Scenedesmus obliquus (0.746)	Laboratory centrifuge, 3000 g/ 10 min	99.3	[51]
Chlorococcum sp. (1.54)	Disc-stack (continuous) centrifuge, 3000 rpm/2.23 L/min	na	[30]
Chlorella vulgaris Chlorella kessleri Chorella sorokiniana Botryococcus braunii Scenedesmus. obliquus Ankistrodesmus falcatus Neochloris oleabundas (0.34-0.49)	Laboratory centrifuge, 1000 rpm, 10 min 1000 rpm, 7.7 min 1000 rpm, 8.7 min 1000 rpm, 7.7 min 1000 rpm/2.9 min 1000 rpm/10 min 1000 rpm/9.3 min	80.0 90.0 90.0 90.0 90.0 78.0 90.0	[52]
Chlorella vulgaris (2.25)	Laboratory centrifuge, 5000 rpm/ 5 min	97.0	[53]
Microalgae consortium ( <i>Desmodesmus</i> spp., <i>Scenedesmus</i> spp., <i>Dictyosphaerium</i> spp., and <i>Klebsormidium</i> sp.) (0.286)	Disc separator (continuous) centrifuge, 7550 g/ 200 L/min	92.1	[54]

<sup>a</sup> Initial biomass concentration (dry weight). <sup>b</sup> Optical density at 690 nm. <sup>c</sup> cells/mL. <sup>d</sup> g volatile solids/L.

na = not available. CTAB = cetyltrimethylammonium bromide. MPOE = Moringa protein extract-oil emulsion. BCBD = N,N'-bis(cetyldimethyl)-1,4-butane diammonium dibromide. IAF = induced air flotation. DAF = dissolved air flotation. M = microfiltration. U = ultrafiltration. PVDF = polyvinylidene fluoride. PES = polyethersulfone. PET = polyethylene terephthalate. PSF = polysulfone. TMP = transmembrane pressure. CFV = crossflow velocity. EFA = effective filtration area.

## 2.5. Chemical coagulation/flocculation

Coagulation/flocculation, induced by the addition of coagulants/flocculants in the microalgae suspension, is conventionally used in the densification of microalgae because it is easy to apply, does not require complex equipment and is not energy expensive. The mechanisms involved in coagulation are related to the reduction or elimination of the electrostatic force of repulsion through the interaction between the added chemicals and the carboxyl and sulfate groups responsible for the negative surface charge of the cells. In flocculation, the neutralized microalgae cells aggregate by the bridging phenomenon that occurs mainly due to the addition of polymeric substances that cause the entrapment of algal cells in the resulting molecular net [55].

Several studies have proven the high harvesting efficiency by coagulation/flocculation of various marine or freshwater microalgae and cyanobacteria using different coagulants. The main inorganic coagulants used in the densification of algal biomass are iron and aluminum salts, which lead to harvesting efficiencies that vary according to the concentration used, the initial density of the culture, and the pH of the medium. Chatsungnoen and Chisti [56] obtained harvesting efficiencies of 95% of different microalgae species with adequate concentrations of aluminum sulfate  $(Al_2(SO_4)_3)$  or ferric chloride (FeCl<sub>3</sub>).

Highly charged cationic polymers also neutralize negative charges in cells. Noh et al. [57] evaluated the polymer  $\alpha$ -poly-L-lysine ( $\alpha$ PLL) with different molecular weights as a cationic flocculant to concentrate *Chlorella ellipsoidea*. The authors observed that the higher the molecular weight of the polymer, the lower the concentration to reach a high biomass density. The predominant mechanism for low molecular weight polymers was charge neutralization since the zeta potential was close to zero at maximum efficiency. While for polymers of higher molecular weight, the bridging mechanism occurred along the polymer chain. Vu et al. [58] observed a similar mechanism, evaluating polyacrylamidebased flocculants in the flocculation of the red microalgae *Porphyridium purpureum*.

Organic coagulants are gaining more space and are becoming more advantageous because they are environmentally sustainable and do not contaminate concentrated biomass. Among these stands out: chitosan, a polymer derived from the deacetylation process of chitin from crustaceans [59]; plant tannins extracted from species such as *Acacia mearnsii* [60]; tannin-derived cationic flocculants - Ecotan and Tanfloc [3]; and plant species such as *Moringa oleifera* [61]. All these products have a high coagulation capacity at low concentrations and the advantage of not being corrosive or toxic, ensuring that their presence in the concentrate does not harm, for example, the biogas production in the anaerobic digestion stage [3]. Table 2 presents the harvesting efficiencies of different microalgae species with cationic polymers and organic and inorganic coagulants.

Specie	Coagulant/concentration	Conditions	Efficiency (%)	Reference
Chaetoceros gracilis	FeCl <sub>3</sub> 20-50 mg/L + chitosan 10-50 mg/L, salinity 20 g/L	1000 rpm/4 min, 100 rpm/1 min, pH 5-9	95.1-100.0	[62]
Chlorella sp.	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 152 mg/L FeCl <sub>3</sub> 143 mg/L	100 rpm/2 min, 25 rpm/20 min, settling time 40- 60 min, 0.12 g/Lª, pH 6-8.1	100.0 100.0	[63]
Chlorella sp.	FeCl <sub>3</sub> 122 mg/L Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 140 mg/L Cationic polymer (Zetag) 34 mg/L <i>Moringa oleifera</i> 4.7 g/L	70 rpm/1 min, 25 rpm/15 min, settling time 1 h, 0.62 g/L <sup>a</sup> , pH >6.0 for all tests	93.0 91.0 98.0 85.0	[64]
Chlorella sp. KR-1	FeCl <sub>3</sub> 200 mg/L	100 mL mess cylinder, agitation/30 s, settling time 20 min, 1.7 g/Lª, pH 3	90.0	[65]
Chlorella sp. KR-1	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 900 mg/L	Vortex 5 min, settling time 30 min, 1.5 g/L <sup>a</sup> , pH 5.4	98.0	[66]
<i>Chlorella</i> sp. NCQ <i>Micractinium</i> sp. NCS2 <i>Scenedesmus</i> sp. CBIIT(ISM)	Cationic biopolymer 30-55 mg/L	200 rpm/3 min, 60 rpm/10 min, settling time 35 min, 0.79, 0.86, 1.32 g/L <sup>a</sup> , pH 7.3-8.2	96.9-97.4	[55]
Chlorella ellipsoidea	Biopolymer 5 kDa 50 mg/L Biopolymer 5 kDa 2 mg/L Biopolymer 5 kDa 0.5-1 mg/L	400 rpm/5 min, 80 rpm/10 min, settling time 1 h, 1 g/L <sup>a</sup> , pH 7.8 pH 8.6-8.7	80.2 88.2 98.3	[57]
Chlorella pyrenoidosa	CaCO <sub>3</sub> 100 mg/L Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 100 mg/L Bioflocculant 100 mg/L	Agitation at 300 rpm, settling time 1 h, pH 8 pH not informed pH 4	95.0 99.0 99.0	[67]
Chlorella sorokiniana	FeCl <sub>3</sub> 6 mg/L + Chitosan 200 mg/L FeCl <sub>3</sub> 6 mg/L + Chitosan 200 mg/L + PAA <sup>b</sup> 100 mg/L FeCl <sub>3</sub> 6 mg/L + PAA 100 mg/L	Settling time 10 min, pH 6 Settling time 10 min, pH 5.7 Settling time 24 h, pH 10	53.0 97.0 79.0	[68]
Chlorella vulgaris	Cationic polymers 5 mg/L Chitosan 10 mg/L	10 mL glass tubes, settling time 1 h, 0.3 g/Lª, pH 6.5 Settling time 4 h	97.0 90.0	[69]
Chlorella vulgaris	Al2(SO4)3         2,500 mg/L           Chitosan         250 mg/L	150 rpm/1 min, 25 rpm/15 min, settling time 15 min, 1.2 g/L <sup>a</sup>	92.4 91.9	[70]

**Table 2** Harvesting of microalgae by chemical coagulation/flocculation with different coagulants

Chlorella vulgaris	Cationic polymer 7 g/kg algae FeCl <sub>3</sub> 4.1 g/kg algae Biopolymer 4.7 g/kg algae	75 rpm/ 1 min, pH 7	94.2 81.0 94.6	[71]
Chlorella vulgaris Choricystis minor Cylindrotheca fusiformis Neochloris sp. Nannochloropis salina	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> or FeCl <sub>3</sub> 75 mg/L Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> or FeCl <sub>3</sub> > 200 mg/L Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> or FeCl <sub>3</sub> 150 mg/L Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 25 FeCl <sub>3</sub> 55 mg/L Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> or FeCl <sub>3</sub> > 200 mg/L	80 rpm/2min, 20 rpm/30min, settling time 30 min, 1.0 g/L <sup>a</sup>	95.0	[56]
Chlorococcum sp. R-AP13	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 180 mg/L           FeCl <sub>3</sub> 114-130 mg/L           Chitosan         40 mg/L	250 rpm/ floc. added slowly, stirring for 2 min, settling time 10 min	87.0 92.0 84.0	[72]
Nannochloropsis sp	Bioflocculant160 mg/L $Al_2(SO_4)_3$ 320 mg/L	120 rpm/1 min, 20 rpm/20 min, settling time 2 h, 1.0 g/L <sup>a</sup> , pH 5 pH 7	92.4 94.1	[60]
Nannochloropsis sp. BR2	Al2(SO4)3         87.5 mg/L           FeCl3         87.5 mg/L           Chitosan         22 mg/L	100 rpm/ 15 min, settling time 30 min, 0.28 g/L <sup>a</sup> pH 10	95.2 95.6 97.9	[59]
Nannochloropsis oculata	FeCl <sub>3</sub> 400 mg/L           Fe2(SO4) <sub>3</sub> 600 mg/L           ZnCl <sub>2</sub> 600 mg/L           ZnSO <sub>4</sub> 600 mg/L           AlCl <sub>3</sub> 600 mg/L           Al2(SO4) <sub>3</sub> 800 mg/L	50 mL glass tubes, 250 rpm, settling time 3 h Settling time 3 h Settling time 3.5 h Settling time 4 h Settling time 4 h Settling time 4 h	93.8 87.3 89.1 84.2 85.5 82.3	[73]
Phaedactylum tricornutum	Polyaluminium chloride40 mg/LAl2(SO4)330 mg/LChitosan20 mg/L	150 rpm/2 min, settling time 30 min, pH 7.5 pH 5.9 pH 9.9	66.6 82.6 91.8	[74]
Porphyridium purpureum	Cationic polymers 21.43 g/kg algae	200 rpm/1 min, 50 rpm/15 min, settling time 1 h, 0.7 g/L <sup>a</sup> , pH 8.9	> 99.0	[58]
Scenedesmus quadricauda	FeCl <sub>3</sub> 700 mg/L	Vortex 30 s, settling time 10 min, 0.58 g/L <sup>a</sup> , pH 11 / pH 7	95.8 / 85.0	[75]
Scenedesmus sp.	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 1500 mg/L	120 rpm/1 min, 25 rpm/12 min, settling time 10 min, pH 8.5	97.9	[49]

Scenedesmus sp.	FeCl <sub>3</sub> 96 mg/L	Agitation by air for 2 min, settling time 10 min, $0.53 \text{ g/L}^{a}$ , pH 6.4	91.4	[76]
Tetraselmis sp.	FeCl <sub>3</sub> 100 mg/L Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 100 mg/L	Agitation by air for 2 min, settling time 30 min, $0.68 \text{ g/L}^{a}$	95.8 95.4	[29]
Spirulina platensis	Cationic polymer 7 g/kg algae FeCl <sub>3</sub> 2.1 g/kg algae Biopolymer 2.2 g/kg algae	75 rpm/ 1 min, pH 8	95.2 87.9 94.9	[71]
Algal bloom	FeCl <sub>3</sub> 20 mg/L Alumen 30 mg/L Chitosan 30 mg/L Alumen 10 mg/L + chitosan 1 mg/L FeCl <sub>3</sub> 5 mg/L + chitosan 2.5 mg/L	100 rpm/1 min, 30 rpm/ 15 min, settling time 30 min, pH 8.2	98.6 96.3 87.3 97.6 97.2	[77]
Mixed microalgae cultures	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> 40 mg/L	200 rpm/1min, 30 rpm/30 min, settling time 30 min, pH 6.5	96.0	[78]
Mixed microalgae culture	Biopolymer Aflok 1.2 mL/L FeCl <sub>3</sub> 200 mg/L	0.55 g/Lª, pH 6, settling time 30 min Settling time 150 min	98.7 88.3	[79]
Consortium of <i>Chlorella sorokiniana</i> and <i>Scenedesmus</i> sp.	Cationic polymer 27 mg/g algae Cationic biopolymer 30 mg/g algae AlCl <sub>3</sub> 100 mg/g algae	100 rpm/2min, 20 rpm/10 min, settling time 30 min, 0.29-0.37 g/L <sup>a</sup> , pH 6.84-7.95	92.0 84.0 97.0	[80]
Consortium of <i>Chlorella</i> sp., <i>Scenedesmus</i> sp., <i>Cynecocystis</i> sp., and <i>Spirulina</i> sp.	Moringa oleifera 8 g/L Moringa oleifera 4 g/L + chitosan 0.75 g/L	150 rpm/2 min, 30 rpm/20 min, 0.5 g/L <sup>a</sup> Settling time 100 min, pH 7.5-7.8 Settling time 20 min, pH 7.5-7.8	75.5 95.8	[61]
Consortium of microalgae and bacteria	Natural flocculant Ecotan 10 mg/L Natural flocculant Tanfloc 50 mg/L	200 rpm/1 min, 35 rpm/15 min, settling time 15 min, 2.8 g/L <sup>a</sup> , pH 7.7-7.9	91.8 90.2	[3]

<sup>a</sup> Initial biomass concentration (dry weight). <sup>b</sup> Polyacrilamide.

#### 2.6. Autoflocculation

Autoflocculation occurs due to changes provided by the addition of salts or bases and changes in the pH of the microalgae culture. The phenomenon is closely related to the presence of  $Ca^{+2}$  and  $Mg^{+2}$  ions, which neutralize the charges of cells in suspension and induce adsorption and agglomeration, forming flocs that precipitate. Precipitation occurs mainly at pH values between 9.5 and 11. It is a low-cost method, as it does not use large volumes of chemical coagulants and does not require complex equipment [58,81].

Vandamme et al. [82] investigated the role of calcium and magnesium precipitation in the flocculation of *Chlorella vulgaris* in the pH range of 9 to 12. The authors observed that in a medium with low phosphate concentration (< 0.1 mM), magnesium plays a more relevant role than calcium because magnesium promotes the formation of structures that carry a positive charge, whereas calcium precipitates as calcium carbonate, a neutrally charged structure.

Increasing the pH forms magnesium hydroxide, in whose crystal structure bivalent magnesium cations are replaced by trivalent aluminum or iron cations. These positively charged structures neutralize the negative charge of microalgae, causing destabilization and flocculation of the biomass.

Salinity also influences autoflocculation. Pérez et al. [83] evaluated the effect of salinity on the flocculation efficiency of the marine species *Skeletonema costatum* at a pH range of 10.5 to 12. For high time intervals (8.5 h), the salinity had no significant effect on the flocculation efficiency at pH values of 11 to 12. For shorter time intervals (1 h), the effect of salinity was accentuated, obtaining higher recoveries with the lowest salinity value at the same pH values.

Although most of the species studied form flocs in the presence of strong bases, some species show such behavior when the suspension has pH values between 3 and 4 [76,84]. Table 3 presents more results of biomass concentration by changes in pH with bases and acids.

#### 2.7. Bioflocculation

Bioflocculation is a densification process by adding microorganisms or metabolites to a microalgae suspension. When added to the microalgae suspension, the microorganisms secrete substances that neutralize the cell charges, destabilizing the suspension and forming flocs that settle [85]. Among the microorganisms evaluated are species of bacteria [23], fungi [86], cyanobacteria [87], and even microalgae [32]. In addition to microorganisms, metabolites are also used for bioflocculation, such as compound poly ( $\gamma$ -glutamic acid), produced by the bacterium *Bacillus licheniformis* [23].

One of the most efficient bioflocculation techniques is inoculating fungal pellets into the microalgae suspension to form dense agglomerates that settle quickly [88,89]. Another technique studied is the co-cultivation of bacteria and fungi with the microalgae of interest, which allow the formation of aggregates that settle [90].

Flocculation of microalgae by the addition of bacteria and fungi may require the addition of carbon and energy sources to allow their growth, increasing contamination in microalgae cultures. Thus, the concentration of non-flocculating microalgae by the addition of flocculating microalgae is a promising alternative, which allows the total reuse of the culture medium as it does not require the addition of carbon sources and different cultivation conditions, resulting in more savings [2]. Table 3 presents some results of microalgae concentration by bioflocculation.

# **Table 3** Harvesting of microalgae and cyanobacteria by biological methods

Specie (Initial conc. g/L ª)	Conditions	Efficiency (%)	Reference		
Autoflocculation					
Chlorella sp. (0.12)	NaOH 5 mol/L, pH 10.2, settling time 24 h KOH 5 mol/L, pH 10.2, settling time 24 h	45.8 35.1	[63]		
Chlorella vulgaris (0.5)	NaOH 0.5 mol/L/pH 10.5-12, settling time 30 min, No Ca or Mg	< 20	[82]		
	+ Ca 0.025-2.5 mM/pH 10.5-12	< 20			
	+ Mg 0.015 mM/pH 10.5-12	< 20			
	+ Mg $\ge$ 0.15 mM/pH 10.5-12	90-100			
Chlorococcum sp. R-AP13 (na)	NaOH 0.04 g/L, pH 12, settling time 10 min	94	[72]		
Chlorococcum nival, Chlorococcum ellipsoideum, Scenedesmus sp. (1.11-1.35)	$HNO_3$ 1 mol/L, pH 1.5-4.5, settling time 10 min	> 90	[91]		
Desmodesmus communis (2.0)	HCl 2 mol/L, pH 4, settling time 60 min	> 95	[84]		
	NaOH 1 mol/L, pH 11-12, settling time 20 min	> 90			
Dunaliella salina (0.4-0.6)	NaOH 8.5 mmol/L/pH 11, settling time 10 min	80	[92]		
Nannochloropsis oculata (na)	KOH or NaOH 1 mol/L/pH 10.5, settling time 60 min	98	[81]		
Chlorella minutissima		84-86			
Phaedactylum tricornutum (0.1)	NaOH 1 mol/L/pH 11, settling time 60 min	98	[74]		
Porphyridium purpureum (0.7)	NaOH, KOH, Na <sub>2</sub> CO <sub>3</sub> , 1 mol/L, pH 10.5, settling time 60 min	91-98	[58]		
Scenedesmus sp. (0.53)	H <sub>2</sub> SO <sub>4</sub> 1 mol/L, 0.19 g/L, pH 3, settling time 30 min	99.5	[76]		
	NaOH 1 mol/L, 0.28 g/L, pH 11.5, settling time 30 min	70.6			
Tetraselmis sp. (0.68)	NaOH 250 mg/L, pH 9.5, settling time 30 min	93.2	[29]		
Skeletonema costatum (na)	HCl 2 mol/L or NaOH 5 mol/L, pH 2/ pH 11-12	60/100	[83]		
Chaetoceros gracilis		60/100			
Chlorella vulgaris, Pseudanabaena CY14-1, Chlamydomonas reinhardtii, Scenedesmus obliquus, Phaeodactylum	NaOH 0.5 mol/L, 18-150 mg/L, settling time 30 min	> 80	[93]		

tricornutum, Diacronema lutheri, Tetraselmis suecica, Nannochloropsis oculate, Dunaliella salina (0.25-0.47)			
T- Isochrysis lutea (0.21)	NaOH 0.5 mol/L, 209 mg/L, settling time 30 min	< 40	
Chlorella vulgaris, Scenedesmus sp., Chlorococcum sp. (0.7-0.8)	NaOH 1 mol/L, pH 10.5-12.5, settling time 10 min	> 90	[94]
Nannochloropsis oculata, Phaeodactylum tricornutum (1.6- 1.8)	NaOH 1 mol/L, pH 9.0-9.3, settling time 10 min	> 90	
Bioflocculation			
Chlorella vulgaris (0.5)	Ettlia texensis0.86 cAnkistrodesmus falcatus0.66 cScenedesmus obliquus0.77 cSettling time 3h	60 50 31	[32]
Chlorella vulgaris (na)	Aspergillus oryzae(HC)1.2×104 spores/mL,20g/Lglucose, pH 4-5	99.2 93	[88]
Chlorella vulgaris (5.9×10 <sup>6 b</sup> )	Aspergillus niger (AC) 7.6×10 <sup>3</sup> spores/mL, pH 4, 3 days Aspergillus niger (HC) 7.6×10 <sup>3</sup> spores/mL, 12 g/L PDB, 15 g/L glucose, pH 5, 3 days	60 25	[86]
Chlorella vulgaris (0.57) Chlorella protothedoides (0.6)	poly(γ-glutamic acid - γ-PGA) 20 mg/L, 11.6 g/L salinity, pH 7.5, 2h poly(γ-glutamic acid - γ-PGA) 20 mg/L, pH 7.5, 2h	82 90	[95]
Chlorella pyrenoidosa (3.3×10 <sup>7</sup> b)	<i>Citrobacter freundii</i> 1.6 <sup>c</sup> + <i>Mucor circinelloides</i> 0.003 <sup>c</sup> , 1.47 g/L glucose, pH 7, 24 h	97.5	[90]
Chlorella pyrenoidosa (na)	Aspergillus fumigatus (unwashed pellets 1:5 dry weight basis), BG11 broth, 4 h	>90	[89]
Chlorella sp. (na)	<i>Pleurotus ostreatus</i> (filtered pellets 57 g/L <sup>d</sup> ), pellets cultured under 100 rpm, 2.5 h	64.9	[96]
Desmodesmus sp. (na)	Bacillus licheniformis γ-PGA (2.5 mL/L), modified Bold 3 N medium, pH 3, 1 min	99.5	[97]

Desmodesmus brasiliensis (0.5 and 1.0)	<i>Bacillus licheniformis</i> γ-PGA (2.5 mL/L), modified Bold 3 N medium, pH 3, 1 min	>99	[23]
Desmodesmus sp. (1.2×10 <sup>8</sup> <sup>b</sup> )	Monoraphidium sp. $1.4 \times 10^8$ cell/mL, Coculture in BG-11 medium, 4h	85.3	[98]
Nannochloropsis oceanica (na)	Solibacillus silvestris 3:1, Culture supernatant (48 h) mixed with microalgal culture (f/2 medium), pH 8, 10 min	90	[99]
Consortium composed mostly with <i>Chlorella sorokiniana</i> and <i>Scenedesmus</i> sp. (0.45 and 0.28)	Bacterial biomass 1 g /0.1 g algae biomass, 10 min Bacterial biomass + cationic polymer 16 mg/g algae biomass, 30 min	40 97	[80]

<sup>a</sup> Initial biomass concentration (dry weight). <sup>b</sup> cells/mL. <sup>c</sup> R<sub>fnf</sub> = ratio in concentration of the flocculating specie and the non-flocculating microalgae. <sup>d</sup> value for 10 g (wet weight) fungal aggregates into the working volume of 175 mL of a 250 mL Erlenmeyer flask.

na = not available. HC = heterotrophic culture, AC = autotrophic culture, PDB = potato dextrose broth.

## 2.8. Electrocoagulation

The biomass concentration can be obtained by destabilizing the repulsive forces naturally present in the microalgae suspension through electricity. This method usually occurs with the immersion of two electrodes, a cathode, and a sacrificial anode, in the suspension containing the microalgae cells. These electrodes can be made of aluminum, iron, copper, or zinc, and also carbon as a non-sacrifice electrode [100].

When using metal electrodes, two phenomena occur: the electrolysis of pure water produces oxygen gas at the anode and hydrogen gas at the cathode. The microbubbles ( $O_2$  and  $H_2$ ) adhere to the surface of the microalgal cells or flocs and float to the top. This phenomenon is called electroflotation. Simultaneously, the anode is oxidized, and the metallic cations become available in the microalgae suspension, destabilizing the electrostatic forces, culminating in the formation of flocs, which precipitate and sediment to the bottom, a phenomenon known as electrocoagulation [101]. Table 4 presents some results of microalgae concentration by electrocoagulation.

The most relevant parameters to the method are the type of electrode used, the distance between the electrodes, the electric current density, the pH of the medium, temperature, salinity, and the time required for sedimentation or flotation of the concentrated biomass. Some obstacles to be overcome are its application on a larger production scale since most studies are on a bench scale and the guarantee of non-contamination of the concentrated biomass as the electrodes are primarily of metals [102,103].

Specie (Initial conc. g/L ª)	Condition	Efficiency (%)	Reference
Microcystis aeruginosa (1.2-1.4 × 10 <sup>9 b</sup> )	Fe electrode with effective area 60 cm <sup>2</sup> , 1 mA/cm <sup>2</sup> , pH 7, time 45 min Al electrode with effective area 60 cm <sup>2</sup> , 1 mA/cm <sup>2</sup> , pH 7, time 45 min	78.9 100.0	[104]
Chlorella pyrenoidosa (2.2 ± 0.15)	Electrodes of parallel flat metals (10*6*0.5 cm), two cathodes 6 cm apart and one anode in the middle. Output voltage 5 V, current density 10 mA/cm <sup>2</sup> , time 5 min Al/C Cu/Zn	95.83/79.16 93.75/83.33	[100]
Scenedesmus	Fe Carbon electrodes - cathodes plates (12*10*2 cm) 6 cm apart	70.83 83	[105]
<i>obliquus</i> (2.4 ± 0.01)	and an anode plate. 1.5 A, initial pH 9, 6 g/L NaCl, time 60 min		
Chlorella vulgaris (3.63 × 10 <sup>10 b</sup> )	Al electrode plates ( $10^{*}3^{*}1$ cm) and a flat stir paddle for mixing, initial pH 8.6, 66.7 A/m <sup>2</sup> , time 4 min	98	[106]
<i>Tetraselmis</i> sp. (0.68)	Two vertically placed Al electrodes plates (total surface area 250 cm <sup>2</sup> and thickness 5 mm), 2 V, 1 A	89.16	[29]
Spirulina platensis	Al Electrodes (52 cm <sup>2</sup> ), pH 4.5, 30 A/m <sup>2</sup> , 30 min	99.9	[107]
(1.30)	Carbon Electrodes (96.7 cm²), pH 4.5, 80 A/m², 30 min	86.9	
Chlorella vulgaris (0.2-1.8)	Fe electrodes (2*10 cm), 0.02 A, 5 V, 400 rpm, time 6 min, pH 7	> 95	[108]
Nannochloropsis	Fe electrode (10*4.5 cm), pH 8, 49.2 mA/cm <sup>2</sup> , 150 rpm/7.7 min	97.2	[109]
oceanica (2.0)	Zn electrode (10*4.5 cm), pH 7, 95.5 mA/cm <sup>2</sup> , 150 rpm/8.9 min	98.5	
Chlorella vulgaris (0.23)	Pilot-scale (111 L), electrolizer, flow rate 240 L/h, Fe electrode	> 85	[110]

Table 4 Harvesting of microalgae and cyanobacteria by electrocoagulation

a Initial biomass concentration (dry weight). b cells/L.

## 3. Comparison of densification methods

Table 5 summarizes the advantages and disadvantages of densification methods. Spontaneous sedimentation is the most economically attractive method for harvesting and concentrating algal biomass on a large scale due to its low energy demand, simple operation, non-use of flocculant, and non-toxicity [2,111]. However, few species (such as *Ettlia texensis, Ankistrodesmus falcatus, Scenedesmus obliquus* AS-6-1, *Chlorococcum* sp. GD, *Tetraselmis suecica, Chlorella vulgaris* JSC-7, and *Skeletonema marino*) are self- settling microalgae [8].

Chemical coagulation/flocculation can concentrate algal biomass on a larger scale due to its cost-effectiveness and operational proficiency. It can significantly increase concentration efficiency when used as a pre-concentration technique before centrifugation, flotation, or membrane filtration [17,57]. Autoflocculation and bioflocculation are low-cost and energy consumption methods, non-toxic to microalgae, and do not use flocculants, allowing the reuse of the culture medium. However, these methods are not yet used on an industrial scale because they work with specific strains, are unreliable for controlled flocculation, and can cause changes in cell composition [2].

Centrifugation is the most conventional method, providing biomass concentrations up to a dehydration level of 20%. However, it is highly energy-intensive, especially in a large-scale environment, and is only suitable for high-value products [112]. Membrane filtration is more favorable than centrifugation due to its lower energy consumption and carbon footprint for microalgae harvesting. Additionally, membrane filtration makes it possible to harvest microalgae in a highly efficient manner, and biomass quality remains unaltered. Both microfiltration (MF) and ultrafiltration (UF) membranes separate microalgae from water. However, membrane filtration is a technology that still needs to overcome the issue of membrane fouling and high maintenance cost. And it is adequate for harvesting microalgae with long lengths or formation of large-colony [47,113].

Harvesting by flotation and electrocoagulation achieves efficiencies similar to those obtained by centrifugation with lower energy consumption and total costs. Flotation is an established method to remove algae from suspension, which is more advantageous and effective than sedimentation. However, it is limited in its technical and economic viability. In addition, flotation requires injection of air and chemical flocculation pre-step, which increases energy consumption and harvesting costs compared to gravity sedimentation [2,118].

Electrocoagulation for harvesting microalgae has several benefits in terms of costs and safety. Although operating at low energy input and does not require the addition of any chemical floculants, it is an unusual approach due to the depletion of the metallic electrodes and possible contamination of microalgal biomass [105]. Compared with chemical coagulation, electrocoagulation also has high harvesting efficiencies but offers more advantages. It does not introduce sulfates and chlorides in the medium; the coagulants produced are highly efficient at lower concentrations, and pH adjustment is unnecessary. In addition, during the process, do not occur alkalinity consumption and the microbubbles produced at the anode and cathode can also contribute to the separation of pollutants through flotation [104].

Figure 1 shows reported efficiencies for the different densification methods of microalgae and cyanobacterial biomass presented in Tables 1 to 4 and cost and energy consumption data collected in some studies. All densification methods can achieve maximum concentration efficiencies (Fig. 1a).

Although membrane filtration, centrifugation, and chemical flocculation maintain high efficiencies under more diverse situations, the spontaneous settling method also attains high efficiencies but is limited to flocculating microalgae harvesting. In contrast, autoflocculation and bioflocculation are the methods that promote more extensive ranges of concentration efficiency. However, along with spontaneous settling, they have lower energy consumption. While centrifugation, chemical flocculation, and membrane filtration are the methods with the highest energy consumption (Fig. 1b). Therefore, the conclusion is that, in general, densification methods that result in high biomass concentration efficiencies for different species and conditions also lead to increased operating and maintenance costs.

Method	Pros	Cons	References
Spontaneous settling	Does not require significant capital investment, energy consumption is negligible, nontoxicity (without chemicals addition).	Slow process, few microalgae (green unicellular and diatom) and cyanobacteria precipitate spontaneously (floc sizes > 100 μm are required), low concentration of the algal cake.	[2,11,13,15, 32,111,114]
Flotation	High efficiency, suitable for large scale, less energy intensive, low space requirement, short operation time, high flexibility.	High efficiency depends on the addition of flocculant/ surfactant, which can be costly, high dosage of flocculants can contaminate the biomass, flotation time varies from a few min to more than 2 h, unfeasible for marine microalgae harvesting.	[2,16,17, 18,42]
Membrane filtration	Less energy-intensive than centrifugation, long membrane lifespan makes recovery relatively more cost-effective in the long term, water reuse, no cell damage, suitable for large algae such as <i>Spirulina</i> sp.	High-energy requirements, problems with membrane fouling and replacement of clogged membranes, inefficient for small algae such as <i>Chlorella</i> sp., harvested product too dilute and may need additional concentration, potential problems with scale-up.	[25,113, 115,116]
Centrifugation	High efficiency, rapid and reliable method, biomass can remain fully contained during recovery.	High financial and energy costs for applying high g-force, low scalability, centrifugal effect damages cells.	[25,115, 117,118]
Chemical coagulation/ flocculation	Low energy process under optimum conditions, simple and fast method, high efficiency, treats large culture volumes, large number of organic and inorganic coagulants.	Depends on the algal concentration, media pH, and coagulants dosage, process is expensive for costly coagulant with high dosage, contamination of the biomass with metals restricts its further application, removal of excess coagulants from the medium leads to extra operational costs and energy, some coagulants adversely affect biochemical components (proteins, starch, and lipids).	[2,72,111, 119,120]
Autoflocculation	Easy and low-cost method, high efficiency, allows culture medium recycling, non-toxic to microalgal biomass, negative effect on pathogenic microorganisms.	Greater influence in the recovery of marine microalgae, changes in cellular composition.	[2,6,10]
Bioflocculation	Energy required is reduced, no extra chemical, no extra operational costs and energy for downstream processing or medium reuse, added microorganisms may contribute to increased methane and lipid yields.	Bioflocculant production requires different cultivation conditions (additional medium costs and increased risk of microbial contamination), recoveries achieved by bioflocculation are lower than autoflocculation and chemical flocculation.	[2,32]
Electro- coagulation	High efficiency, process is usually fast, good results with carbon electrodes, which do not cause contamination of the biomass, non-species specific.	Energy intensive, best results with Al and Fe electrodes, possible contamination of biomass, replacement of electrode and fouling of cathodes, increase in temperature and changes in pH of algal suspension.	[5,100,105, 107,109]

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Table 5 Characteristics of	f different methods o	f concentration (	of microalgae and	cvanohacterial	sucnensions
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Figure 1 Comparison of concentration efficiency (A), energy consumption (B) and costs (C) of algal biomass densification methods (based on the data shown in Tables 1 to 4) and references [23,82,121]

Virtually all microalgae densification studies cite Grima et al. [117], which estimated that costs associated with microalgae harvesting represent more than 20–30% of the total costs of microalgal production. Harvesting costs are between 0.2 and 18 US\$/kg dry mass produced (Fig. 1c) and show a marked correlation with energy consumption, except for chemical coagulation/flocculation. Coagulation/flocculation costs and flotation are related to the type of coagulant or surfactant used. For example, Behera and Balasubramanian [61] cite costs of US\$9.02, US\$0.28, and US\$0.037 to recover 1 MT of wet algae under optimal conditions with chitosan, alum, and a natural coagulant (*Moringa oleifera* extract), respectively. Alkarawi et al. [40] mention the cost of US\$ 0.179 to concentrate 1 m<sup>3</sup> of biomass (*Chlorella vulgaris, Isochrysis galbana*, and *Tetraselmis suecica*) with cetyltrimethylammonium bromide (CTAB) surfactant (35 mg/L) and to obtain concentration efficiency greater than 89%. Regarding the cost of the process, it is worth noting that a flocculant with an excellent cost-benefit ratio to concentrate specific microalgae or cyanobacteria may present a low cost-benefit ratio for another species. Lama et al. [93] calculated the costs of coagulation/flocculation with chitosan and NaOH for several species and obtained cost/concentration factor ratios ranging from 0.4 for *Nannochloropsis oculata* with 0.03 g FeCl<sub>3</sub>/g biomass to 103.7 for *Pseudanabaena* CY14- 1 with 0.33 g chitosan/g biomass.

Several researchers report that the commercialization of algal biomass, concentrated with high-energy technologies, is only viable for high-value products [29,31]. For low-value products obtained from densified algal biomass, such as biofuels, the harvesting energy should not exceed 1.8 kWh/kg dry mass [122]. This limit corresponds to 0.5 kWh/m<sup>3</sup> to a microalgal suspension with 0.3 kg dry mass/m<sup>3</sup>, enabling only the spontaneous settling, autoflocculation, and bioflocculation technologies and very specific cases of the other technologies.

An alternative to reduce densification costs is a multi-step process, combining low-cost pre-concentration steps with high-efficiency and cost-effective techniques. Ríos et al. [48] proposed to reduce microalgae densification costs from US\$750/kg (with cross-flow filtration) to US\$8.5/kg by adding a pH-induced flocculation-sedimentation pre-concentration step before membrane filtration. Wang et al. [51] reduced *Scenedesmus obliquus* densification costs by almost 50% (from 9.63 to US\$5.47/kg of dry biomass) by combining a pre-concentration by flocculation with ferric sulfate and centrifugation. Lucakova et al. [108] used an electrocoagulation pre-concentration step before centrifugation, reducing total energy costs to 14% of the centrifugation cost alone.

# 4. Conclusion

The efficiency is not a problem as all densification methods achieve maximum concentration efficiencies with specific species and optimized conditions. However, low-cost methods (spontaneous settling, autoflocculation, and bioflocculation) do not work well for all species. Some species require high-cost densification methods, leading to a constant search to reduce energy consumption and harvesting costs of these methods. Without cost-effective methods, research should direct towards selecting species that combine the desired characteristics for the downstream processes and the compounds extracted from the algal biomass.

# **Compliance with ethical standards**

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# Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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