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Review

Recent Advances of Microalgae Exopolysaccharides for Application as Bioflocculants

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Abstract: Microalgae are used in flocculation processes because biopolymers are released into the culture medium. Microalgal cell growth under specific conditions (temperature, pH, luminosity, nutrients, and salinity) provides the production and release of exopolysaccharides (EPS). These biopolymers can be recovered from the medium for application as bioflocculants or used directly in cultivation as microalgae autoflocculants. The optimization of nutritional parameters, the control of process conditions, and the possibility of scaling up allow the production and industrial application of microalgal EPS. Therefore, this review addresses the potential use of EPS produced by microalgae in bioflocculation. The recovery, determination, and quantification techniques for these biopolymers are also addressed. Moreover, other technological applications of EPS are highlighted.

Keywords: autoflocculation; bioflocculation; biopolymers; microalgal biotechnology; recovery process; identification techniques

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1. Introduction

Microalgae are photosynthetic microorganisms cultivated in marine, hypersaline, brackish, freshwater, or wastewater for the production of high value-added compounds (pigments, proteins, lipids, polyunsaturated fatty acids, and intracellular and extracellular polysaccharides) [1–3]. Thus, the biomass of these microorganisms is of industrial interest in the development of pharmaceuticals, nutraceuticals, cosmetics, and food/feed [4]. However, the recovery of microalgal biomass is costly (20–30% of total production costs), limiting the commercialization of these bioproducts on a large scale [5].

Moreover, there is growing interest in alternative methods of harvesting microalgae biomass at low cost and energy. Microalgae exopolysaccharides (EPS) have been highlighted for promoting autoflocculation or acting as bioflocculants. Thus, these compounds can act in the process of microalgal biomass recovery and treatment of industrial effluents, with the additional advantage of low energy consumption, low environmental impact, and reduced production of toxic compounds [6–9].

In this sense, Yang et al. [10] reported bioflocculant activity of EPS from *Scenedesmus acuminatus* in the recovery of the biomass of this same microalga. The results showed that the addition of microalgae EPS (3.2 mg g^{-1}) significantly reduced the use of aluminum coagulant (Al^{3+}) from 77.6 to 4.5 mg g^{-1}. The authors noted that this result potentially reduced the chemical cost by up to 75%. Aljuboori, Uemura, and Thanh [11] reported that

EPS from *Scenedesmus quadricauda* showed bioflocculant activity in the biomass recovery of this same microalga with flocculation efficiency of up to 86.7%.

Microalgal EPS production depends on the microalgae species, cultivation conditions, or nutrient limitations such as nitrogen and phosphorus deprivation [12–14]. Furthermore, polysaccharides produced by microalgae can be classified based on their functionality: (i) structure of cell walls, (ii) storage (intracellular), and (iii) extracellular or exopolysaccharides, which are released into the environment. In addition to being a flocculating agent, microalgal EPS may have immunomodulatory [15], antioxidant [16], anti-inflammatory [17], anticoagulant [18], antibacterial [19], and anticancer properties [20], and may act as a gelling and thickening agent [12].

The most prominent families of microalgae in the production of EPS were Desmidiaceae, Chlamydomonadaceae, Chlorellaceae, Porphyridiaceae, and Glaucosphaeraceae [1]. However, there are few studies about other microalgae diversities in the production of this metabolite and its flocculating efficiency. In this context, this review reports the potential use of EPS produced by microalgae in bioflocculation. The recovery, determination, and quantification techniques concerning these biopolymers are also addressed. Moreover, other technological applications of EPS are highlighted.

2. Potentiality of Microalgal Polysaccharides in Bioflocculation

The use of autoflocculating microalgae to induce flocculation of non-flocculating species is considered one of the most promising methods in bioflocculation [21]. EPS can create a viscous coating around cells [12]. This coating in bioflocculation allows the formation of an aggregate consisting of microalgae–microalgae, or microalgae with another microorganism, leading to adherence of microalgae to the flocculent moss surface [22] or flocculent sludge surface [23,24].

Wang et al. [25] examined the algal-bacterial bioflocculation induced by strains of *Scenedesmus obliquus*, *Botryococcus braunii*, *Chlorella* sp. BWY-1, *Haematococcus pluvialis*, *Dictyosphaerium ehnenbergianum*, and *Chlorella vulgaris*. The microalgae *Scenedesmus obliquus* and *Botryococcus braunii* did not allow flocculation. *Chlorella* sp. BWY-1, *Haematococcus pluvialis*, and *Dictyosphaerium ehnenbergianum* showed high flocculation activity with 67.8, 50.9, and 43.2%, respectively. The concentration of chlorophyll a was approximately 5.5 mg L^{-1} for *Chlorella* sp. BWY-1, 3.0 mg L^{-1} for *Haematococcus pluvialis*, and 4.9 mg L^{-1} for *Dictyosphaerium ehnenbergianum*. *Chlorella vulgaris* exhibited the best flocculation activity (86.6%). However, the concentration of chlorophyll a decreased rapidly, reaching the lowest value in the experiment (1.6 mg L^{-1}). The authors attributed these results to the high energy required for the production of EPS.

EPS represent carbon and energy reserves for cells and are often excreted by microalgae as part of physiological processes or under stress conditions [12,21,26–28], such as light intensity and light continuity, temperature [29,30], pH [31,32], excess, limitation or absence of nutrients, such as carbon, nitrogen, phosphorus, sulfur, sodium, potassium, iron, magnesium and calcium [14,33,34], and toxic substances [35,36], such as cadmium [37], copper, lead, chromium, nickel [31,38], and silver [39].

Koçer et al. [40] investigated the potential for EPS production using the microalgae *Chlorella minutissima*, *Chlorella sorokiniana*, and *Botryococcus braunii*. The authors analyzed the effects of nitrogen and carbon concentrations in the culture medium and light intensity on EPS production. *Chlorella minutissima* produced the highest concentration of EPS (0.245 ± 0.003 g L^{-1}) compared to *Chlorella sorokiniana* (0.163 ± 0.002 g L^{-1}) and *Botryococcus braunii* (0.117 ± 0.001 g L^{-1}). Regarding the effects of nitrogen ($NaNO_3$) and carbon (Na_2CO_3) concentration in the BG-11 medium and lighting time on EPS production, the best conditions for three microalgae were nitrogen reduction (0.2 g L^{-1}) and carbon (0.02 g L^{-1}) and 12 h of lighting time. Under these conditions, *Chlorella sorokiniana*, *Botryococcus braunii*, and *Chlorella minutissima* produced 0.183, 0.120, and 0.215 g L^{-1} EPS, respectively. Thus, the authors observed an inverse relationship between the supply of these nutrients and the concentration of EPS produced.

Surendhiran and Vijay [41] analyzed the flocculation efficiency of the *Chlorella salina* using a microbial flocculant. The authors found that flocculation was improved with zinc chloride ($ZnCl_2$) as a cationic inducer. Moreover, the flocculation obtained maximum efficiency (98.6%) with the following conditions: temperature (30.6 °C), pH (10.4), flocculation time (6.2 h), the volume of bioflocculant (0.34 mL), and cationic inductor concentration (0.031 mM).

Thus, in addition to contributing to biomass recovery and mitigation of industrial effluents, the use of microalgae for the production of EPS proves to be an efficient and low environmental impact way to reduce costs in the flocculation process.

3. Recent Advances in Harvesting Algae and Pretreatments for the Extraction of Cell-Bound EPS

Studies on optimization strategies for the recovery of microalgae biomass are increasing since it demands high energy and operating costs (20 to 30% of the total production cost) [42–44]. In this way, it is necessary to define the recovery method to process high biomass production (Table 1). Thus, physical and chemical characteristics of the culture medium, such as pH, salinity, and cellular structure of microorganisms, must be analyzed and linked to the chosen method [44–46].

Table 1. Technoeconomic analysis of microalgae biomass recovery (adapted from Valdovinos-García et al. [47]).

Drying Process	Harvest Method	Responses			
		Electrical Power (kWh Year^{-1}) *	Unit Production Cost (US $ kg^{-1}) *	Operating Cost (US $ Year^{-1}) *	Biomass Production (kg Year^{-1})
Spray drying	Auto-flocculation followed by vacuum filtering	13,988	1.25	61,000	48,145.6–53,495.11
	Auto-flocculation followed by plate press filter	8738	1.19	58,000	
Drum dryer	Auto-flocculation followed by plate press filter	8297	0.85	42,000	48,145.6–53,495.11
	Auto-flocculation followed by vacuum filtering	13,597	0.91	45,000	
Spray drying	Adding an iron salt followed by vacuum filtering	13,987	1.21	59,000	48,145.6–54,306.51
	Adding an iron salt followed by plate press filter	8737	1.15	56,000	
Drum dryer	Adding an iron salt followed by plate press filter	8296	0.81	40,000	48,145.6–54,306.51
	Adding an iron salt followed by vacuum filtering	13,546	0.87	43,000	
Spray drying	Flocculation with chitosan followed by vacuum filtering	13,988	1.26	62,000	48,145.6–54,306.51
	Flocculation with chitosan followed by plate press filter	8728	1.20	59,000	
Drum dryer	Flocculation with chitosan followed by plate press filter	8297	0.86	43,000	48,145.6–54,306.51
	Flocculation with chitosan followed by vacuum filtering	25,597	0.92	46,000	

* The values presented are the sum of respective data obtained from harvesting and drying processes.

Traditionally, methods used in biomass recovery include coagulation, flocculation, flotation, gravity sedimentation, and centrifugation [45,46,48]. In flocculation methods, chemical compounds such as sodium hydroxide (NaOH), magnesium sulfate ($MgSO_4$), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), sodium alginate ($NaC_6H_7O_6$), tannin, and other polymers can be used (Table 2). However, these can be combined to

optimize the processes in the recovery of larger volumes of biomass [43,45,49]. In recent years, combined methods such as sedimentation–flocculation–coagulation, flocculation–centrifugation, and electrocoagulation–flotation have been used and show promise concerning cost and energy efficiency [43–46,49,50]. Moreover, natural (including EPS) and synthetic flocculating agents are applied in microalgal recovery [42,51].

Table 2. Comparative yield of microalgae flocculation/coagulation using different substances.

Microalgae	Recovery Process	Experimental Conditions	Substance Used in Biomass Recovery	Process Yield	Reference
Chromochloris zofingiensis	Flocculation and alkaline sedimentation	Bold's Basal Medium, biomass concentration 0.5, 1.0, 1.5 g L^{-1}, 200 L working volume, air sparged (~10 L min^{-1}), centrifugation 12,000× *g* for 10 min, pH 7.0	NaOH (4.6 and 8 mM) and $MgSO_4$ (6, 8 and 10 mM)	Sedimentation yield above 90%	[52]
Chlorella vulgaris UTEX 395	Flocculation with naturally available magnesium in brackish water	BG-11 medium, biomass concentration 0.3% *v* v^{-1}, stirring at 700 rpm for 5 min, pH 9.0	Mg^{2+} (0.3 mM) and $MgCl_2$ (9.6 mM)	Sedimentation yield of 100 cm h^{-1}	[53]
Chlorella marinha sp.	Flocculation induced by NaOH	F/2 medium Guillard, 5000× *g* for 5 min	NaOH (5 and 7 mM)	Flocculation yield of 90%	[54]
Chlorella vulgaris	Reversible flocculation	Wright's Cryptophyte medium, 10 L working volume, centrifugation 20 min of stirrung at 250 rpm, pH 8.5	Mg (2.5 mM) and NaOH (4 mM)	Maximum flocculation efficiency of 90%	[55]
Phaeodactylum tricornutum				Maximum flocculation efficiency of 73%	
Chlorella salina	Alkaline autoflocculation	Guillard's F/2 media with artificial seawater media, optical density of 0.1, 11,500× *g* for 12 min, pH 8.0	NaOH (4 mM)	Biomass recoveries greater than 95% efficiency	[56]
Scenedesmus sp., *Kirchneriella* sp., and *Microcystis aeruginosa*	Induced flocculation	Medium mixture, 50 L working volume, 200 rpm for 1 min, pH 7.7	$CaCl_2$ (20, 60, 120 and 180 mg L^{-1}), $NaC_6H_7O_6$ (10 and 20 mg L^{-1}) and Tannin (10 and 20 mg L^{-1}).	Maximum flocculation efficiency for Tannin 95.35%, sodium alginate 90.49% and, calcium chloride 84.04%	[57]
Chlorella vulgaris	Induced natural flocculation	N8 medium, 1 L working volume, 100 rpm for over 24 h, pH 6.8	Chitosan (0.25 g L^{-1}) and aluminum sulfate (2.5 g L^{-1})	Flocculation yield of 90%	[58]
Chlorella vulgaris	Induced flocculation	MLA medium, 350 L working volume, 100% CO_2 for 1 min d^{-1}, pH 9.0	Cationic polyacrylamide polymer (2 g L^{-1})	Flocculation yield of 97%	[59]

Nguyen et al. [42] develop cationic polymers (poly[2(acryloyloxy) ethyl]trimethylammonium chloride and poly(3acrylamidopropyl) trimethylammonium chloride) for the harvest of *Chlorella vulgaris* and *Porphyridium purpureum*. The polymers show excellent flocculation performance for both microalgae with stable floc formation. Similar recovery strategies were also observed by Zhu et al. [51] when they analyzed three types of sulfates (aluminum sulfate, aluminum potassium sulfate, and ferric sulfate) as flocculants for harvesting *Chlorella vulgaris*. The results showed the flocculate potential of the chemical agents at a dosage of 2.5 g L^{-1} and speeds for coagulation and flocculation (150 and 25 rpm), and time of 10 min. The biomass recovery efficiency found ranged from 83 to 90%.

After recovery, it is important to pretreat the biomass to obtain EPS bound to microalgal cells [12,60]. Researchers describe that up to 50% of the total EPS can remain bound to the cell of these microorganisms. However, there are no standard methods for this extraction. The use of chemical reagents such as formaldehyde, ethylenediaminetetraacetic acid, sodium hydroxide, as well as sonication, heating, and washing with distilled wa-

ter and/or complexation/treatment with ionic resins, were performed to recover these polysaccharides from the surfaces of microalgal cells [12,60,61].

Furthermore, the method used to break EPS and cell wall interactions must not promote cell lysis to avoid contamination by intracellular compounds and compromise the entire EPS recovery process [60,61]. Thus, some chemical agents such as formaldehyde and glutaraldehyde were used to protect the microalgal cell from lysis during EPS isolation [60,61]. These fixing agents chemically react with hydroxyl, sulfhydryl, carbonyl, or amino groups present in microalgae cell membranes and prevent cell lysis during EPS extraction. However, they can compromise the method if they react with the extracted EPS [12,60,61]. The washing of microalgae cells with water demands temperature (30–95 °C) and time (1–4 h), which can promote cell lysis and consequent contamination with intracellular constituents [60]. In this sense, in most studies, microalgae EPS were isolated without biomass treatment since these treatments add a high cost to the processes [12,60,61].

4. Techniques for Recovery/Identification of Microalgae Polysaccharides

The recovery of intracellular and extracellular compounds from microalgae cultures is the bottleneck to applying this sustainable technology [62]. Recently, several studies have investigated microalgae recovery and sedimentation methods from flocculants as an alternative with high energy efficiency (Table 3) [43].

Table 3. Techniques for recovering and identifying of microalgal EPS.

Techniques	Microalga	Process Conditions	Objective	Responses	Reference
Membrane filtration	*Porphyridium cruentum*	Permeate fluxes of 49.8, 68.9 and 81.9 L h^{-1} m^{-2} and 4 bar for, respectively, cross-flow velocities of 2.5, 3.3 and 4.2 m s^{-1}; 49.7 L h^{-1} m^{-2}.	Influence of cross-flow velocities on filtration performances.	EPS concentration at 6.3 to 10.4 times reaching from 1.74–2.26 g L^{-1} (80% (*w* w^{-1}) recovery).	[63]
Membrane filtration	*Botryococcus braunii* CCALA778	Culture flow circulating in 110 cm^2 area 0.2 µm microfiltration hollow fiber membrane (GE Healthcare®, CFP-2-E-35MA).	Optimization and efficiency of extraction and recovery, ensuring high efficiency without compromising the viability of the culture.	Increased EPS productivity by 25% (4 g m^{-2} d^{-1}). Daily EPS extraction rate of 0.36 g m^{-2} d^{-1}.	[64]
Ultrafiltration (polymeric membrane)	*Porphyridium cruentum*	PES 50 kDa flat membrane in full recirculation mode, with permeate flow transmembrane pressure (TMP) curves (0.10–1.06 kg GlcEq m^{-3}), tangential fluid velocity (0.3–1.2 m s^{-1}), and temperature (20 and 40 °C).	Parametric study of ultrafiltration of EPS solutions in organic membrane.	The concentrated solution of 0.10 kg GlcEq m^{-3} (moderate fouling, portion of irreversible/reversible fouling was 88 and 12%).	[65]
Diafiltration	*Flintiella sanguinaria*	Vivaflow ultrafiltration system (Sartorius) and 100 kDa NMWCO membranes.	Native EPS extraction.	EPS solution was concentrated (volume reduction factor of 5).	[66]
High Pressure Anion Exchange Chromatography (HPAEC)	*Flintiella sanguinaria*	The quantification of monosaccharides was achieved by injecting different concentrations of monosaccharides and plotting the response area as a function of concentration.	Quantification and identification of native EPS extract.	Identification of galactoxylan, with rhamnose and glucuronic acid, low content of sulfate groups (0.6%), and methylated and acetylated compounds (5.1 and 3.2%, *w* w^{-1}).	[66]

Table 3. *Cont.*

Techniques	Microalga	Process Conditions	Objective	Responses	Reference
Molecular weight (Mw)	*Chlorella zofingiensis*	Determination with gel permeation chromatography and refraction detector (RI), and at a flow rate of 0.5 mL min^{-1} using 0.2 M sodium nitrate as the mobile phase.	Investigation of the physicochemical characteristics of EPS.	EPS yields were 208.4 and 364.3 mg L^{-1} with average molecular weights of 2.66×10^4 and 1.88×10^4 Da, respectively.	[20]

Several species of algae can act as flocculants, where the process allows the advantage of recycling the medium [67]. The flocculation capacity of autoflocculating microalgae is closely related to EPS secretion [6]. With the optimization of cultivation conditions, the extraction of EPS becomes advantageous since it promotes higher productivity of the biomass and biocompound. In this way, increases in the extraction yield and sustainability of the process are reached. The implementation of recovery and identification protocols varies according to the location of the polysaccharides in the culture [68,69].

Among the classic methods of extracting EPS are centrifugation and microfiltration. These procedures separate the biomass from the EPS-constituted precipitate [20]. After this step, the centrifuged material must be precipitated using methanol, alcohol, ethanol, or isopropanol. With this method, the selective concentration of EPS is possible [70]. As an alternative to the classical methods described, the recovery of EPS can be carried out during the downstream and upstream processes, without the need for chemical additives [12,71]. Methods such as sonication and heating are also used to extract microalgal EPS [72]. Filtration modules from 1 kDa to 500 kDa have been used for the concentration of extracellular compounds present in the culture medium. The ultrafiltration technique can be performed in the following forms: rotating devices, tubular, flat, or spiral plate and hollow fiber, where the liquid medium flows parallel to the ultrafiltration surface and the fraction of interest is permeated through the membrane [12,69].

To increase the performance of filtration techniques, the use of synthetic material is necessary, such as nanocomposite membranes consisting of nanoparticles in a polymeric membrane (SiO_2, TiO_2) [73]. The identification of EPS can be performed through Fourier transform infrared spectroscopy from functional compound determination [68]. Gas chromatography with mass spectrometry has shown excellent results in the identification of microalgae EPS. Other techniques, such as ion-exchange chromatography, size exclusion chromatography, and affinity chromatography, are widely used to purify and fractionate microalgal polysaccharides [68,69].

5. Application of Microalgal Bioflocculants in Microalgae Harvesting

5.1. Bioflocculation

Bioflocculation is considered a sustainable method that occurs from the aggregation of microalgal cells in the presence of biopolymers synthesized by microorganisms. Biopolymers are mainly composed of extracellular polymeric substances, which contain polysaccharides, proteins, lipids, and nucleic acids in their structure [21,24,74,75].

In addition to the presence of metabolites synthesized by microorganisms, the bioflocculation processes of non-flocculating microalgae can occur in the presence of other microorganisms, such as fungi, bacteria, and other microalgae [21,75]. This process was demonstrated by Guo et al. [7], using supernatant and cell suspension from the autoflocculating *Scenedesmus obliquus* AS-6-1 culture for the recovery of non-flocculating microalgae biomass. Furthermore, to increase the efficiency of bioflocculation processes, other flocculants such as Al^{3+} and Fe^{3+} can be added together with extracellular polysaccharides [7,10,76]. According to Yang et al. [76], the extracellular polymeric substance co-extracted in the Al^{3+} recovery process after the primary flocculation step contributed to the clotting process of *Scenedesmus acuminatus*. There was an increase in the process' efficiency when extracellular substances ($\geq$0.430 mg L^{-1}) were added.

Bioflocculation has been considered a promising strategy for cost reduction in the recovery of microalgal biomass. Among the advantages of this method, there is the absence of chemical flocculants, ease of operation, and an ecologically correct and sustainable approach [24].

5.2. Autoflocculation

Unlike bioflocculation processes, autoflocculation can occur naturally from cell adhesion and aggregation. The autoflocculation of microalgae cells is a phenomenon caused by the secretion of flocculating substances (e.g., glycosides or polysaccharides) which adhere to the microalgal cells. Under alkalinity conditions, autoflocculation occurs from positive precipitates formed by calcium and magnesium ions that neutralize the negative charge of microalgal cells. The other mechanism is related to the EPS produced by microalgae during their physiological activities, which induce flocculation [74]. The autoflocculation process is dependent on the cellular characteristics of the microalgae and other factors such as the composition of available nutrients (e.g., the concentration of Ca, Mg, N, and P), type and concentration of precipitates formed, and pH value [21,24,74,77]. Some autoflocculating species have been reported, such as *Scenedesmus rubescens* SX [68], *Scenedesmus obliquus*, *Chlorella vulgaris*, *Ettlia texensis*, *Ankistrodesmus falcatus* [78], and *Neocystis mucosa* SX [60], among others. Although the mechanisms of autoflocculation are still not well understood, it has been shown that extracellular polymeric substances can influence the autoflocculating capacity of microalgae [21,77]. According to Wan et al. [79], autoflocculation can occur when flocculants produced by the microalgae neutralize charges, forming bridges or patching adjacent cells. Additionally, the hydroxyl and carboxyl groups in the polysaccharide are strongly related to microalgae flocculation. They serve as binding sites during this process. These characteristics were demonstrated in studies by Alam et al. [9], Guo et al. [7], and Lv et al. [60].

Some microalgae produce extracellular polymeric substances in significant amounts during physiological activities, especially at the end of the growth phase when the extracellular polymer acts as a flocculant [74,80]. In these cases, the parameters used in cultivation tend to influence this process, as they affect the production and composition of extracellular polymeric substances [81].

Guo et al. [7] determined that the autoflocculant activity of *Scenedesmus obliquus* AS-6-1 occurred until the end of the exponential phase and increased with the time of cultivation and the cell concentration of the medium. Autoflocculation occurred from the presence of extracellular biopolymers, which formed a membrane on the cell surface, forming aggregates and sedimenting. Alam et al. [9] studied the spontaneous flocculation of *Chlorella vulgaris* JSC-7 and the addition of medium from this strain in non-flocculent microalgae. According to the authors, spontaneous microalgae flocculation was associated with an extracellular polysaccharide composed of glucose, mannose, and galactose. *Chlorella vulgaris* JSC-7 was also able to improve the biomass recovery of the other microalgae tested. In another study, polymeric substances synthesized by *Chlorella vulgaris* (FACHB-31) and bound to the cell were responsible for increasing autoflocculation. The production of polymeric substances was influenced by glycine added in the medium with light intensity and mixing time. As the concentration of polymeric substances is higher at the end of the cultivation, this period was also responsible for the higher solid concentration rates achieved in the flocculation. However, cultivation time is a parameter that must be considered, as it can increase the costs of harvesting microalgae [80]. Table 4 presents some studies on the production and potential application of extracellular polymeric substances in microalgae harvesting. According to Ummalyma et al. [24], more studies are needed to understand the mechanisms involved in microalgae autoflocculation. The development of research based on the mechanisms of autoflocculation will contribute to cost reduction, ensuring sustainability in downstream processes.

Table 4. Production and potential application of microalgal bioflocculants in microalgae harvesting.

Microalga	Description of the Experimental Set	Chemical Composition and Identification of Bioflocculants	Produced and Applied Concentration of Bioflocculants	Bioflocculant Application	Reference
Spirulina sp. LEB-18	Outdoor cultivation, using a raceway (250 L), Zarrouk medium, under natural light for 30 d (probable stationary phase), and pH 9.8–10.5.	Sugars composition: glucose, galactose, fructose, and organic acids were glucuronic, galacturonic and pyruvic.	9.5 g L^{-1} was the highest production of extracellular polymeric substances.	Possible application as a bioflocculant and/or other industrial applications.	[82]
Scenedesmus obliquus AS-6-1	Cultivation to stationary phase, 28 °C, 14/10 h light/dark cycle, 60 mol m^{-2} s^{-1}.	Cell wall-associated polysaccharides. Monomers consist of glucose, mannose, galactose, rhamnose and fructose.	0.6 mg L^{-1} of bioflocculant was responsible for 88% of the flocculant activity of *Scenedesmus obliquus* FSP-3.	Bioflocculation of *Chlorella vulgaris* CNW-11, *Scenedesmus obliquus* FSP-3 and *Nannochloropsis oceanica* DUT01.	[7]
Chlamydomonas reinhardtii	Cultivation at 5 to 25 °C, pH 6–10, 40–60 µmol photons m^{-2} s^{-1}, 30 d of cultivation.	Bioflocculant composition: proteins (42.1% *w* w^{-1}), carbohydrates (48.3% *w* w^{-1}), lipids (8.7% *w* w^{-1}), and nucleic acid (0.01% *w* w^{-1}).	4 mg L^{-1} of bioflocculant was responsible for 96.6% of the flocculant activity of *Chlamydomonas reinhardtii*.	Microalgae bioflocculation	[83]
Ettlia texensis	Cultivation in a 4 L photobioreactor, batch mode, 24-h lighting, 300 rpm, 26 °C, pH 6.5, and 300 µmol m^{-2} s^{-1}.	Bioflocculants containing mainly glycoproteins patched to the cell surface.	–	Microalgae autoflocculation and bioflocculation.	[6]
Desmodesmus sp. ZFY and *Monoraphidium* sp. QLY-1	Microalgae were used in co-culture, cultivated in mixotrophic medium BG-11 + ammonium nitrate and glucose, pH 6.8, 300 mL, 25 °C, 120 rpm, 3500 lux, and 7 d of cultivation (stationary phase).	Bioflocculant consisted mainly of polysaccharides and proteins. The levels of polysaccharides in co-culture were 46.53% in substances loosely bound to cells (LB-EPS).	Concentration of total extracellular polymeric substances was 368.40 mg L^{-1}.	Microalgae bioflocculation.	[84]
Scenedesmus acuminatus	Cultivation performed in 15 L photobioreactors at 25 °C, modified BG-11 medium (NO_3 reduction), 180 µmol m^{-2} s^{-1}, light period 24 h d^{-1} and pH 6.5–7.0.	High (>50 kDa; 35.1%) and low molecular weight (<3 kDa; 46.1%) polymeric substances were identified; being composed of galactose, glucosamine, mannose.	3.2 mg g^{-1} of extracellular polymeric substances were added in the harvesting process together with Al^{3+} (4.5 mg g^{-1}).	Microalgae bioflocculation.	[10]
Chlorella vulgaris JSC-7	Modified Bold's Basal Medium with nitrogen supplementation was used, pH 6.9, 28 °C, 13/11 h light/dark cycle and 25 µmol m^{-2} s^{-1}.	The bioflocculant is a cell wall polysaccharide; The monomers consist of glucose, mannose, and galactose.	47 mg of the bioflocculant was extracted from 4 L of culture; Addition of 0.5 mg L^{-1} of the bioflocculant was responsible for >80% of the flocculation of the suspended microalgal cells.	Bioflocculation of *C. vulgaris* CNW11 and *Scenedesmus obliquus* FSP.	[9]

6. Other Applications of Microalgal EPS

EPS produced by microalgae have specific structural and physicochemical characteristics that allow industrial and environmental application (Figure 1). The use of these biopolymers as biosurfactants and heavy metal biosorbents is an innovation in environmental biotechnology. These approaches are economically and ecologically sound strategies for reducing environmental pollution [85]. EPS are also crucial for biological soil crust (biofilm) development. This application reduces water infiltration into the soil by inducing surface sealing and clogging of the pores. Therefore, there is an increase in the availability of nutrients and improvement in the soil's aggregate stability [86,87]. In addition, the different biological activities presented by EPS, such as antiviral and antibacterial [88], antioxidant [89], anti-inflammatory [90], immunomodulatory [91], and anticancer, indicate the potential of these compounds for application in various sectors such as food, cosmetics, pharmaceuticals, and biomaterials [14,20].

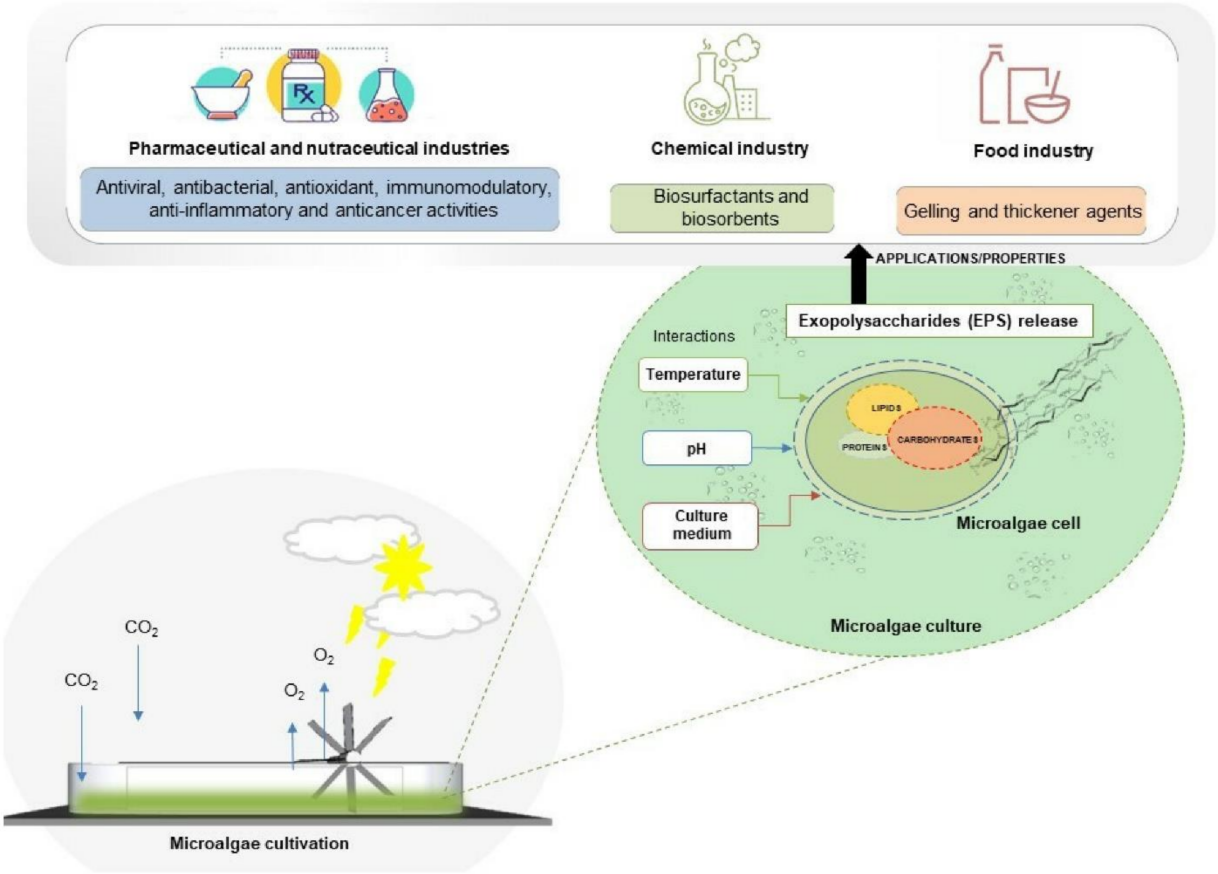

Figure 1. Interactions and structure of EPS with microalgae cultivation and their applications.

7. Conclusions

Microalgae exhibit rapid growth to produce metabolites under specific cultivation conditions, contributing to a more ecological approach to biomass and EPS production. In addition, to improve the economic competitiveness of innovative products derived from microalgae, industries must seek in scientific research the effectiveness and advantages of using these biotechnological processes. Microalgal EPS have been explored in the field of flocculation due to the need for new products obtained from sustainable alternatives to petroleum-based compounds. The main future challenges in the bioremediation sector will be EPS production on an industrial scale. In addition, the cost reduction of the identification and recovery processes of these biopolymers also deserves further investigation. However, the structural diversity of EPS produced by microalgae provides different properties that imply alternative and integrative applications. Moreover, EPS have antioxidant, anti-

inflammatory, anticancer, antiviral, antimicrobial, and immunomodulatory activities, which boost the development of natural pharmaceuticals and nutraceuticals.

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