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Microalgae harvesting for wastewater treatment and resources recovery: A review

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ABSTRACT

Microalgae-based wastewater treatment has been conceived to obtain reclaimed water and produce microalgal biomass for bio-based products and biofuels generation. However, microalgal biomass harvesting is challenging and expensive, hence one of the main bottlenecks for full-scale implementation. Finding an integrated approach that covers concepts of engineering, green chemistry and the application of microbial anabolism driven towards the harvesting processes, is mandatory for the widespread establishment of full-scale microalgae wastewater treatment plants. By using nature-based substances and applying concepts of chemical functionalization in already established harvesting methods, the costs of harvesting processes could be reduced while preventing microalgae biomass contamination. Moreover, microalgae produced during wastewater treatment have unique culture characteristics, such as the consortia, which are primarily composed of microalgae and bacteria, that should be accounted for prior to downstream processing. The aim of this review is to examine recent advances in microalgal biomass harvesting and recovery in wastewater treatment systems, considering the impact of consortia variability. The costs of available harvesting technologies, such as coagulation/flocculation, coupled to sedimentation and differential air flotation, are provided. Additionally, promising technologies are discussed, including autoflocculation, bioflocculation, new filtration materials, nanotechnology, microfluidic and magnetic methods.

Introduction

Microalgae-based wastewater treatment offers an opportunity to generate bio-based products and recover bioenergy from microalgal biomass. This alternative can reduce the requirement for freshwater and nutrients in microalgal culture, while recycling nutrients from wastewater. In this way, the generation of bio-based products from microalgal biomass turns into an environmentally friendly option [1]. The costs of producing microalgal biomass in freshwater supplied with synthetic medium can only be justified for high value-added products where the return on investment makes the production process economically viable. During the recent decades, there has been a resurgence of interest in microalgae production for bioenergy generation, including biogas, biodiesel, bioethanol, and bio-hydrogen [2]. This continues to be a research subject, particularly in Europe, where biofuels are a strategic priority for energy independence. At the COP 27 climate change meeting, Europe set a target of increasing the use of renewable energy in transportation to at least 14% by 2030.

Growing microalgae as a by-product of wastewater treatment can reduce the cost of microalgae production, but economic and energy assessments have identified several limitations that must be addressed [3]. Thus, innovative cultivation techniques and harvesting methods are

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Abbreviations: DAF, dissolved air flotation; EPS, extracellular polymeric substance; HRAP, high-rate microalgal pond; NMP, nano magnetic particle; PHA, polyhydroxyalkanoate.

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necessary for advancing bioenergy production. The cost of microalgae harvesting, which constitutes up to 20–30% of the final production cost, presents a significant challenge to the industrialization of microalgal biofuels [4]. Within the context of wastewater treatment, harvesting is of utmost importance as it affects the final discharge and potential reuse of treated water.

As unicellular organisms, many microalgae present difficulties in settling due to their small size (1–10 µm), similar density to water, negative surface charge (from −7.5 to −40 mV), and low settling velocity (10^{-5}-10^{-6} m/s) [5,6]. The settling capacity of microalgae can be significantly influenced by a range of factors, including surface charge, size, shape and the presence of extracellular polymeric substances (EPS) [7,8]. Several mechanical, electrical, biological, and chemical-based harvesting techniques are currently used to concentrate microalgae from 0.02% to 0.25% (w/w) to 1–5% (w/w) [9]. In commercial systems, commonly used harvesting techniques include filtration, centrifugation, sonication, electrocoagulation, and chemical-induced flocculation [5, 10–12].

Despite their potential application, some major constraints as high energy requirements, changes in cell composition, and high costs (i.e. electrode and membrane replacement or flocculant costs), have hindered their use in wastewater treatment plants [10]. Indeed, in the context of wastewater treatment, only low-cost techniques capable of handling large volumes of water and biomass can be applied. Ideally, the solid concentration should be between 1% and 5% w/w for downstream processes such as biogas production [13]. Furthermore, the energy requirements for the harvesting step should be low to ensure the net energy production and self-sustainability of the wastewater treatment process.

Several studies in the literature have focused on microalgae harvesting, as it is a crucial step in the biomass production chain. However, the specificities of wastewater treatment consortia, and particularly the importance of biomass recovery to guarantee the treated water quality and biomass downstream processing, have not been thoroughly covered. The aim of this review is to provide an overview of recent advances in biomass harvesting and their application to the recovery/reuse of microalgae biomass from wastewater treatment plants. To this end, the influence of consortia, microalgae production systems for wastewater treatment, and costs of different harvesting technologies are discussed.

Microorganisms co-occurring in wastewater treatment systems

Microalgae-bacteria consortia are capable of treating wastewater and producing valuable biomass for bio-based products and bioenergy generation [14]. The utilization of microalgae and other microorganisms naturally occurring in wastewater is the most effective method for large-scale wastewater treatment [1]. Indeed, heterotrophic bacteria degrade organic matter and release CO_2, improving microalgae cell growth and harvesting efficiency due to spontaneous flocculation [14]. The synergistic cooperation among these microorganisms can enhances the settling velocity and nutrients uptake (nitrogen and phosphates) [15].

In natural consortia, microorganisms are already adapted to the prevailing ambient conditions, including the chemical composition of the medium. In these systems, microalgae are closely associated with other microorganisms that coexist in a mixed consortium composed by bacteria, protozoa, and other organisms [1,16,17]. The interaction between microalgae and other microorganisms during the wastewater treatment process can also enhance the harvesting process. Research has shown that the production of significant sticky extracellular polymeric substances (EPS) by bacteria leads to the formation of microbial aggregates that enhance biomass harvesting, reducing by 30% the energy input and global cost of harvesting process [19] due to spontaneous flocculation. On the other hand, bioflocculation involves co-culturing flocculating microalgae, such as *Scenedesmus obliquus* and *Skeletonema* sp., with non-flocculating microalgae like *Chlorella vulgaris* [18] for improved biomass harvesting.

Seasonal variations in weather conditions and wastewater composition can have a significant impact on the formation and composition of the microbial consortia. Such changes can provide the microbial culture with greater resilience and higher ability to eliminate contaminants due to its adaptability according to environmental conditions [18]. The growth rate of microalgae can be boosted due to stimulation from their interactions with bacteria [14]. This makes the microalgae-bacteria consortia a promising platform for advanced wastewater treatment and bioproducts recovery. By manipulating engineering parameters, the symbiotic relationship between these microorganisms can be improved, leading to an increase in lipid or carbohydrate content through nutrient competition, metabolite exchange, and signal metabolite molecule transport.

In microalgae-bacteria interactions, three main types of consortia are commonly found due to the bacterial types present in the culture: I) bacteria naturally associated with microalgae, from consortia isolated from unsterilized wastewater; II) bacteria in activated sludge, with a high capacity of phosphorus and nitrogen removal; and III) bacteria with known identities, that are strains with specific functions, including pollutant-resistant and growth-promoting bacteria, present or added in the consortia [19]. Table 1 shows the potential effect of bacteria and microalgae co-culture.

Positive impacts of bacteria on microalgae in consortia include: (I) nutrient exchange: bacteria can release organic nutrients, cofactors, vitamins, and chelators that promote the growth of microalgae. In return, microalgae produce metabolites such as polysaccharides, amino acids, enzymes, and organic acids that can be utilized by bacteria [13]; (II) enhancement of growth and metabolite production: certain bacterial strains can stimulate the growth and metabolite production of microalgae, such as pigments, carbohydrates, proteins, lipids, and vitamins

Table 1
Effect of microalgae co-culturing with other microorganisms.

Microalgae/Bacteria	Effect	Ref.
Microalgae Aerobic granular sludge enriched by *Nitrospirae* and *Bacillariophyceae*	Improved nitrogen and carbon removal	[22]
Lobomonas rostrata *Mesorhizobium loti*	Secretion of Vitamin B12 allowing growth of dependent microalgae	[23]
C. vulgaris *B. licheniformis* *G. lucidum*	High growth performance. COD, TN, and TP removal rates higher than 80%.	[24]
C. vulgaris Aerobic granular sludge	Boosted biolipid production/auto-flocculation of microalgae	[25]
O. lucimarinus *Pseudomonas sp.* TW7	Methabolism of B_1 used by microalgae	[26]
C. vulgaris *A. beijerinckii*	Microalgae biomass production increased by 71.8% and the protein content increased by 28.2%. COD, P-PO_4^{3-} and NH_4^+–N removal rates increased by 20.8%, 18.5% and 8.9%	[27]
C. vulgaris *B. licheniformis*	Bacterial stimulation of microalgae genes expression related to chlorophyl metabolism	[28]
Microalgae wastewater bacteria	High lipid content in microalgae	[29]
Chlorococcum robustum activated sludge	$NH4^+$–N removal rates 2.58 higher. Improvement on expression of photosynthesis genes	[30]
Scenedesmus sp. *Chlorella* sp. Activated sludge	Increased lipid content in microalgae by 26.6%	[31]
Chlorella sp. *B. fluminensis*	Change in the fatty acid composition	[32]
C. vulgaris *B. licheniformis*	Secretion of Vitamin B_{12} which can enhance the microalgae cell concentration	[33]

COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus; NH4 $^+$N: ammonium; P-PO_4^3: phosphate

[6]; (III) pollutant removal: bacteria in the consortia have the ability to adsorb and decompose organic matter and toxic substances present in wastewater, thus aiding in pollutant removal [11,12]; (IV) carbon fixation: microalgae play a crucial role in the carbon cycle and CO_2 sequestration. Bacteria can contribute to carbon fixation by providing CO_2 to microalgae through respiration and utilizing the oxygen produced by microalgae during photosynthesis [18].

On the other hand, depending on the bacterial strains present in the consortia, some negative impacts on microalgae can occur, including: (I) inhibition of growth: some bacteria can secrete metabolites that inhibit microalgal growth, including proteins, peptides, alkaloids, amino acids, pigments, and fatty acids [30]; (II) competition for resources: in environments with limited nutrient supply, bacteria and microalgae may compete for resources, which can hinder the growth of microalgae [13]; (III) microalgal cell lysis: certain bacterial strains can break down microalgal cells through the production of enzymes, resulting in the lysis of microalgae [31]. In these cases, strategies to prevent the growth of unwanted strains must be designed.

The interactions between microalgae and bacteria in microalgae-bacteria consortia are complex and involve nutrient exchange, signal transmission, and competition. Nutrient exchange enables mutualistic relationships between microalgae and bacteria, where both parties benefit from the exchange of essential substances [13,32–35]. Signal transmission involves the secretion and recognition of signaling molecules, such as indole-3-acetic acid (IAA) and quorum-sensing (QS) signal molecules, which regulate the growth and behaviour of microalgae and bacteria [36,37]. However, competition between bacteria and microalgae can also occur when resources are limited in the environment [13].

Understanding the interactions between microalgae and bacteria in consortia is crucial for optimizing the selection of bacteria and establishing more robust microbiomes for applications in pollution remediation and greenhouse gas mitigation [19].

Surpassing wastewater contaminants that can hinder microalgal growth

Microalgal cultivation can be affected by various contaminants found in wastewater. Heavy metals such as lead, cadmium, and copper can be toxic to microalgae, hindering their vital processes. Organic pollutants like pesticides and industrial chemicals may interfere with metabolic functions, while pathogens and viruses can infect and damage microalgae cells. Fluctuations in pH, temperature, and salinity levels, as well as suspended solids and oxygen depletion, can also stress or harm microalgae growth. In wastewater treatment plants, the pre-treatment step is intended to remove large particles, debris, and solids with high settling capacity. In microalgae-based wastewater treatment, this would help minimize the physical interference and fouling of microalgae cultures, which may impede light penetration and hinder the photosynthesis, hence microalgae growth [38,39].

Contaminants in wastewater can also cause extreme fluctuations in pH levels, which can adversely affect microalgae growth. To address this, CO_2 injection may be considered in order to ajust the pH within the range for microalgae cultivation (pH 6–8) [40]. In cases where wastewater contains high concentrations of toxic compounds, dilution with other non-toxic effluents can be an effective strategy to reduce their impact on microalgae cultivation. This helps lower the concentration of contaminants to a level that is less harmful to microalgae.

Certain contaminants can be pretreated chemically to transform them into less toxic forms or remove them from wastewater. Examples of pretreatment processes include chemical precipitation, oxidation, reduction, and adsorption, which can target specific contaminants and reduce their negative impact on microalgae growth [41,42]. Among these pretreatments, biological processes such as activated sludge treatment, constructed wetlands, or biofiltration can be employed to degrade or remove contaminants through the action of microorganisms before using the effluent as a microalgae culture medium. These biological treatment methods effectively reduce the concentrations of various pollutants [43,44], boosting the suitability of wastewater for microalgae cultivation.

Although membrane filtration techniques such as microfiltration, ultrafiltration, nanofiltration or reverse osmosis are effective at removing toxic particulates and even some pathogens from wastewater [45], they are not yet economically feasible for the treatment of large volumes. Nonetheless, these techniques can significantly improve the quality of treated water used for microalgae cultivation. Furthermore, advanced oxidation processes such as ozonation, UV irradiation, or advanced oxidation with hydrogen peroxide can be employed to break down and remove persistent organic contaminants in wastewater, mitigating their toxic effects on microalgae [46].

Harvesting methods

When developing a harvesting method for microalgae in a wastewater treatment system, the aim should be to target non-specific strains, improve biomass recovery, reduce operating and maintenance costs, and minimize environmental impact [57]. The impact of the harvesting technique on treated water quality and eventual reuse should also be considered [55]. Other crucial factors affecting the harvesting efficiency include cell dimensions, metabolic activity, and cell density [58].

Several reactor designs have been proposed for the treatment of wastewater with microalgae, and they can be divided into two categories: fixed cell/biofilm photobioreactors (PBR) and suspended cell photobioreactors. Fixed cell photobioreactors provide a unique opportunity to address the cost challenges associated with microalgae harvesting. These reactors enable the simultaneous production of a clarified effluent and easily harvestable microalgae [11]. Different configurations, designs, and geometries of biofilm photobioreactos have been studied for the production of microalgal biomass and the removal of nutrients from wastewater. However, the development of microalgal biofilm systems is still in its early stages compared to suspend cell systems, and it remains costly due to the high cost of materials used for microalgae immobilization, such as carrageenan, chitosan, and alginate. Furthermore, the structural weakness of these photobioreactors during long-term operation, especially in wastewater treatment, is a concern due to the high phosphate concentration in some effluent streams [47]. In this case, suspended cell PBR, despite their limitations in harvesting, are still the most commonly used. The most prevalent is the high-rate microalgal pond (HRAP), which was first implemented in California in 1950 [48].

The initial recovery stage can vary significantly and is typically conducted by gravity settling (concentrating from 10 to 20 times), thickening (concentrating around 10 times), dewatering (to produce a paste with a solids content of 10–25%), and drying [49]. Dewatering and drying steps are energy-intensive and costly, and they present the greatest technological challenges in producing microalgae biomass for bioproducts recovery [32]. Existing techniques are based on chemical, mechanical, electrical, and biological principles [48,49].

Centrifugation is a fast and efficient method for microalgae harvesting, but its use is limited due to the high investment and operation cost, in terms of equipment and energy consumption. Also, care must be taken to avoid mechanical damage to microalgae cells during the process [35]. For these reasons, sedimentation and flotation are considered more economic and suitable alternatives to harvest microalgae from wastewater, particularly when the end product does not have a high market value.

Sedimentation occurs naturally, but is a slow process. To reduce the settler volume and hydraulic retention time, it is typically preceded by coagulation/flocculation [50,51], or just coagulation [52]. Clarifiers or settlers are commonly used to separate microalgal biomass through sedimentation.[51,53] Recovering microalgae cells through flocculation by increasing the pH can reduce residual nutrients, yet it increases salinity and residual organic matter in the biomass [51].

Coagulation/flocculation techniques

The implementation of a pre-concentration harvesting step can significantly reduce energy consumption in downstream processes by reducing volume and increasing biomass concentration [53]. Research has been focusing on techniques with low energy requirements and cost. Among conventional methods, sedimentation, coagulation/flocculation, flotation, and filtration are still being studied [31]. Table 2 shows microalgal biomass recovery methods by pre-concentration techniques.

Chemical coagulants used in this step are based on metals or synthetic polymers and are known for their high efficiency [30]. However, there is still no commercial large-scale demonstration of their economic feasibility for harvesting microalgae for biofuels [60]. Additionally, concerns about environmental pollution and contamination of biomass with metals from chemical agents may limit their application [71]. As a result, there is a growing trend towards developing renewable, biodegradable, and highly efficient coagulants [72,73].

Coagulation /flocculation is considered the preferred method for large-scale harvesting of microalgae for use in biomass applications and biofuel production. This method offers advantages such as high harvesting efficiency, low cost, well-defined operational guidelines, and scalability for the volumes required in wastewater treatment plants [74–76]. Coagulation involves destabilizing a colloidal suspension by adding [77]chemicals, namely coagulants. Inorganic coagulants include salts of polyvalent cations such as aluminum (Al^{3+}) or iron (Fe^{3+}) [50]. The most commonly used metal-based coagulants are aluminum sulfate ($Al_2(SO_4)_3$), polyaluminum chloride (PAC), ferric chloride ($FeCl_3$), and ferric sulfate ($Fe_2(SO_4)_3$) [55,78]. On the other hand, flocculation consists of the aggregation of individual particles into flocs, which can be assisted by the addition of organic or inorganic substances known as polielectrolytes [81].

Microalgal biomass harvesting has been optimized through coagulation-flocculation with PAX-18 (aluminum salt) followed by sedimentation at a demonstrative scale, providing simple operational guidelines [50]. Harvested biomass reached a total solids concentration ranging from 5 to 20 g/L, and turbidity below 8 NTU. Furthermore an optimized two-stage gravity thickening process reached a biomass concentration of 26.5 g/L [81].

However, the use of chemicals for coagulation-flocculation may limit the reuse of wastewater and harvested biomass. While metal-based salts are cost-effective, they can also contaminate the effluents due to their no-biodegradability [76]. Additionally, they should not interfere with biomass downstream processing, for instance the anaerobic digestion to generate biogas/biomethane, as metal salts can lower the pH [37]. In this respect, organic flocculants [34] have emerged as an environmentally friendly alternative, offering several advantages. Some of these agents include biodegradable polymeric flocculants such as chitosan [49], starch [64], modified cationic starch [62], tannins [77], and commercially available products like Ecotan, Tanfloc [63], Greenfloc 120, Drewfloc 447, Flocudex CS/5000, Flocusol CM/78, and Chemifloc CV/300 [78].

Studies have shown that harvesting microalgal biomass through coagulation-flocculation can be effective for pre-concentrating biomass. Combining these technologies with a low-cost physical harvesting method (i.e. gravity settling) can enhance the energy yield in the process of microalgae cultured in wastewater biomass recovery. However, scaling-up from experimental results may not be straightforward, as some parameters like the viscosity of the flocculant can interfere with the full-scale harvesting set-up [33].

Table 2
Microalgal biomass recovery by pre-concentration techniques.

Pre-concentration technique	Microalgae	Procedure	Recovery	Ref.
Flocculation	*C. vulgaris P. purpureum*	Polyacrylamide	83.9% 95.5%	[59]
Flocculation	*C. vulgaris* CS-41	Cationic polyacrylamide	97%	[60]
Flocculation	*Chlorella vulgaris*	Ferric sulfate	85%	[61]
Autoflocculation	*Scenedesmus* sp. NC1	- pH	> 88.32% 94.95%	[62]
Autoflocculation	*Ettlia* sp.	pH	91%	[63]
Autoflocculation	*C. vulgaris P. purpureum*	pH	88.4% 58.1%	[59]
Autoflocculation	*P. tricornutum*	- pH	73% 95%	[64]
Bioflocculation	*Scenedesmus* sp.	*Aspergillus niger*	99.4%	[65]
Bioflocculation	*C. pyrenoidosa*	*Citrobacter* *Mucor* pool	97.45%	[66]
Bioflocculation	*C. pyrenoidosa*	*Citrobacter* W4	87.37%	[67]
Bioflocculation	*C. sorokiniana*	*A. niger*	90%	[68]
Bioflocculation	*C. vulgaris* SAG 211–19	Bacterial pool	92%	[69]
Bioflocculation	*C. vulgaris*	Cationic starch grafted tannin	90.8%	[40]
Bioflocculation	*Euglena* sp.	EPS *Skeletonema* sp.	93.4%	[70]
Flocculation-sedimentation	Mixed culture	10 mg/L Ecotan 50 mg/L Tanfloc	91.8% 90.2%	[41]
Flocculation-sedimentation	Mixed culture	25 mg/L potato starch	95%	[71]
Flocculation-sedimentation	Mixed culture	20–40 mg/L Tanfloc	90–94%	[72]
Sedimentation	Mixed culture	Biomass recirculation	94%	[73]
Electrocoagulation-flocculation	*S. almeriensis*	12 V 23 mA•cm^{-2}	99%	[74]
Electrocoagulation-flocculation	*C. vulgaris*	pH	99.55	[75]

Bioflocculation and autoflocculation

The process of spontaneous flocculation, also known as autoflocculation, occurs when CO_2 in the cells is depleted and the culture pH increases, leading to the precipitation of carbonate salts and co-precipitation of magnesium and calcium ions, which neutralize the negative charges on the cells and cause them to coagulate [80]. Harvesting efficiencies of up to 95% can be achieved when supported by an artificial increase in pH (Table 2). Autoflocculation can occur either spontaneously in cultures cultivated under sunny conditions with limited CO_2 supply, or through the addition of an alkali to increase the pH of the medium. The discovery of strains with outstanding autoflocculation abilities, such as *Scenedesmus* sp. NC1 [56] and *P. tricornutum* [59], have further enhanced this approach, reaching harvesting efficiencies of 88% and 73% respectively, without the need for alkali addition (Table 2).

Several studies have demonstrated the impact of pH on microalgae harvesting, with a clear effect observed when the culture pH was induced to an alkaline value [57]. The autoflocculation process was triggered by increasing the pH to an alkaline value through the addition of bases, resulting in high efficiencies. *C. vulgaris* cultured in synthetic medium reach 88.4% of recovery using polyacrylamide, alkaline addition [58]. *P. tricornutum* reach 95% of recovery by induced $Mg(OH)_2$ floculation in seawater improved by the addition of small amount of NaOH [59] (Table 2). The addition of bases has been shown to enhance spontaneous autoflocculation by 22% [59].

Bioflocculation, in contrast to autoflocculation, is the process by which target microalgae are caused to flocculate primarily through the use of EPS secreted by microorganisms present in the medium (Fig. 1). Microalga or diatom species that naturally flocculate can be added after cultivation, grown in consortium [81], or their metabolic by-products such as EPS can be utilized to enhance flocculation.

For instance, a harvesting efficiency of 93.4% for *Euglena* sp. was

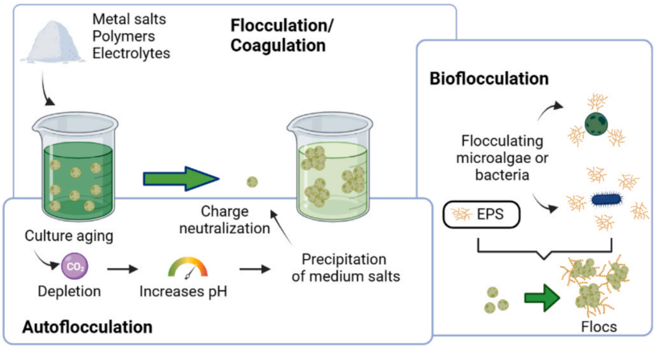

Fig. 1. Diagram of the different coagulation/flocculation approaches.

accomplished through the use of *Skeletonema* sp. as a bioflocculant [65] (Table 2). This method has also been proposed as a mechanism to remove up to 83% of the harmful *Cyclotella* diatoms through *Scenedesmus* bioflocculation [82], suggesting the use of this strain in the harvesting approach with other microalgae.

Consequently, autoflocculation is a cost-effective alternative to chemical flocculants as it consumes low energy, does not involve the use of chemical additives, and can be easily scaled up [34,83]. Many microalgae are capable of secreting soluble EPS that can function as bioflocculants due to their molecular structures that facilitate flocculation [84].

When cultivating large volumes of microalgae in HRAP using wastewater, a substantial amount of soluble EPS will be present in the supernatant after harvesting, which can be utilized to improve subsequent harvesting steps or extracted and purified for other applications [17]. For instance, *Scenedesmus acuminatus* supernatant EPS has been reported to enhance the harvesting efficiency and reduce the need for chemical flocculants in the process [85].

The cultivation of microorganisms capable of producing bioflocculants, either individually or in isolation before their addition to the system, is another possibility, such as the use of bioflocculant-producing bacteria. In this regard, the impact of EPS produced or secreted by these microorganisms on biological flocculation is well established. For instance, one study used seafood wastewater to cultivate microalgae and achieved 92% harvesting efficiency through bioflocculation due to the adhesion of microalgae to EPS produced by bacterial cells grown simultaneously [64]. Additionally, others, produced *A. niger* pellets for use as bioflocculants and obtained a harvesting yield of 99.4%, highlighting the significant role of tyrosine and tryptophan present in the EPS produced by these fungal pellets in the harvesting process [86]. Other examples of bioflocculation include the use of *Citrobacter freundii* (No. W4) and *Mucor circinelloides* to harvest 97.45% of *Chlorella pyrenoidosa* [61], as well as EPS-based bioflocculant extracted from anaerobic sludge, which was used to harvest 91.8% of *Chlorella sorokiniana* [87] (Table 2).

Flotation

Conventional and non-conventional flotation technologies, including electroflotation and dissolved air flotation (DAF), are emerging as promising harvesting methods. DAF, which is widely used for sludge thickening in wastewater treatment plants, has also been applied to microalgal biomass due to its ability to exploit the microalgae's natural self-floating tendency and the low-density flocs they form through coagulation. This method has a fast reaction time, small footprint, moderate operational costs [7,88] and is considered one of the most cost-effective harvesting methods. The general mechanism of DAF is illustrated is Fig. 2.

Research suggests that the integration of microbubbles in DAF can enhance the separation efficiency by expanding the surface area and reducing ascent velocity, leading to better adhesion of the microbubbles to the microalgal biomass [89]. The use of cationic surfactants and other synthetic and natural molecules can produce positively charged bubbles (functionalization) that adhere to the negatively charged flocs of the microalgal biomass, causing them to float to the surface and improving the flotation process, as demonstrated in [90].

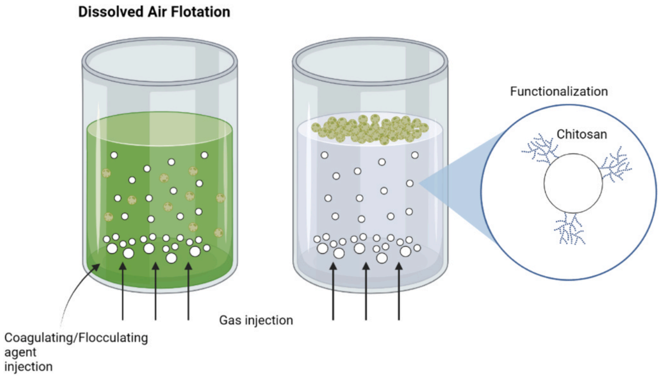

Fig. 2. Dissolved Air Flotation mechanism.

This specificity of flotation as a harvesting method is not shared by the natural sedimentation process [89]. For example, flocculation with the natural flocculant Tanfloc at a concentration of 500 mg/L followed by DAF achieved a harvesting efficiency of 94.5% in *C. sorokiniana* biomass [91], and the use of $Al_2(SO_4)_3$ in *Microcystis flos-aquae* resulted in a harvesting efficiency of 95.5% [92] (Table 3).

The largest wastewater treatment plant utilizing microalgae is located in Chiclana (Spain) with a hydraulic retention time of only 2 days and requires 3 m^2 per person equivalent [1,97]. The harvesting stage of this plant has two goals: (1) to increase the content of suspended solids to 4% for feeding the anaerobic digesters and producing biomethane, and (2) to produce an effluent that complies with the quality standards set by the European Directive 91/271/CE. To minimize energy consumption and make the wastewater treatment economically feasible, it was recommended to use a combination of DAF and a low-energy pressurization pump that consumes 40 Wh/m^3 for the entire system. To further enhance the harvesting efficiency, DAF should be combined with coagulation followed by flocculation [98]. This combination represents one of the few large-scale harvesting systems in microalgae-based wastewater treatment plants. Furthermore, a recent publication reported that DAF is the method of choice in a pilot-scale wastewater treatment plant to control the overgrowth of microalgae

Table 3
Performance of microalgae harvesting by dissolved air flotation.

Method	Microalgae	Procedure	Recovery	Ref.
Coagulation	*C. sorokiniana*	10 mg/L Zetag 75 mg/L Tanfloc 0.5 g/L $Al_2(SO_4)_3$ 1 g/L $FeCl_3$	98.4% 94.5% 95.4% 96.7%	[94]
Flocculation	*Nannochloropsis* sp.	0.16 g/L AFlok-BP1	53.3%	[96]
Coagulation	Microcystis flos-aquae	$Al_2(SO_4)_3$, pH 6.2	95.5%	[95]
Coagulation	Mixed culture	105 mg/L tannin	> 80%	[97]
Autoflocculation	*C. sorokiniana*	pH 12, 20% recirculation rate	96.5–97.9%	[98]
Functionalyzed dissolved air flotation	*C. vulgaris*	Polyoctyl-chitosan	60%	[93]
Flocculation	*C. vulgaris*	12–18 mg/L Chitosan	> 95%	[99]

and concentrate biomass (50 g /L) with an energy consumption below 0.1 kWh/m^3 [99].

Filtration and microfluidic methods

Filtration is a harvesting method based on the physical separation through exclusion, resulting in an effective separation of the culture medium for various types of microalgae [53,90–96]. This makes filtration a promising alternative for cost-effective and environmentally sustainable microalgae downstream processing [100]. The method offers advantages in terms of cost, energy demand, simplicity of operation, and biomass recovery rate [101,102].

However, the major limitation of filtration is fouling and clogging, which increase operational, energy, and maintenance costs [103]. These issues are exacerbated in HRAP that are subject to long periods of cultivation and contamination. New materials have been developed to improve filtration efficiency, reduce filter pore fouling (allowing multiple use cycles) and facilitate the removal of collected biomass [53,103, 104].

Some innovative technologies have demonstrated energy efficiency in microalgae harvesting or the potential for cost reduction, but further feasibility studies are needed to determine their integration into HRAP due to their unique design compared to conventional applications. For example, the microfluidic centrifugal separator can physically separate cells with low energy input, with reported performances between 0.0077 kWh/m^3 [105] and 1.1 kWh/m^3 [106]. However, this approach is not yet practical due to the non-modularity and high cost of testing. This issue may be addressed in the future by using 3D printing for harvesting devices [107]. Another approach being explored is the development of nanocomposite membrane filtration [108] to overcome fouling.

Magnetic and nanotechnological methods

The use of micro or nano magnetic particles (NMP), such as those produced with Magnetite (Fe_3O_4), has emerged as a new approach in microalgae harvesting. These particles can adhere to cells, allowing the aggregates to be recovered through the application of an external magnetic field, such as magnets [109,110] (Fig. 3). This leads to fast, automated, scalable, and efficient separation [111]. Efforts are underway to improve the durability and harvesting capacity of these magnetic particles by functionalizing them with tannins [112], ammonium quaternaries [113], and other hydrophobic compounds [114].

This method offers several advantages, such as the ability to reuse nanoparticles multiple times [115,116], ease of biomass removal [109], simplicity of re-functionalizing particle surfaces which can result in energy savings compared to traditional centrifugation and filtration [117]. However, cost-effective technologies for mass production and recovery of these micro and nanoparticles are still required, as many current methods use solvents and bases to separate microalgae and metallic particles [118].

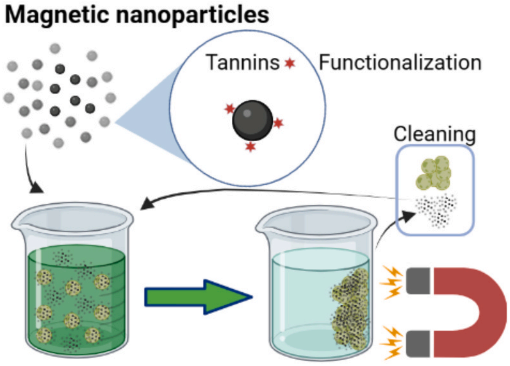

Fig. 3. Harvesting through magnetic nanoparticles.

Despite the advances in microalgae harvesting, the commercial application of magnetic, filtration, and microfluidic approaches is not still feasible. Traditional coagulation/flocculation methods using metallic salts or organic compounds, which have well-established guidelines and life cycle assessment studies, are still the preferred choice for microalgae harvesting. Currently, efforts are focused on making established technologies more affordable, rather than rapidly implementing new ones.

Biochemicals from harvested microalgae

Biochemicals obtained from microalgae harvested in wastewater treatment plants have a potential application across various industrial sectors that do not require a high level of purity or absence of harmful compounds. This includes the production of biofuels and structural polymers such as bioplastics. For instance, microalgae are currently used to produce biogas and biomethane in wastewater treatment plants, which is consumed as vehicle biofuel within the facility [97–99]. In addition, microalgae have the potential to be used for biodiesel production due to their ability to accumulate lipids within their biomass, as these lipids can be extracted and converted into biodiesel [119,120] through the transesterification process. Besides, their biomass can serve as a sustainable source of biodegradable polymers such as bioplastics. Certain cyanobacteria produce significant amounts of polyhydroxyalkanoates (PHA) [121], which are biopolymers that can replace petroleum-based plastics. Utilizing microalgae cultivated in wastewater for PHA production offers an environmentally friendly alternative to traditional plastics. Furthermore, pigments produced by these photosynthetic microorganisms (chlorophyll, carotenoids and phycobiliproteins) have several potential applications [122].

However, for applications requiring a high level of purity, certain considerations need to be accounted for, given the potential presence of contaminants, toxins, and impurities in wastewater. While wastewater treatment processes aim to remove a significant portion of these substances, some of them may accumulate in the biomass posing a risk [123]. To ensure the safety of microalgae-based nutraceuticals or food supplements, several steps should be taken:

I) pre-treatment of wastewater: prior to microalgae cultivation, effective pre-treatment processes should be implemented to remove or reduce contaminants. This may involve physical, chemical, and biological treatments to enhance the quality of wastewater and minimize the presence of harmful substances [124]; II) purification techniques: if contaminants are detected in the microalgae biomass, purification techniques should be employed to eliminate or reduce them to safe levels. Examples of purification methods include membrane filtration or ion exchange extraction methods [125].

Once the bottleneck of ensuring high purity usage of microalgae biomass has been addressed, additional applications can be considered. Microalgae biomass is rich in various nutritional and bioactive compounds, including proteins, pigments, omega-3 fatty acids, antioxidants, and vitamins. These compounds have applications in the production of nutraceuticals and pharmaceuticals, as exemplified by the red pigment astaxanthin, which is a potent antioxidant commonly used in supplements and skincare products [126]. Also, microalgae biomass cultivated in wastewater can be processed and used as a nutrient-rich feed supplement for livestock, poultry, and aquaculture. Microalgae offer a sustainable and protein-rich alternative to conventional feed sources, reducing reliance on fishmeal and soybean-based feeds [126,127]. Finally, microalgae-derived bioactive compounds, including proteins, polysaccharides, and lipids, find application in the cosmetic and personal care industry. Skincare products, hair care formulations, and colour cosmetics incorporate ingredients derived from microalgae due to their moisturizing, anti-aging, and antioxidant properties [128].

Harvesting costs

The cost of microalgae harvesting can be influenced by various factors, such as the technique used, biomass dilution, moisture content, and cell growth phase [129]. While there is a lack of consensus in the literature, it is estimated that harvesting costs can account for 20–30% of the total costs of microalgae biomass production [130–132]. In non-automated centrifugation and filtration systems, costs are typically attributed to labour (58–68%), consumables (30–17%), and equipment (18%) [53].

This represents a significant bottleneck in some products' obtention from microalgae, mainly due to the high operational costs. This is largely due to the low concentration of microalgae biomass (0.2–2.0 g/L) [67,133], and the large volumes of biomass to harvest [101]. Likewise, several cost analyses reported by the US Department of Energy's Aquatic Species Program indicate that there are few viable HRAP options, especially considering the low cost of fuels [134]. Among the microalgae harvesting methods, few approaches are feasible for generating profits due to the lack of scalability in high-capacity harvesting systems such as those required for wastewater treatment [135]. A cost analysis of various approaches for microalgae production and refining in a biorefinery concept found that only 27 out of the 2000 proposed solutions were economically viable [135]. The study concluded that flocculation with aluminium sulfate was the optimal and economically sustainable harvesting method for biomass, especially if electricity generators from green sources (e.g. wind, solar) are installed [135].

In terms of environmental impact, it has been reported that flocculation is the least impactful method and a feasible harvesting candidate for biofuel production [136]. Most of the technologies reported in the literature (bioflocculation, autoflocculation, new flocculation substances, microfiltration, microfluidic separation, magnetic approach and nanotechnological methods) were only validated on a bench scale, with few examples of large-scale tests [137] and its economic viability evaluation.

On the other hand, traditional methods such as coagulation-flocculation have well-defined protocols for large-scale use. The cost effectiveness of the coagulation-flocculation process may depend on the coagulant cost. In this sense, we are witnessing a slow transition from metallic flocculants to natural organic ones, which should benefit the microalgae industry production costs. For instance, the lowest theoretical prices of microalgae harvesting with biodegradable flocculation substances are: Polyacrylamide – 37.5/Ton [138], Tanfloc – \$38/Ton [139] and Cathionic Starch grafted tannin – \$27.4/Ton [36] (Table 4), which are biodegradable and affordable for low-value applications.

Bioflocculation techniques are promising for microalgae harvesting from wastewater, yet the economic viability it still to be determined. Only a few studies have reported the costs of bioflocculation processes (\$1350 per ton) [62]. Currently, there is no comprehensive analysis that compares the cost-effectiveness of these methods to traditional flocculation, so further research is needed. It is estimated that a producing less than \$40 per ton of harvested microalgae may be possible through the use of the coagulation-flocculation technique [143]. However, the high cost of centrifugation, which is at least \$480 per ton, makes it an uneconomical method for low-value applications [131]. The cost of centrifugation can be reduced by incorporating a pre-flocculation stage in the process [35].

The total cost of harvesting and dewatering is estimated to be around \$160 per ton and \$1100 per ton, respectively, with an energy consumption of 4.5 kWh/kg. These values apply to the production of biodiesel, which is estimated as \$2180 per ton using centrifugation as the sole harvesting method, but can be reduced to \$14.5 per ton when combined with the slow sedimentation method [89].

Although the high cost of the natural flocculant chitosan (\$20–50/kg) may limit its large-scale application, studies have indicated its theoretical economic viability as a flocculant through its use as a coating for micro and nanospheres functionalization, optimization of flocculation, and dual harvesting with other flocculants such as clay [34,20,21,54,79]. Harvesting through chitosan has been reported to cost \$7280 per ton of microalgae, which is reduced to \$4920 with double flocculation using $Al_2(SO_4)_3$ [140]. On the other hand, modifying of chitosan to nano-chitosan can reduce the harvesting cost to \$24.6 per ton [141], which would enable the economically viable biodiesel production from microalgae biomass below a threshold of \$80 per ton [141,149].

Table 4
Microalgae harvesting cost.

Method	Estimated cost	Ref.
Cationic polyacrylamide	\$37.5/Ton	[141]
Tanfloc	\$38/Ton	[142]
Cathionic starch grafted tannin	\$27.4/Ton	[40]
Bioflocculation	\$1350/Ton	[67]
Centrifugation	€480/Ton	[134]
Chitosan	\$7280/Ton	[143]
$Al_2(SO_4)_3$-chitosan	\$4920/Ton	[143]
Nano chitosan	\$24.6/Ton (4 g/L culture)	[144]
Fe_3O_4 Arginine magnetic nanoparticles	\$347/Ton	[111]
$Al_2(SO_4)_3$	\$28/Ton	[145]
Coagulation-flocculation	\$40/Ton	[146]
Cationic polyacrylamide	\$37.5/Ton	[141]
Cationic polyacrylamide Benthonite recycling	\$15.9/Ton	[141]
Fe_3O_4-chitosan	\$3548.64/Ton	[119]
Ferric chloride/chitosan	\$7925/Ton	[143]
Flopam	\$157Tton	[142]
Ultrafiltration	\$300 (50x concentration)	[147]
Filtration-centrifugation	\$5.35/m^3 (770–1086x concentration)	[58]
Flocculation-centrifugation	\$4.52/m^3	[58]
Fe_3O_4 magnetic nanoparticles	\$1830/Ton	[116]
Fe_3O_4- amine functionalized nanoparticles	\$450– 520/m^3	[148]
Fe-based nanomaterials	\$505/Ton	[149]
Filtration	\$206/Ton	[150]
Flocculation-filtration	\$139/Ton	[150]
Bioflocculation	\$1950/Ton	[90]
Flocculation-filtration	€160/Ton	[151]

Despite having low energy expenditure, harvesting using functionalized NMP can be prohibitive for large-scale applications due to the cost of reagents and energy required for preparation. However, recent progress has been reported in the manufacture of nanoparticles that can be produced at a temperature of 20 °C and are effective at pH 8.0, reducing the cost of use and making them attractive for industrial applications [150]. Among the NMP, the lowest cost so far reported is for functionalization with arginine (\$347/ton) [73], but it is still much higher than the results currently reported for coagulant polymers.

Among the various harvesting methods described in the literature, aluminium sulfate harvesting continues to be the most cost-effective, with a cost of \$28 per ton. While cationic starch grafted tannin and nano-chitosan have a total cost of \$27.4 per ton and \$24.6 per ton, respectively, there are no reports of their commercial use. In terms of cost and biodegradability, Tanfloc may be a cost-effective alternative for harvesting microalgae for low-value applications, such as biodiesel production. Although ultrafiltration approaches have been reported to be less expensive [144], their cost and performance are still not comparable to those of flocculant polymers.

In light of the information presented, the use of metallic and organic flocculating agents for harvesting microalgae remains the best option despite its current uneconomic viability. However, advancements in nanotechnology and materials engineering hold a great potential for improving its application. The harvesting process remains a challenge for the growth of the microalgae industry, which is estimated to be worth \$10 billion (https://www.fnfresearch.com/microalgae-market) and projected to reach \$18 billion by 2028. Therefore, there is significant interest from the industry in improving harvesting techniques and reducing costs to make the production of bioproducts from microalgae economically feasible.

Concluding remarks and future prospects

The techniques used to harvest biomass from microalgae-based wastewater treatment systems must be cost-effective and environmentally friendly. The aim of this technology is not only to treat water and make it safe for disposal or reuse, but also to recover biomass for bio-based products and biofuels generation. As this review shows, there are some cost-effective methods for harvesting microalgal biomass in wastewater treatment systems, which can be suitable for in applications such as biofuels generation. Economic feasibility is expected provided that the cost of biomass harvesting does not exceed 30% of the overall bioenergy production cost.

A combination of a low-cost pre-concentration techniques, such as autoflocculation, bioflocculation, or coagulation, and a solid-liquid separation method, such as gravity sedimentation or flotation, can be cost-effective for biomass thickening. Although new approaches like magnetic and nanotechnological methods have been explored, they still need to be evaluated for their economic feasibility on a large scale before they can be considered as viable alternatives.

The natural consortia characteristics of microalgae-based wastewater treatment should be further explored for harvesting purposes. There have been few studies focused on inducing autoflocculation in cells, and even fewer in the context of wastewater treatment. In this system, cells with natural flocculation capabilities can be selected based on their physical characteristics. For example, continuous recirculation of cells separated by a gravity settler can result in a higher concentration of autofloccultant cells in the media, which is a simple physical approach. This can result in the majority of the culture being composed of autofloccultant cells.

Efficient, sustainable biomass harvesting in microalgae-based wastewater treatment is crucial for the process to be cost-efficient and environmentally friendly. While new approaches hold promise, their economic feasibility on a large scale requires further evaluation. Exploring the natural characteristics of microalgae consortia in wastewater treatment offers opportunities to enhance harvesting capabilities.

Declaration of Competing 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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