Paredes-Molina *et al.*
Biotechnology for Biofuels and Bioproducts (2025) 18:17
https://doi.org/10.1186/s13068-024-02595-1

Biotechnology for Biofuels and Bioproducts

RESEARCH

Open Access

Lactic whey as a potential feedstock for exopolysaccharide production by microalgae strain *Neochloris oleoabundans* UTEX 1185

Daniel Moisés Paredes-Molina[1†], Miguel A. Cervantes-López[1,2†], Domancar Orona-Tamayo[2], Nancy E. Lozoya-Pérez[2], Flora I. Beltrán-Ramírez[2], Juan Vázquez-Martínez[3], Karla L. Macias-Sánchez[1], Sergio Alonso-Romero[2] and Elizabeth Quintana-Rodríguez[2*]

Abstract

Background Lactic whey, a significant agro-industrial byproduct, poses environmental risks due to its chemical composition. Despite various valorization efforts, effective utilization remains a challenge. This study explores the potential of *Neochloris oleoabundans*, a microalgae known for its metabolic versatility and resilience to adverse conditions, to produce exopolysaccharides (EPS) using lactic whey as a substrate. We compared EPS production from lactose, the primary sugar in whey, with whole lactic whey. Characterization of the EPS was performed using Fourier transform infrared spectroscopy (FT-IR) and gas chromatography–mass spectrometry (GC–MS), while morphological analysis was conducted via scanning electron microscopy (SEM). This research aims to assess the feasibility of converting lactic whey into valuable EPS, providing a sustainable approach to managing this agro-industrial waste.

Results Lactic whey has produced the highest EPS and the FT-IR spectra revealed structural variations in the monomers which compose these polymers. Galactose and glucose were shown to be the primary monomers, according to GC–MS EPS analysis. SEM revealed a homogenous matrix and *N. oleoabundans*'s bioflocculant characteristics.

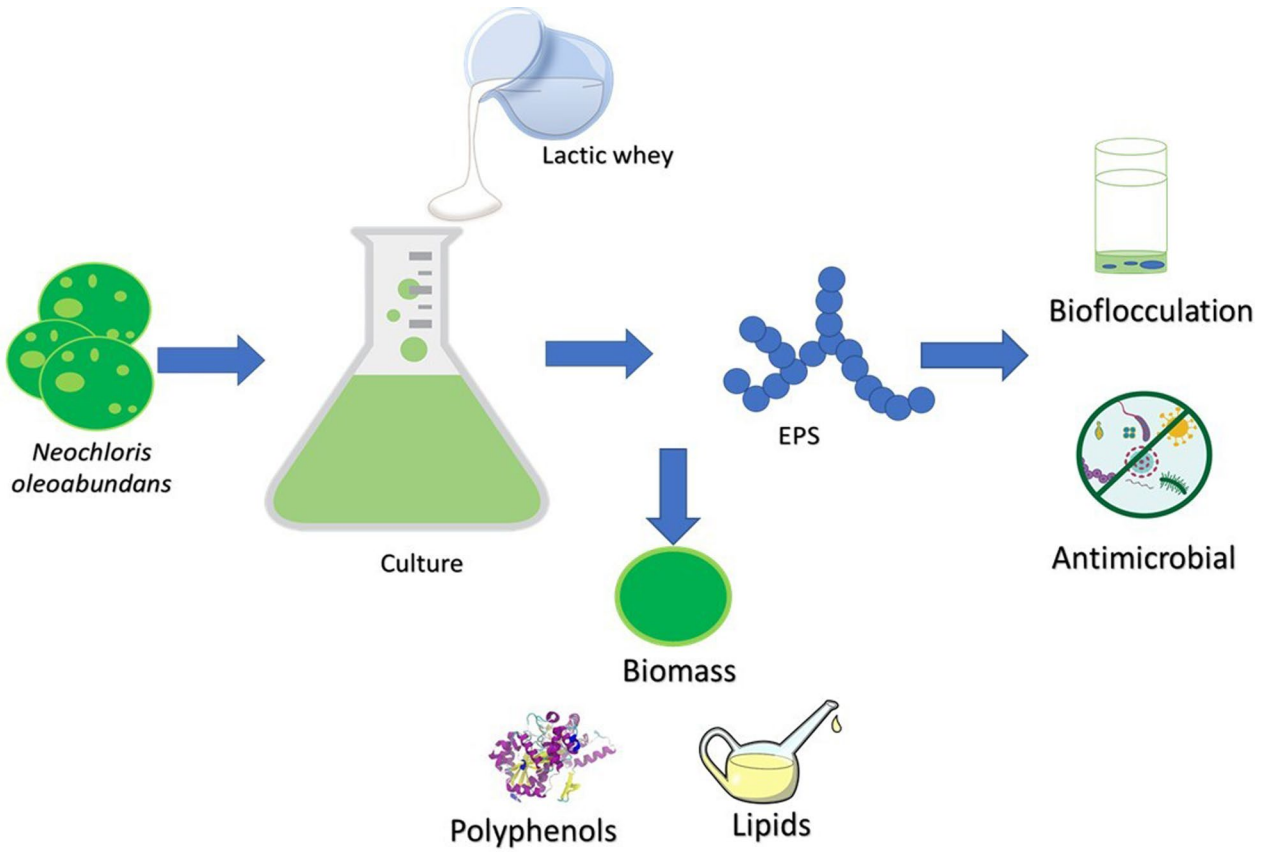
Conclusions Microalgae *N. oleoabundans* can produce EPS using lactic whey as feedstock and it has the potential to be employed as a wastewater treatment.

Keywords Agro-industrial residue, Lactic whey, Microalgae, Exopolysaccharides, Bioflocculant, *Neochloris oleoabundans*

†Daniel Moisés Paredes-Molina and Miguel A Cervantes-López is equally contributed to this paper.

*Correspondence:
Elizabeth Quintana-Rodríguez
equintana@ciatec.mx
Full list of author information is available at the end of the article

Graphical Abstract



Background

Globally, cheese manufacturing produces approximately 180-190 million tonnes of whey derived annually [1] with Mexico contributing around 2.4 million tonnes of lactic whey from dairy and artisanal cheese company each year [2]. This substantial volume of whey presents significant environmental challenges and underscores the need for innovative valorization strategies to manage this dairy industry byproduct sustainably. Presumably, only half of the lactic whey is likely to be reused, while the other half is discarded down the drain, contaminating rivers and soil.

Furthermore, a cheese industry that produces 40,000 L of unrefined lactic whey is estimated to pollute an equivalent amount of a population of 1,250,000 inhabitants [3, 4]. The discharge of lactic whey into aquatic ecosystems and soil matrices poses significant environmental challenges due to its high biochemical oxygen demand (BOD) and its chemical oxygen demand (COD) which are attributed to its substantial content of soluble solids, primarily lactose and proteins [5]. Lactic whey is rich in nutrients (mainly lactose and proteins), thus, it could be evaluated through value-added product acquisition or used as feedstock to produce valuable products [6]. Microalgae have been used as a feedstock, as various residues, and for the manufacture of other useful products. Microalgae have also been utilized to create biomolecules with biotechnological uses and to remove dangerous elements (e.g., nitrogen and phosphorus) from a variety of wastewater sources, including municipal, agro-industrial, and livestock waterways. [7]. There is a substantial variety of biomolecules derived from microalgae, such as proteins, pigments, lipids, and carbohydrates. Additionally, microalgae can secrete extracellular polymeric substances into their immediate environment, a complex mixture of high molecular weight polymers composed of polysaccharides named exopolysaccharides (EPS) [8]. EPS can be released in stress conditions and have diverse functions, for instance, chelation of metals, flocculation, transport, transformation of organic matter and cellular

communication, among others [9, 10]. According to their chemical composition and molecular weight, EPS differ in bioactivity, for example, the molecular weight directly influenced the reducing power. As the molecular weight decreased, the polysaccharides exhibited a stronger reducing power [1]. EPS could be used for emulsifying, as coagulants, as antioxidants, and they can possess anti-inflammatory, antitumoral and antimicrobial properties which may have a wide variety of industrial applications [11]. *Neochloris oleoabundans* is a microalgae that has shown excellent growth flexibility and has been studied mainly for its ability to produce metabolites of interest [12]. Several studies have reported the capacity of *N. oleoabundans* to grow in adverse conditions, in addition to its capability of tolerating high salinity attributable to its marine origin and recently its capacity to withstand low pH [2, 13]. According to published research, *N. oleoabundans* can grow at pH 5, however, this has a detrimental effect on the production of proteins and carotenoids [2]. Lactic whey has a pH of roughly 5, allowing us to investigate the possibility of employing it as a feedstock to produce EPS. In this study, we examined the ability to produce EPS from lactic whey under continuous and half-lightning conditions, as well as EPS characterization using FT-IR spectra, SEM, and gas chromatography coupled with mass spectrometry (GC/MS). Furthermore, we studied its potential use as a bioflocculant.

Results

Lactic whey characterization

Whole lactic whey was subjected to pasteurization to avoid the growth of other microorganisms that can compete with *N. oleoabundans*. Pasteurization was carried out at 75 °C for 15 min. After heat treatment, we observed an increase in components such as lactose (18.25%), total solids (27.52%) and in conductivity (19.42 mS/cm) that could be associated with the protein and lipid degradation (Table 1). Moreover, it decreased the pH making the lactic whey acidic.

Table 1 Properties of whey before and after treatment

Property	Fresh whey	Treated whey
Conductivity (mS/cm)	6.67 ± 0.01	19.42 ± 0.01*
pH	6.27 ± 0.01*	5.31 ± 0.01
Lactose (%w/v)	2.83 ± 0.03	18.25 ± 1.47*
Proteins (%w/v)	0.66 ± 0.02*	0.33 ± 0.06
Lipids (%w/v)	0.64 ± 0.08*	0.24 ± 0.019
Total solids (%w/v)	6.19 ± 0.07	27.52 ± 1.15*
Ashes (%w/v)	0.52 ± 0.04	2.78 ± 0.04*

The number represents mean ± SD, asterisks significant differences (ANOVA, $P < 0.05$) n = 6

* represent statistically significant differences

N. oleoabundans growth using lactic whey as a carbon source

In *N. oleoabundans* kinetics growth cultures with the control medium (C12), and with the medium supplemented with lactose (L12) as a carbon source, there is a long lag phase that spans from day 0 to day 5, during which the cells adapt to the culture media used. Starting on day 6, the exponential phase begins in both media; however, for the medium supplemented with whey as a carbon source (W12), it was not possible to observe growth. On the other hand, kinetics growth with continuous lighting results in a shorter lag phase, which lasts from day 0 to day 2 for the L24 and C24 media and until day 3 for the medium W24, suggesting that continuous luminosity contributes to improving the adaptability of the cells in the culture media used. The exponential phase begins on day 3 in cultures C24 and L24 and lasts until days 6 and 7, respectively; for the W24 medium, the exponential phase begins on day 4 and lasts until day 5. The L24 medium had the highest specific growth rate (μ) (0.52 d-1), followed by the C24 medium (0.42 d-1), and the W24 media (0.22 d-1), as shown in Fig. 1b.

N. oleoabundans grew quicker than the control group in cultures supplemented with lactose as a carbon source during the 12:12 photoperiod (Fig. 1a). The lactose photoperiod culture (L12) showed an increase in growth of around 50% when compared to the control photoperiod culture (C12) at the end of the experiment, whereas the lactose in continuous lighting culture (L24) showed 76% when compared to the control in continuous lighting culture (C24) (Fig. 1b). Whole lactic whey under the photoperiod (W12) culture was unable to grow (Fig. 1a). While whole lactic whey in continuous lighting (W24) exhibited a 55% decrease in growth compared to the control group (Fig. 1b).

Medium viscosity change using lactic whey as a carbon source

The EPS fraction denominated soluble fraction or polysaccharides release, increases the viscosity of the medium in which they are dissolved [14]. This viscosity increment depends on the concentration and the molecular weight of the polysaccharide in the solution [15]. *N. oleoabundans* did not produce EPS with a 12:12 photoperiod under any treatment (Fig. 2a). In continuous lighting, the production of soluble EPS production significantly different was detected on day 3 in

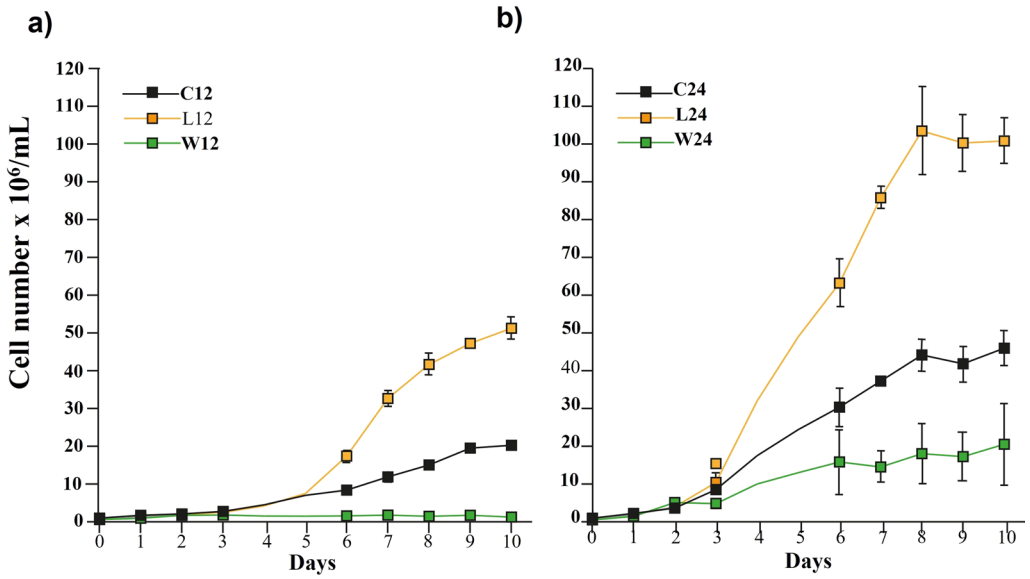


Fig. 1 Growth curves of *N. oleoabundans* cultured with two photoperiods: **a** 12:12 using lactose (L12), lactic whey (W12) and control (C12) and **b** continuous illumination using lactose (L24), lactic whey (W24) and control (C24) (mean ± SD) n = 6

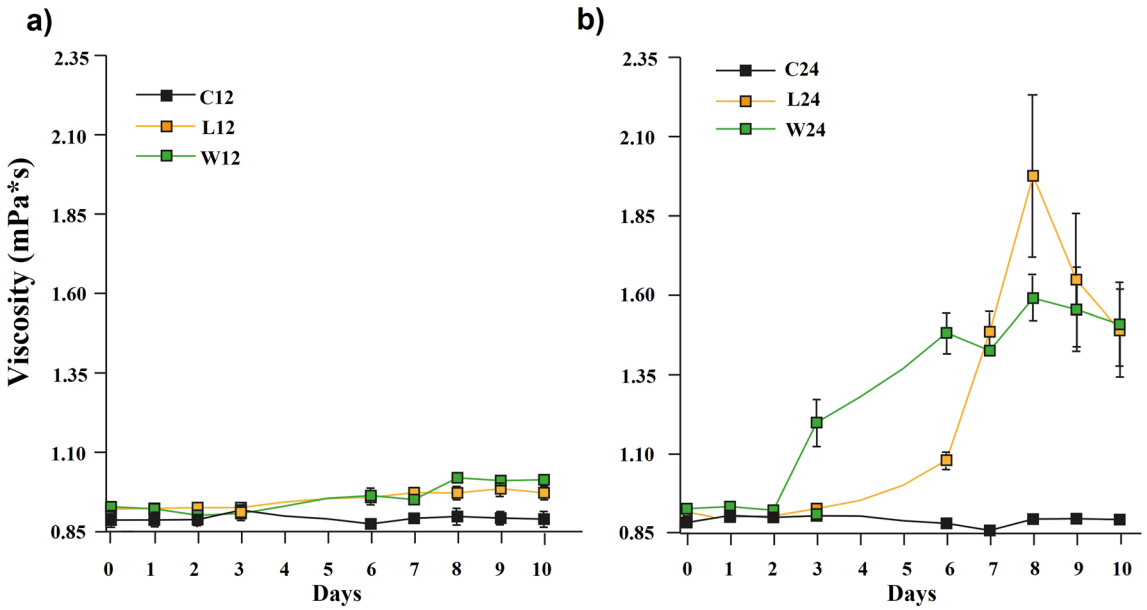


Fig. 2 Viscosity of the medium throughout the culture of *N. oleoabundans* with 2 photoperiods: **a** 12:12 using lactose (L12), lactic whey (W12) and control (C12) and **b** continuous lighting using lactose (L24), lactic whey (W24) and control (C24) (mean ± SD) n = 6

the culture with whole lactic whey (W24) and on day 6 in the culture with lactose (L24) (Fig. 2b). On day 3, the W24 culture began to have the consistency of a viscous gel. For the L24 culture, the gel appearance was observed on day 6.

EPS production

EPS production was measured per day during 10 days showing an appreciable production on whey on the fifth day under continuous lighting (Fig. 3). Since day nine and ten no significant differences were found in EPS production in whey treatment (Fig. 3). The data show that *N. oleoabundans* is not able to produce EPS significantly in autotrophic mode (Fig. 3). The W24

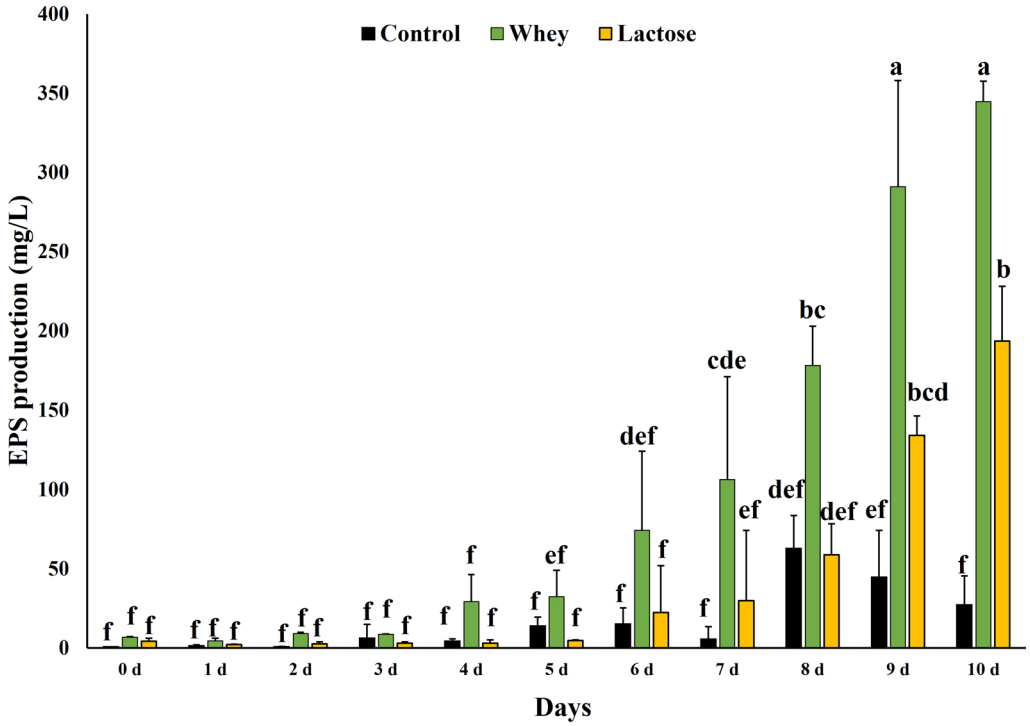


Fig. 3 Kinetic of EPS production by *N. oleoabundans* under continuous lighting. Each bar represents mean ± SD, each letter means a significant difference (post hoc Tukey, $P < 0.05$) n = 6

culture showed the highest EPS production and is also the condition in which there was a decrease in growth compared to the control (Fig. 1).

EPS characterization

Carbohydrate and protein content

Increasing illumination time had no statistically significant influence on carbohydrate concentration in N. oleoabundans (Fig. 4a). Similarly, protein content was not statistically significant with increased illumination time (Fig. 4b). EPS from lactic whey and lactose as a carbon source presented more carbohydrate content than protein.

FT-IR spectra

In order to determine the primary functional groups of the various EPS produced, their infrared spectra were obtained (Fig. 5). The band between 3500–3300 cm-1 in polysaccharide spectra has been assigned to the stretching vibration of the abundant OH present in these polymers [16]. This band accompanied by the medium peak between 1650 and 1580 cm-1, present in all spectra, can also be assigned to stretching the N–H bond of an amine of protein content in the extracts [17]. The ~ 1650 cm-1 band can also be assigned to a carboxylic acid (-COOH) group [18]. The 1500–1200 cm-1 region mainly includes vibrations of the -CH2 and C–OH groups, present in carbohydrates. However, this region presents bands that generally overlap and relating these bands to a specific group is currently a challenge [17]. Some EPS characterization works have related the signals of this region with the presence of sulfate groups; particularly ether sulfate (RO-SO-3) at 1315–1220 cm-1 [18] and the stretching of the S=O bond at 1270–1120 cm-1 [19]. In previous research, the presence of these groups has been related to the absorption of potentially toxic inorganic compounds, such as metals [8, 20]; these bands are present in both spectra of the EPS of cultures with whey and not in the control cultures, which suggest the presence of these groups as a response to toxic whey compounds. In the EPS of cultures with lactose, these bands only appear in the culture with continuous lighting. Similar to earlier studies, the sulfate groups may be associated with cell adhesion in these conditions because of the large number of cells in the culture (Fig. 1) [21].

In all the spectra an intense peak between 1100 and 1000 cm-1 can be observed, which corresponds to the C–O–C and C–O bonds and confirms the presence of carbohydrates [17]. Although it is not possible to determine the type of carbohydrate that makes up the EPS from FT-IR spectra, both the EPS spectra obtained from the L12 and C12 cultures show a peak similar in 1054 and

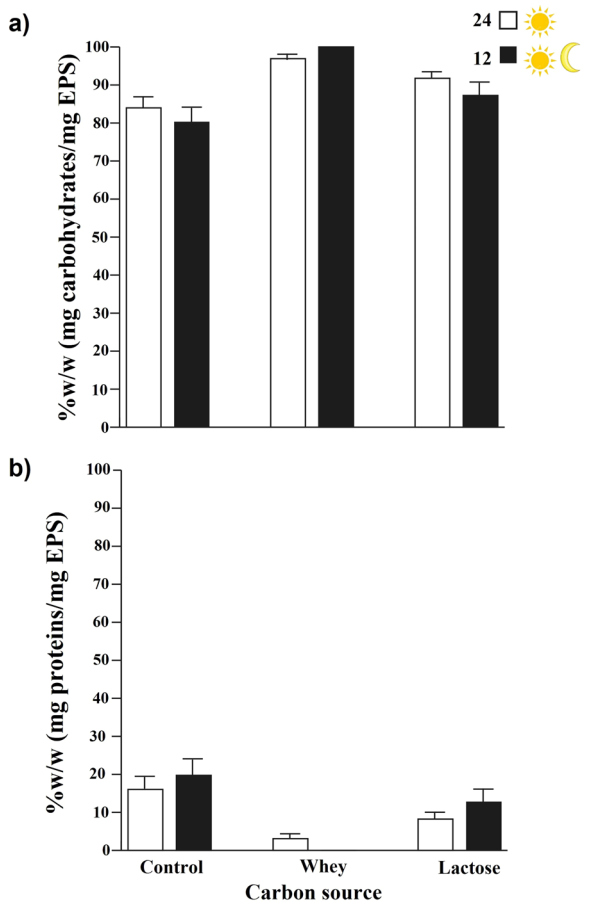


Fig. 4 **a** Carbohydrate and **b** protein content in EPS produced by *N. oleoabundans* with 2 photoperiods: 12:12 using lactose (L12), lactic whey (W12) and control (C12) and continuous lighting using lactose (L24), lactic whey (W24) and control (C24) (mean ± SD, $P < 0.05$) n = 6

1055, respectively; this could suggest a similarity in the type of monosaccharides in EPS, since it was determined that the carbohydrate content is not significantly different (Fig. 4). In addition, EPS production was very low in these conditions (Fig. 3) and culture W12 showed a different peak in this region. The increase in the photoperiod induced a differential peak of the finger-print zone in the EPS of all cultures (Fig. 5).

Gas chromatography with electron impact mass spectrometry (GC–EIMS)

Glucose (55%), levulinic acid (22%) and galactose (15%) were the main monomers identified in the polymer using lactic whey as a carbon source (Table 2). Our results show that *N. oleoabundans* maintain the EPS production from the fifth to the tenth day under continuous lighting (Fig. 3) which could be related to cellular maintenance.

EPS morphology

Figure 6 shows the surface morphology of the EPS obtained through scanning electron microscopy at different magnifications. EPS present a smooth surface like a film, however, fibers of irregular size and shape are also observed (Fig. 6). On the other hand, at high magnifications 2000x, 2500× and 5000x, a film with a rough surface can be observed.

Flocculation activity

In flocculation assays, we did not observe a dosage effect (Fig. 7). The optimal concentration was 25 mg/L showing more than 50% of bioflocculation for all the times tested. However, two hours resulted in a better time for

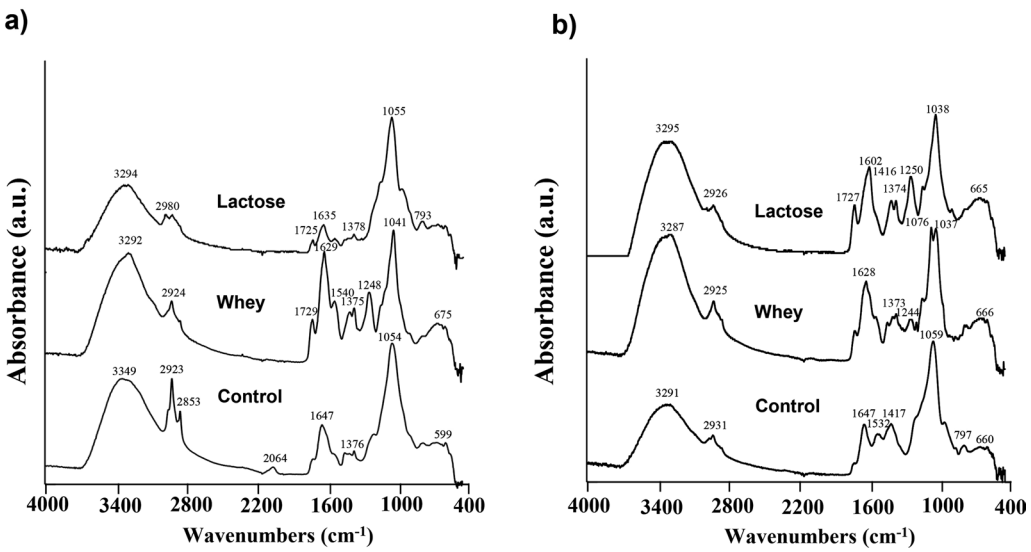


Fig. 5 FT-IR spectra of EPS produced by *N. oleoabundans* with 2 photoperiods: **a** 12:12 using lactose (L12), lactic whey (W12) and control (C12) and **b** continuous illumination using lactose (L24), lactic whey (W24) and control (C12)

Table 2 Percentage monomer composition in EPS from N. *oleoabundans* identified by GC/MS

Compound	Lactic whey (%)	Lactose (%)
Laevulinic acid	22.0 ± 0.6	21.0 ± 0.7
Fructose	6.8 ± 0.2	5.9 ± 0.4
Galactose	15.0 ± 1	14.3 ± 0.4
Glucose	55.0 ± 0.9	58.8 ± 0.6

flocculation. EPS from *N. oleoabundans* is an effective bioflocculant agent and can be a sustainable choice for cell harvesting; however, additional research is needed into its efficacy and cost. Some of the factors that influence flocculant activity are pH, temperature and concentration of the bioflocculant, with the results obtained it can be established that the maximum concentration that allows greater flocculation is 25 mg/L.

Antimicrobial activity

EPS were characterized by their antimicrobial activity against Gram-negative bacterial strains: *Klebsiella pneumoniae* ATCC 13884 and *Pseudomonas aeruginosa* ATCC 10145. The Gram-positive bacterial strain *Staphylococcus aureus* ATCC 6538 and *Nocardia brasiliensis* ATCC 19296 were also tested. We found that EPS had superior antimicrobial activity outcomes with bacteria Gram positive, *S. aureus* and *N. brasiliensis*. For *S. aureus*, all EPS concentrations examined demonstrated antibacterial action, with no significant differences between them (Table 3). While with *N. brasiliensis,* only 30 mg/mL and 20 mg/mL showed inhibition halo of 0.65 cm and 0.46 cm, respectively. No inhibitory halo was observed for *K. pneumoniae* or *P. aeruginosa* which are Gram-negative bacteria, regardless of the EPS concentration that was examined (Table 3 and Fig. 8). EPS offers a great deal of promise for the polymer's use in pharmaceuticals and cosmetics to prevent the growth of Gram-positive bacteria.

Biomass characterization

After EPS is extracted from the supernatant, biomass can be utilized to extract additional valuable biomolecules such as proteins, lipids, polyphenols, and pigments. Given that N. oleoabundans naturally accumulates lipids, this parameter was assessed. Whey and lactose did not induce lipid productivity compared to the control group (Fig. 9a). We found no significant differences between cultures that used continuous lighting or 12 h of lighting, regardless of the carbon source (Fig. 9a).

Total polyphenol content was analyzed since these biomolecules have a significant rise in popularity for their antioxidant properties with multiple applications in the food, cosmetic and pharmaceutical industries. However,

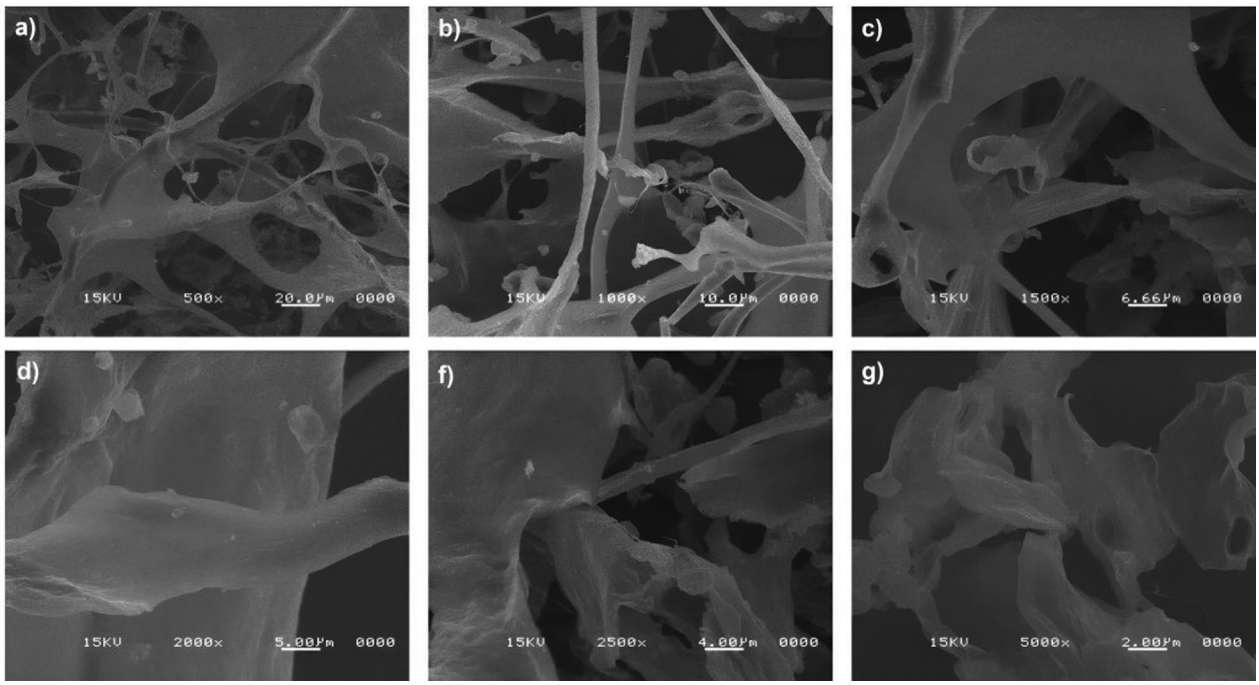


Fig. 6 SEM images corresponding to EPS from *N. oleoabundans* using lactic whey as a carbon source at different magnifications: **a** 500x, **b**1000x, **c**1500x, **d** 2000x, **f** 2500 and **g** 5000x

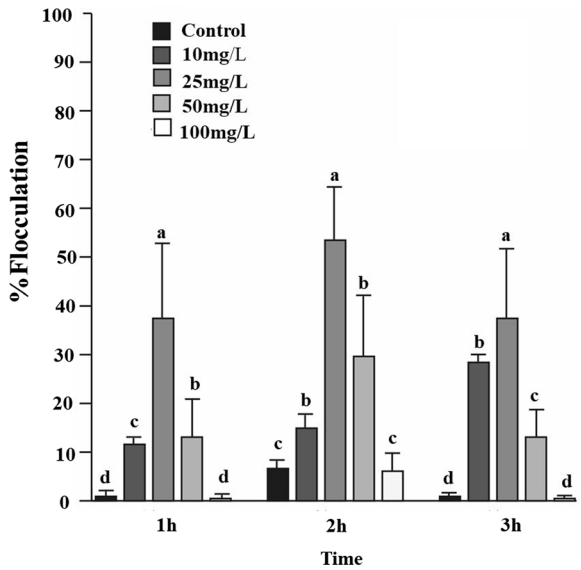


Fig. 7 Dosage effects in the percentage of flocculation assays. Each bar represents mean ± SD, each letter means a significant difference (post hoc Tukey, $P < 0.05$) n = 6

whey and lactose seem to inhibit polyphenol production compared with the control group (12/12 photoperiod) (Fig. 9b). Additionally, it was observed that continuous lighting did not favor polyphenol production. We found significant differences in the control cultures under the photoperiod (12/12).

Discussion

The exponential growth of the dairy industry has been observed yearly. The global dairy market was worth 893 billion dollars in 2022, and market analysts predict that it will rise at a CAGR of 5.79% between 2023 and 2028 [22]. The global cheese industry produced 3.5 million tonnes in 2021, yielding an estimated 31.5 million liters of lactic whey [23]. Lactic whey is a valuable residue with a high nutrient content, including lactose and proteins, which microorganisms like microalgae can exploit as sources of carbon and nitrogen. Microalgae have been used to treat sewage water and it is possible to obtain value-added products using the effluents as a growth medium [24]. Microalgae *Chlorella pyrenoidosa*, *Anabaena ambigua* and *Scenedesmus abundans* were cultivated in dairy wastewater in a proportion 3:1. All of the strains effectively reduced BOD (biochemical oxygen demand) and COD (chemical oxygen demand) and enhanced lipid production [25]. *Scenedesmus* sp. was grown in wastewater from cheese processing, and a high lipid production and high assimilation of nitrogen and phosphorus were achieved [26]. However, few studies have been conducted using the lactic whey residue as a carbon source in microalgae cultivation and for EPS production. Other studies have reported that *N. oleoabundans* can grow using lactose [27, 28] (the main sugar in lactic whey), however, in the present study, whole lactic whey was utilized instead. A higher biomass production of *N. oleoabundans* in the autotrophic mode was reported compared to the mixotrophic, using lactose and glucose as a carbon source supplementing the autotrophic culture with air enriched with 5% CO2 (v/v) [29].

Cultures on 12:12 photoperiod required longer time for adaptation, with a significant lag phase compared to the culture exposed to continuous lighting, which was expected given the autotrophic activity of *N. oleoabundans*. Additionally, on the eighth day, L24 and C24 entered the stationary phase; in contrast, W24 exhibited lower growth. The results showed that lactose increased growth significantly compared with the control culture, and the lactic whey (Fig. 1) growth was higher with continuous light. Growth reduction in *N. oleoabundans* when utilizing lactic whey could be due to diverse factors; such as low pH, high salt content and turbidity in the medium; however, we were unable to determine which factor caused the decrease in growth. Garcia et al. (2000), found that *Chlorella* showed a reduction in cell numbers and photosynthetic efficiency when cultivated using cheese whey from ricotta effluent caused by high salt concentration [30]. The ability of *N. oleoabundans* to grow in different residual effluents has been reported previously in chicken manure [31], swine wastewater [32], and general municipal waste [33]. *N. oleoabundans* has shown superior efficacy in growing in mixotrophic and heterotrophic

Table 3 Bacterial growth inhibition diameter (cm) caused by varying EPS concentrations

EPS concentration				
Strains	**Ampicillin**	**10 mg/mL**	**20 mg/mL**	**30 mg/mL**
Klebsiella pneumoniae ATCC 13884	0.5 ± 0.06^{a}	0^{b}	0^{b}	0^{b}
Pseudomonas aeruginosa ATCC 10145	0.23 ± 0.06^{a}	0^{b}	0^{b}	0^{b}
Staphylococcus aureus ATCC 6538	1.53 ± 0.06^{a}	0.47 ± 0.06^{b}	0.31 ± 0.03^{b}	0.37 ± 0.06^{b}
Nocardia brasiliensis ATCC 19296	0.93 ± 0.08^{a}	0^{d}	0.46 ± 0.05^{c}	0.65 ± 0.05^{d}

The number represents inhibition diameter (cm, mean ± SD), each letter represents significant differences (post hoc Tukey, $P < 0.05$) n = 6

^{a}, ^{b}, ^{c}, ^{d}Letters are representing significant differences too. Only that in this case the analysis was made between all the treatments and comparing among them

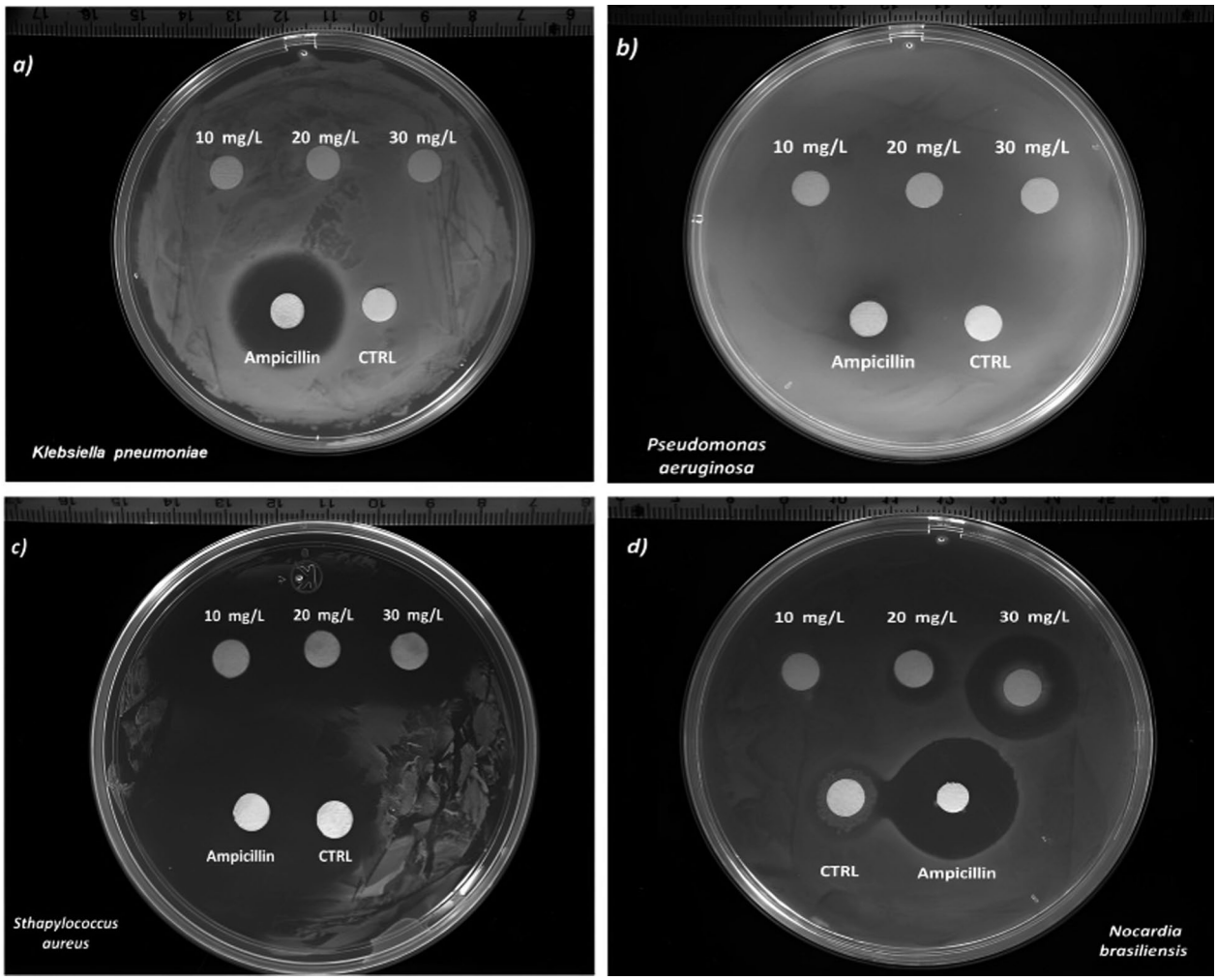


Fig. 8 Antimicrobial effects of EPS on bacterial strains. Bacterial strains Gram negative tested in antimicrobial assays were: **a** *K. pneumoniae* ATCC 13884 and **b** *Pseudomonas aeruginosa* ATCC 10145. While bacterial strains Gram positive were **c**) *S. aureus* ATCC 6538 and **d**) *N. brasiliensis* ATCC 19296. As positive control was used ampicillin, as negative control (CTRL) was used water and EPS extracted were tested at 10, 20 and 30 mg/mL

conditions, a characteristic that contributes notably to its value [12]. In the present study, we report the growth capacity of *N. oleoabundans* in lactic whey diluted 35% (V/V), despite the high content of organic matter and acidic pH (Table 1). The majority of research demonstrated the need to dilute the lactic whey residue to be examined, with dilutions ranging from 1 to 20%; we employed a higher dilution of 35% (V/V) [34–36]. Until previously, only *Desmodesmus* sp. had been reported to grow in pure whey under conditions comparable to the residue used in the present study [37]. Although the growth of *N. oleoabundans* decreased in lactic whey, the EPS production increased compared to the lactose culture and the control under continuous light (Figs, 3 and 4). EPS are polymers released by microorganisms to cope with harsh environment; such as in our study where lactic whey has a low pH, high conductivity due to salt content, and turbidity in the media, all of which function as stressors for the microalgae.

Previous research has demonstrated light influence and nutrients as the most likely factors affecting EPS production [38]. The concentration of nutrients affects photosynthetic activity, which in turn affects how much EPS is produced by carbon fixation and utilization [39]. Glycosyltransferases are mainly photosynthetic enzymes that produce microalgal polysaccharides [11]. Thus, lighting is an important factor involved in EPS production. The lighting regime and a high salinity concentration (lactic whey is a significant source of salts) are two methods used to improve EPS synthesis [40].

Salt stress and mixotrophic culture conditions increased EPS production in *Nostoc flagelliforme* [41], however, EPS production is accompanied by a decrease in growth [42]. It is widespread knowledge that abiotic

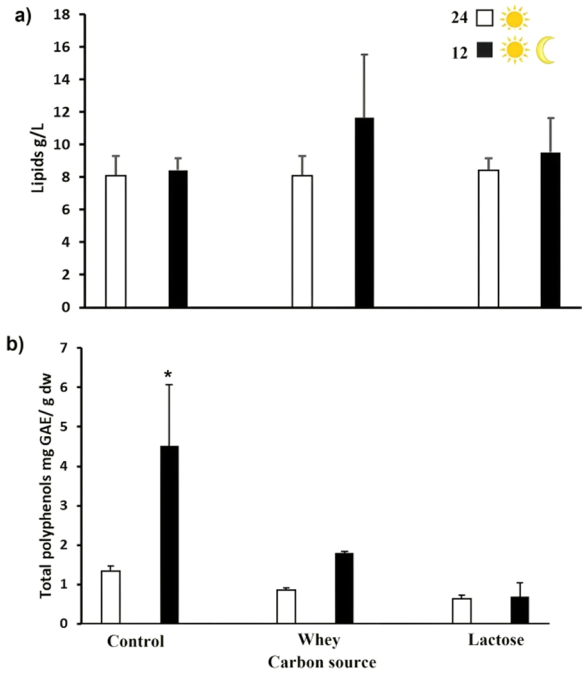


Fig. 9 Biomass characterization by *N. oleoabundans* with two photoperiods: 12:12 using lactose (L12), lactic whey (W12) and control (C12) and continuous lighting using lactose (L24), lactic whey (W24) and control (C24). **a** Lipid production and **b** polyphenol content. Bars represents (mean ± SD, $P < 0.05$) n = 6

stress has an impact on microalgae's ability to produce biomass and affects cell growth [43]. We found that introducing lactic whey as a carbon source inhibited growth and may also be linked to stress caused by high salt concentration. EPS production was only stimulated under continuous light. It is important to note that the production of EPS in W24 was detected on the same day that *N. oleoabundans* began to grow, suggesting a role of EPS in the protection against possible stress factors from the whey, such as the high load of organic matter, the pH, that was slightly lower than the control and the high content of salts [44]. EPS could have diverse functions; as a protective barrier, absorption of organic and inorganic compounds, and binding enzymes [29]. The data suggest that *N. oleoabundans* W24 produces EPS in response to continuous lighting and higher temperatures and that these EPS likely decrease the toxicity of inorganic chemicals in the effluent [8, 20]. EPS contain a great diversity of functional groups, such as sulfates, which can bind different exogenous organic and inorganic compounds, and reduce their toxicity against microalgae [8]. This hypothesis is better explained when comparing EPS production with the L24 culture; although lactose promotes EPS production, other whey compounds further increase EPS production. Regarding the temperature, a higher temperature in W24 in comparison to W12, increased the photosynthetic activity [45] and the acquisition of nutrients [8]. This facilitates the accumulation of intracellular organic carbon that is subsequently secreted as EPS.

A limited number of research have been conducted to investigate EPS synthesis by microalgae in residual wastewater. The maximum EPS production by *Chlorella sp.* and *Micractinium* sp., grown in different effluents, was only 57.8 mg/L and 59 mg/L, respectively, on day 15 [16]. In the present study, we report almost 6 times more production on day 10. This significant difference is most likely due to the medium used in this study which contains almost 100 times more organic carbon. Previous studies reported that increased organic substrate content in residual effluent increased EPS production [17].

The EPS production was higher than in previous studies where wastewater was used as a carbon source [18] and even higher than *C. vulgaris* and *C. zofingiensis* grown using glucose [19]. Although the EPS production using whey was lower than that reported in previous studies where *N. oleoabundans* was cultivated using pure sugars [27, 28], utilizing a residue as a carbon source may reduce cultivation costs and result in the production of value-added metabolites (such as EPS), which would have a beneficial environmental impact. Studies of lighting effects on EPS generation have focused on production, not composition [46]. Using the same medium, this microalgae has been demonstrated to create EPS (>95% carbs) through autotrophic processes under lighting conditions five times stronger than those utilized in this work [31]. These reports indicate that lighting intensity significantly affects the EPS composition. In that aspect, similar results were obtained when the available organic substrate (lactic whey) was directed to synthesize non-nitrogenous polysaccharides [47].

To our knowledge, this is the first study to compare the EPS composition of *N. oleoabundans* using different carbon sources. Microalgae can modulate the EPS biosynthesis machinery to adapt to environmental conditions and manipulate the chemical EPS composition according to the functions required by the organism [8]. Photosynthetic activity is a key factor affecting EPS synthesis processes [46]. The effect of the carbon source becomes evident in cultures with continuous lighting as observed in L24 and W24 which show different peaks in the finger-print zone. This suggests differences in the type of carbohydrates in EPS. The peak at 1075 cm-1 of the EPS obtained from W24 has been related to the presence of galactans in polysaccharides [48]. Galactose in EPS appears to function as a carbon source in *Botryococcus braunii* in order to keep the organism in a stationary state [49]. Polymers obtained from green microalga are

characterized to have galactose as the main monomers [9]. It is important to mention that in a previous study, EPS production by *N. oleoabundans* was not detected using lactose as a carbon source; however, their mixotrophic cultures were aerated using a 5% CO_2 enriched stream [27].

It has been found that there is a correlation between nitrogen sources and their concentrations and EPS generation. In the presence of nitrogen, there is a major photosynthesis rate which generates biomass and synthesis of storage compounds, like lipids and decreases EPS [49].

As mentioned above, culture conditions can induce EPS production. Furthermore, it has been reported that monomer composition can change according to the culture conditions [50]. However, we did not find drastic changes in EPS composition where lactose or lactic whey were used as a carbon source. The main monomers in EPS for both conditions were galactose, glucose and fructose. Levulinic acid has been found to be generated through the acid-catalyzed dehydration of C6-sugars [51]. Therefore, it is plausible that this molecule originated during extraction and purification. EPS produced by the red microalga *Porphyridium* sp. is composed mainly of D-xylose, D- and L-galactose and D-glucose [52]. While EPS from *Navicula* sp. presented glucose and galactose as main monomers [53]. As a result, glucose makes up a larger percentage of the EPS generated by *N. oleoabundans* in the present study [28].

SEM analysis found that EPS showed a homogenous matrix which is an indicator of structural integrity and is an important property when they are used to make films [54]. Microalgal EPS can be used as polymeric industrial material due to their advantages of being eco-friendly, non-toxic and biodegradable [55]. Microalgal EPS has an enormous potential to be used as bioflocculant due to its ability to create a viscous coating around cells [56]. Microalgal EPS derived from *Chlorella* sp. BWY-1, *Haematococcus pluvialis*, and *Dictyosphaerium ehrenbergianum* showed high flocculation activity with 67.8, 50.9, and 43.2%, respectively [57]. Additionally, we found almost 60% using 25 mg/L at 2 h. Microalgal EPS have the potential to be used as sustainable bioflocculants for biomass separation [58]. EPS are able to establish different bonds, such as London dispersion forces, electrostatic interactions and hydrogen bonding which make them candidates for water treatments [59]. Environmental contamination is increasing in recent years due to wastewater treatment using synthetic polymers and toxic salts [60]. Therefore, EPS from *N. oleoabundans* presents the potential to be studied in wastewater treatment. Several studies demonstrated that EPS had shown unusual antimicrobial activities against a wide spectrum of microorganisms (from viruses to bacteria) [61–64]. Functional groups in the EPS, such as phosphate, hydroxyl, and carbonyl, have been proposed to be crucial for antibacterial activity [65]. EPS produced by the microalga *Grasiella* sp. Showed antibacterial properties against *Vibrio anguillarum* and *Listonella anguillarum* [66], while EPS from the red microalga *Porphyridium* inhibited growth against *E. coli* and *B. subtilis* at low concentration of 0.1% w/v [67]. Contrary to EPS from cyanobacterium *Arthrospira platensis* and microalga *Porphyridium purpureum* which showed antiviral activity against ectromelia virus [68]. The microalgal extracellular polymers (EPS) used in this study are heteropolymers made up of various monomers that are capable of generating distinct activities. *S. aureus* and *N. brasiliensis*, two strains of Gram-positive bacteria, were resistant to the antibacterial action of EPS, but not strains of Gram-negative bacteria. *N. brasiliensis* is a significant skin pathogen given that it can infect wounds, particularly in people with diabetes [69]. The pharmaceutical and cosmetics industries stand to benefit greatly from the antibacterial action of *N. oleoabundans*' EPS. EPS from *N. oleoabundans* can be obtained under the biorefinery concept, using biomass to acquire other biomolecules. The biomass is separated, and the EPS are recovered from the supernatant. While lipids and polyphenols are obtained from biomass, in this way all waste is used. Even though the microalgae produce fewer lipids and polyphenols using whey as a carbon source, the microalgae still produce them. It will be necessary to characterize the lipids and polyphenols obtained to search for a possible application. Subsequent studies will focus on searching for applications for biomass to evaluate biorefinery concept using lactic whey as a carbon source in microalgae.

Conclusion

The microalga *Neochloris oleoabundans* demonstrated the ability to produce extracellular polymeric substances (EPS) utilizing lactic whey, a significant industrial byproduct with potential environmental implications, under continuous illumination conditions. The synthesized EPS exhibited notable flocculation efficacy, indicating its potential application in cell harvesting and wastewater treatment processes. Additionally, the EPS displayed antimicrobial properties, suggesting possible utilization in cosmetic and pharmaceutical formulations. Further investigation into the EPS's antioxidant capacity and anti-aging potential may reveal additional applications in food, cosmetic, and pharmaceutical industries. Concurrently, *N. oleoabundans* demonstrated the capability to produce lipids and polyphenols using lactic whey as a carbon source. To fully realize the economic viability of lactic whey utilization as a substrate for microalgal cultivation, comprehensive biomass characterization studies

are warranted. Such investigations may elucidate additional valuable metabolites and optimize production conditions, thereby enhancing the overall process efficiency and commercial feasibility.

Materials and methods

Biological material

N. oleoabundans strain (UTEX 1185) was obtained from the UTEX algae collection at the University of Texas in Austin. The strain was kept at room temperature and natural lighting in a 500 mL flask with MBB medium composed of (g/L): K_2HPO_4 0.75; KH_2PO_4 1.75; $NaNO_3$ 2.5; NaCl 0.25; $MgSO_4 \cdot 7H_2O$ 0.75; H_3BO_3 0.1142; EDTA 0.5; KOH 0.31; $MnCl_2 \cdot 4H_2O$ 0.0144; $NaMnO_4 \cdot 2H_2O$ 0.0119; $CoCl \cdot 6H_2O$ 0.004; $ZnSO_4 \cdot 7H_2O$ 0.0882; $CuSO_4 \cdot 5H_2O$ 0.0157; $FeSO_4 \cdot 7H_2O$ 0.0498; H_2SO_4 0.00183; $CaCl_2 \cdot 2H_2O$ 0.25; the medium was aerated with a filtered air stream (0.22 µm).

Lactic whey was sourced from a local cheese manufacturer in San Francisco del Rincon, Guanajuato, Mexico. To mitigate microbial interference, the whey underwent pasteurization. This thermal treatment involved heating the whey to 80 °C for 15 min, followed by immediate refrigeration. Prior to refrigeration, the whey was allowed to cool in a temperature-controlled chamber for 30 min to facilitate temperature reduction.

Characterization of whey

The physicochemical characterization of the fresh and treated whey was conducted using standard analytical methods. Reducing sugars were quantified using the Miller method [70], while protein content was determined via the Bradford assay [71]. Total lipids were assessed following the Mexican norm NOM-AA-5-198O. Total solids and ash content were measured according to the Mexican norm NMX-AA-034-SCFI-2015 and by heating samples at 550–600 °C for 2 h, respectively. Conductivity was measured using a HACH® Sension156 multimeter, and pH was determined with a HANNA® HI 2030–01 potentiometer.

Mixotrophic culture conditions

Cultures were developed in 500-mL Scotch flasks with adapted lids; an inlet for aeration of filtered compressed atmospheric air (0.22 µm) and an outlet covered with a filter to release pressure. As control©, cultures were put only with MBB medium. Lactose treatment was a culture supplemented with 10 g/L of lactose (L) (Meyer®). Treatment with lactic whey was a culture with 35% (V/V) of whey (W).

Cultures were subjected to two conditions: A) continuous lighting with lactose and 28 ± 1 °C (L24), lactic whey (W24) and control (C24). B) photoperiod 12:12 and 28 ± 1 °C with lactose (L12), lactic whey (WL2) and control (CL12). To simulate environmental conditions, two Volteck® model LED-60FX4 fluorescent lamps were used for illumination with a total intensity of 101.25 µmol/m2; the initial pH of the cultures was 6.6, except for those containing whey, which was 6.0. The cultures were manually shaken every day until homogeneity and were aerated as described in 4.1.

Culture monitoring

Cell density was quantified spectrophotometrically by measuring the optical density at 750 nm (OD_{750}). A pre-established calibration curve was used to convert OD_{750} values to cell concentration. When necessary, samples were diluted to ensure measurements fell within the linear range of the spectrophotometer. To avoid the interference generated by the turbidity of the culture with whey, the cells were counted directly in the Neubauer chamber. Due to the viscosity, the sample was centrifuged at 10,000RCF using a Hermle model Z383-K centrifuge to separate the biomass. The supernatant was recovered and its viscosity was measured using a Cannon Fenske model CFRC-100 viscometer. 8 mL of sample were placed and the viscometer was kept in a water bath at 24 °C.

Extraction and quantification of EPS

A kinetic of EPS production was conducted over a 10-day period. EPS was extracted daily from cultures C, W, and L maintained under continuous illumination until day 10 of the experiment. We selected continuous lighting due to the viscosity analysis showing perceptible changes only on this condition. To recover the EPS the next methodology was followed: the medium was recovered, it was heated to approximately 80 °C for 20 min. After, the medium was centrifuged at 10,000 rpm for 30 min to remove algal cells. The supernatant was recovered, and 96° ethanol was added to a final volume of 30% ethanol, the mixture was homogenized, kept at 4 °C for 24 h and centrifuged at 10,000 RCF for 15 min. The supernatant was retrieved in a previously cooled beaker and 2 volumes of 96° ethanol were added, homogenized, and kept at 4 °C for 24 h and centrifuged at 10,000 rpm for 15 min. The supernatant was discarded, and the pellet was washed 2 times with 80% (v/v) ethanol. The pellet was resuspended in distilled water and dialyzed in deionized water with a membrane with a molecular weight cutoff of 1 kDa for 6 days at 4 °C with two changes of water. For the quantification, a sample of the dialysate was taken, and the proteins were quantified by the method described in 4.1 and the total carbohydrates by the modified Dubois method [72]. Subsequently, 50 µL of phenol 80% (v/v) and 2 mL of H_2SO_4 were added to 100µL of sample, after

10 min of incubation at room temperature conditions and protected from the light, absorbance at 490 nm was determined using UV–Vis Evolution™ 300 Spectrophotometer (Thermo Scientific™). The quantity of EPS produced was calculated by subtracting the amount of the EPS dosage of the same medium not inoculated with the strain. Also, a control without *N. oleoabundans* was carried out and the medium interference was determined. The experiments were performed sixfold, with data obtained expressed as mean value ± standard deviation.

EPS characterization

Carbohydrate and protein content

Since proteins and carbohydrates are the main component of EPS, they were used to evaluate changes in EPS, comparable to previous studies [73, 74].

Fourier transform infrared spectroscopy

The dialysate was lyophilized for 24 h using the 2.5-L FreeZone equipment at -50 °C from the LABCONCO brand. To increase the purity of the sample, two washes were made with each of the following solvents: 80% ethanol with 0.1% formic acid, (under these conditions the solubility of the contaminating proteins increases) absolute ethanol, acetone and ether (1:1:1). The remaining solvent was evaporated and the mass obtained was analyzed using the Thermo Scientific™ Nicolet iS10 FT-IR Spectrometer in the region of 400 cm^{-1}-4000 cm^{-1} with a resolution of 4 cm-1.

Gas chromatography with electron impact mass spectrometry (GC–EIMS)

To determine the monomeric composition of the EPS, 10 mg were resuspended in 200 µL of 4 M HCl, the mixture was incubated at 99 °C for 2 h, allowed to cool at room temperature and neutralized with a volume of 4 M NaOH, if necessary. pH was further adjusted to 7 using a solution of 1 M NaOH and 1 M HCl. The mixture was filtered (0.22 µ), frozen and lyophilized for 24 h. The obtained powder was stored at −40 °C until its derivatization. The salts formed during the neutralization were removed during the derivatization [75].

For derivatization, 20 µL of pyridine and 80 µL of N, O-bistrifluoroacetamide (BSTFA) with 1% Trimethylchlorosilane Chloride (TMCS) were added to the powder. The mixture was left to react for 30 min at 80 °C and 101RFC. It was then allowed to cool at room temperature. Subsequently, 100 µL of isooctane were added for a final volume of 200 µL and analyzed by GC–EIMS. The GC–EIMS equipment consists of a model 7890A gas chromatograph coupled to a model 5973 electron impact ionization mass detector (EIMS) (Agilent Technologies, Inc., USA). A model 7693A autosampler and model 7683 autosampler (Agilent Technologies, Inc., USA) were also used. The sample (1µL) was injected in non-partition mode. The injection chamber temperature was 250 °C. A constant flow of helium (1 mL/min) was used as carrier gas. The GC oven started at 150 °C for 3 min, then the temperature increased at a rate of 4 °C/min to 280 °C, where it was maintained for 25 min. The transfer line to the MS had a temperature of 250 °C, the ionization source of 230 °C and the quadrupole of 150 °C. Measurements were carried out in SCAN mode with a mass range of 50–550 m/z and at 2.9 scan/s. Spectra were obtained at 70 eV. MassHunter Workstation Software was used for data collection and AMDIS software for determination of retention time and mass spectrum.

Morphology of exopolysaccharides

Morphology analysis was carried out with a TOPCON SM-510 scanning electron microscope (SEM). Images were acquired at an accelerating voltage of 15 kV and a working distance of 15 mm. The samples were previously coated with Au–Pd. All samples were fractured at liquid nitrogen temperature.

Evaluation of flocculation

After 7 days of cultivation, *N. oleoabundans* cultures were harvested and centrifuged at 2800RFC for 10 min. Pellets were washed with distilled water, and cells were resuspended and measured OD_{750} to 0.9. To evaluate the flocculation efficiency, varying concentrations of EPS (10, 25, 50, and 100 mg/mL) were evaluated, with water serving as a control. These solutions were added to cell suspensions, which were then agitated. The optical density at 690 nm (OD_{690}) was measured by sampling 200 µL of cell suspension from a depth of 4 cm below the surface (designated as A) immediately after mixing. Following a 30-min settling period, another 200 µL sample was taken from the same depth to measure OD_{690} (designated as B). The flocculation efficiency (F) was calculated using the following equation:

$$F = \frac{(1 - B)}{A}\ x\ 100\%.$$

Three biological replicas were completed twice.

Antimicrobial activity assays

To determine the antimicrobial activity of *N. oleoabundans* exopolysaccharide, a disc antibiogram assay was performed for the bacterial strains Gram-negative *K. pneumoniae* ATCC 13884 and *P. aeruginosa* ATCC 10145 and the bacterial strain Gram-positive *S. aureus* ATCC 6538 and *N. brasiliensis* ATCC 19296. All strains were grown until an exponential phase in liquid nutrient medium based on growth curves performed for

each microorganism. Petri dishes containing nutrient agar were inoculated with 200 µl from dilution 1:10 of liquid medium samples. Filter paper discs were impregnated with 10 mg/mL, 20 mg/mL and 30 mg/mL concentrations of EPS. Following this, discs were deposited on the surface of the previously inoculated plates. The plates were incubated at 28 °C overnight and the radius of growth inhibition was determined. Ampicillin 10 mg/mL was used as a positive control and sterile water was used as a negative control. The experiment was replicated three times.

Biomass characterization

The recovered biomass was analyzed for lipid and polyphenol content.

Lipid determination

A sample of 30 mL was centrifuged (Optima L-90 K ultracentrifuge: Beckman, Coulter, Fullerton, CA, USA) at 10,000 rpm for 15 min at 4 °C and the supernatant was discarded. The total lipids from the pellets were extracted by mixing 2 mL of chloroform, 1 mL of dichloromethane and 0.05% of food antioxidant butylated hydroxytoluene (BHT). Samples were mixed and incubated at 4 °C for 12 h. After this time, samples were newly centrifuged at 10,000RFC for 15 min at 4 °C and washed twice, mixing 4 mL of dichloromethane–methanol (1:1 v/v). The supernatants were collected and processed as previously mentioned. In a separatory funnel, the samples were collocated, shaken for 5 min and separated. The solvent was evaporated using a rotary evaporator and dry weight of the residue was determined. The pellet is saved and stored at −20 °C to perform hydrolysis for further determinations. The lipid content of the sample is calculated as follows and reported as gr of lipids per liter of culture:

$$\text{Total lipid} = \frac{Weight\ sample\ vial - weight\ empty\ vial}{Volume\ sample}.$$

Polyphenol content

Polyphenols were extracted from 2 mL of culture. Culture samples were centrifuged (Hermle Z 383 centrifuge, Labortechnik, Wehingen, Germany) at 10,000 RFC for 15 min at 4 °C and the supernatant was discarded. Polyphenols were extracted with 2 mL of solvent (70% methanol) incubated for 12 h at room temperature in a rotatory shaker (250 rpm). After this time, samples were centrifuged at 10,000 RFC for 5 min and supernatants were separated in a new 1.5 mL tube and evaporated to dryness in a rotary vacuum evaporator (Büchi Rotavapor R-114 and Büchi Vacobox B-171; Flawil, Switzerland). The polyphenol quantification was performed by the Folin–Ciocalteu method in a microplate format [76]. Samples absorbance were measured at 760 nm in a spectrophotometer (Benchmark plus microplate reader, Biorad, Hercules, CA).

Acknowledgements

We appreciate the gracious donation of the bacterial strains from Drs. Varinina Lopez-Ramirez and Rafael Uzarraga-Salazar. Catalina de la Rosa, Miroslava Silva and Ernesto Ornelas for their support and training given at the "Medio Ambiente and Materiales laboratories" in CIATEC in order to accomplish this research. This research was funded by Ciencia Basica grant 2017–2018 A1-S-29550 to E.Q.-R. from the Consejo Nacional de Ciencia y Tecnología (CONACYT) Mexico.

Author contributions

EQR planned the experiments DMPM, MACL, NELP, FIBR, SAR and JVM performed experimental work DOT, KLMS, JVM and EQR directed experiments DMPM, MACL, DOT, NELP, KLMS and EQR performed statistic analysis DMPM, MACL, NELP, FIBR and EQR prepare figures EQR got financing, wrote the article All authors reviewed the manuscript.

Funding

This research was funded by CONAHCyT México Grant Investigación Científica Básica 2017–2018 A1-S-29550 and Dirección General para el Desarrollo Científico y Tecnológico Sub-modalidad del estado de Guanajuato "Ciencia Productiva" CATEGORIA Impacto a Posgrados de Calidad Apoyo para Publicación de Artículos Científicos IDEAGTO/CONV/208/2022 to E.Q.R.

Availability of data and materials

Data are available on request from the authors.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Investigación y Soluciones Tecnológicas, CIATEC, A.C., PC 37545 León, Guanajuato, Mexico. [2]UPIIG, del Instituto Politécnico Nacional, PC 36275 Silao, Guanajuato, Mexico. [3]Tecnológico Nacional de México/ ITESI Irapuato PC 36821, Irapuato, Guanajuato, Mexico.

Received: 4 October 2023 Accepted: 13 December 2024
Published online: 12 February 2025

References

1. Buchanan D, Martindale W, Romeih E, Hebishy E. Recent advances in whey processing and valorisation: Technological and environmental perspectives. Int J Dairy Technol. 2023;76(2):291–312. https://doi.org/10.1111/1471-0307.12935.
2. Mazorra-Manzano MÁ, Moreno-Hernández JM. Propiedades y opciones para valorizar el lactosuero de la quesería artesanal. CienciaUAT. 2019;14(1):133–44.
3. Valencia Denicia E, Ramírez Castillo ML. La industria de la leche y la contaminación del agua; 2009
4. Torres-Martínez Q, Romero-León K. Alternativas tecnológicas para uso del lactosuero valorización económica de residuos. Estud Soc Rev Aliment Contemp y Desarro Reg. 2020. https://doi.org/10.2483/es.v30i55.908.

5. Ganta A, Bashir Y, Das S. Dairy wastewater as a potential feedstock for valuable production with concurrent wastewater treatment through microbial electrochemical technologies. Energies. 2022;15(23):9084.
6. Sebastián-Nicolás JL, González-Olivares LG, Vázquez-Rodríguez GA, Lucho-Constatino CA, Castañeda-Ovando A, Cruz-Guerrero AE. Valorization of whey using a biorefinery. Biofuels, Bioprod Biorefining. 2020;14(5):1010–27.
7. Srimongkol P, Sangtanoo P, Songserm P, Watsuntorn W, Karnchanatat A. Microalgae-based wastewater treatment for developing economic and environmental sustainability Current status and future prospects. Front Bioeng Biotechnol. 2022. https://doi.org/10.3389/fbioe.2022.904046.
8. Xiao R, Zheng Y. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnol Adv. 2016;34(7):1225–44.
9. Babiak W, Krzemińska I. Extracellular polymeric substances (EPS) as microalgal bioproducts: a review of factors affecting EPS synthesis and application in flocculation processes. Energies. 2021;14(13):4007.
10. Naveed S, Li C, Lu X, Chen S, Yin B, Zhang C, et al. Microalgal extracellular polymeric substances and their interactions with metal (loid) s: a review. Crit Rev Environ Sci Technol. 2019;49(19):1769–802.
11. Franco-Morgado M, Amador-Espejo GG, Pérez-Cortés M, Gutiérrez-Uribe JA. Microalgae and cyanobacteria polysaccharides: Important link for nutrient recycling and revalorization of agro-industrial wastewater. Appl Food Res. 2023. https://doi.org/10.1016/j.afres.2023.100296.
12. Safi C, Pollio A, Olivieri G. Neochloris oleoabundans from nature to industry: a comprehensive review. Rev Environ Sci Bio/Technol. 2021;20(4):943–58.
13. Rashidi B, Dechesne A, Rydahl MG, Jørgensen B, Trindade LM. Neochloris oleoabundans cell walls have an altered composition when cultivated under different growing conditions. Algal Res. 2019;40: 101482.
14. Rossi F, De Philippis R. Exocellular polysaccharides in microalgae and cyanobacteria: chemical features, role and enzymes and genes involved in their biosynthesis. Physiol microalgae. 2016. https://doi.org/10.1007/978-3-319-24945-2_21.
15. Petruccioli M, Raviv M, Di Silvestro R, Dinelli G. Agriculture and agro-industrial wastes, byproducts, and wastewaters: origin, characteristics, and potential in bio-based-compounds production. In: Comprehensive biotechnology. Amsterdam: Elsevier; 2011.
16. Wang M, Kuo-Dahab WC, Dolan S, Park C. Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, Chlorella sp and Micractinium sp, in wastewater treatment. Bioresour Technol. 2014;154:131–7.
17. Ge S, Champagne P. Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. Water Res. 2016;88:604–12.
18. Wang J, Yang H, Wang F. Mixotrophic cultivation of microalgae for biodiesel production: status and prospects. Appl Biochem Biotechnol. 2014;172:3307–29.
19. Zhang J, Liu L, Chen F. Production and characterization of exopolysaccharides from Chlorella zofingiensis and Chlorella vulgaris with anti-colorectal cancer activity. Int J Biol Macromol. 2019;134:976–83.
20. Clément-Larosière B, Lopes F, Gonçalves A, Taidi B, Benedetti M, Minier M, et al. Carbon dioxide biofixation by Chlorella vulgaris at different CO2 concentrations and light intensities. Eng Life Sci. 2014;14(5):509–19.
21. García-Cubero R, Cabanelas ITD, Sijtsma L, Kleinegris DMM, Barbosa MJ. Production of exopolysaccharide by Botryococcus braunii CCALA 778 under laboratory simulated Mediterranean climate conditions. Algal Res. 2018;29:330–6.
22. IMARC. Global Dairy Market: Industry Trends, Share, Size, Growth, Opportunity and Forecast 2023–2028. 2022.
23. FAO. Dairy Market Review: Emerging trends and outlook 2022. Rome; 2022.
24. Choi H-J. Dairy wastewater treatment using microalgae for potential biodiesel application. Environ Eng Res. 2016;21(4):393–400.
25. Brar A, Kumar M, Pareek N. Comparative appraisal of biomass production, remediation, and bioenergy generation potential of microalgae in dairy wastewater. Front Microbiol. 2019;10:678.
26. Mercado I, Álvarez X, Verduga M-E, Cruz A. Enhancement of biomass and lipid productivities of Scenedesmus sp. cultivated in the wastewater of the dairy industry. Processes. 2020. https://doi.org/10.3390/pr8111458.
27. Wu N, Li Y, Lan CQ. Production and rheological studies of microalgal extracellular biopolymer from lactose using the green alga Neochloris oleoabundans. J Polym Environ. 2011;19:935–42.
28. Li Y, Wang C, Liu H, Su J, Lan CQ, Zhong M, et al. Production, isolation and bioactive estimation of extracellular polysaccharides of green microalga Neochloris oleoabundans. Algal Res. 2020;48: 101883.
29. Pierre G, Delattre C, Dubessay P, Jubeau S, Vialleix C, Cadoret J-P, et al. What is in store for EPS microalgae in the next decade? Molecules. 2019;24(23):4296.
30. Nazos TT, Stratigakis NC, Spantidaki M, Lagouvardou Spantidaki A, Ghanotakis DF. Characterization of cheese whey effluents and investigation of their potential to be used as a nutrient substrate for chlorella biomass production. Waste Biomass Valor. 2023. https://doi.org/10.1007/s12649-023-02081-z.
31. Altunoz M, Pirrotta O, Forti L, Allesina G, Pedrazzi S, Obali O, et al. Combined effects of LED lights and chicken manure on Neochloris oleoabundans growth. Bioresour Technol. 2017;244:1261–8.
32. Wang Y, Ho S-H, Cheng C-L, Nagarajan D, Guo W-Q, Lin C, et al. Nutrients and COD removal of swine wastewater with an isolated microalgal strain Neochloris aquatica CL-M1 accumulating high carbohydrate content used for biobutanol production. Bioresour Technol. 2017;242:7–14.
33. Valev D, Silva Santos H, Tyystjärvi E. Stable wastewater treatment with Neochloris oleoabundans in a tubular photobioreactor. J Appl Phycol. 2020;32(1):399–410. https://doi.org/10.1007/s10811-019-01890-x.
34. Wang X, Lin L, Dong J, Ling J, Wang W, Wang H, et al. Simultaneous improvements of Pseudomonas cell growth and polyhydroxyalkanoate production from a lignin derivative for lignin-consolidated bioprocessing. Appl Environ Microbiol. 2018. https://doi.org/10.1128/AEM.01469-18.
35. Christwardana M, Hadiyanto H. Cultivating microalgae botryococcus braunii in tofu whey medium. J Bioresour Environ Sci. 2022;1(1):12–9.
36. Russo GL, Langellotti AL, Oliviero M, Baselice M, Sacchi R, Masi P. Valorization of second cheese whey through cultivation of extremophile microalga Galdieria sulphuraria. AIMS Environ Sci. 2021;8:435–48.
37. Bonett JEA, de Sousa GP, Cardoso PG, de Freitas CF, Duarte WF. Isolation of freshwater microalgae and outdoor cultivation using cheese whey as substrate. Biocatal Agric Biotechnol. 2020;29: 101799.
38. Medina-Cabrera EV, Rühmann B, Schmid J, Sieber V. Optimization of growth and EPS production in two Porphyridum strains. Bioresour Technol Reports. 2020;11: 100486.
39. Abidizadegan M, Blomster J, Fewer D, Peltomaa E. Promising biomolecules with high antioxidant capacity derived from cryptophyte algae grown under different light conditions. Biology. 2022. https://doi.org/10.3390/biology11081112.
40. Velmurugan R, Incharoensakdi A. Overexpression of glucose-6-phosphate isomerase in Synechocystis sp. PCC 6803 with disrupted glycogen synthesis pathway improves exopolysaccharides synthesis. Algal Res. 2021. https://doi.org/10.1016/j.algal.2021.102357.
41. Shen S-G, Lin Y-H, Zhao D-X, Wu Y-K, Yan R-R, Zhao H-B, et al. Comparisons of functional properties of polysaccharides from nostoc flagelliforme under three culture conditions. Polymers. 2019. https://doi.org/10.3390/polym11020263.
42. Potier M, Tea L, Benyahia L, Nicolai T, Renou F. Viscosity of aqueous polysaccharide solutions and selected homogeneous binary mixtures. Macromolecules. 2020;53(23):10514–25.
43. Garza-Rodríguez ZB, Hernández-Pérez J, Santacruz A, Jacobo-Velázquez DA, Benavides J. Prospective on the application of abiotic stresses to enhance the industrial production of exopolysaccharides from microalgae. Curr Res Biotechnol. 2022;4:439–44.
44. Abreu AP, Morais RC, Teixeira JA, Nunes J. A comparison between microalgal autotrophic growth and metabolite accumulation with heterotrophic, mixotrophic and photoheterotrophic cultivation modes. Renew Sustain Energy Rev. 2022;159: 112247.
45. Girard J-M, Roy M-L, Ben HM, Gagnon J, Faucheux N, Heitz M, et al. Mixotrophic cultivation of green microalgae Scenedesmus obliquus on cheese whey permeate for biodiesel production. Algal Res. 2014. https://doi.org/10.1016/j.algal.2014.03.002.
46. Goo BG, Baek G, Choi DJ, Il PY, Synytsya A, Bleha R, et al. Characterization of a renewable extracellular polysaccharide from defatted microalgae Dunaliella tertiolecta. Bioresour Technol. 2013;129:343–50.
47. Zaragoza Carmona JAJ. Caracterización parcial de carbohidratos en exopolisacárido de Neochloris oleoabundans y Chlorella sp. microalgas

útiles para producción de biodiesel. Universidad Autónoma de Nuevo León; 2016
48. Hong T, Yin J-Y, Nie S-P, Xie M-Y. Applications of infrared spectroscopy in polysaccharide structural analysis: Progress, challenge and perspective. Food Chem X. 2021;12: 100168.
49. Díaz Bayona KC, Garcés LA. Effect of different media on exopolysaccharide and biomass production by the green microalga Botryococcus braunii. J Appl Phycol. 2014;26:2087–95.
50. Decamp A, Michelo O, Rabbat C, Laroche C, Grizeau D, Pruvost J, et al. A new, quick, and simple protocol to evaluate microalgae polysaccharide composition. Mar Drugs. 2021;19(2):101.
51. Di Bucchianico DDM, Wang Y, Buvat J-C, Pan Y, Moreno VC, Leveneur S. Production of levulinic acid and alkyl levulinates: a process insight. Green Chem. 2022;24(2):614–46.
52. Bernaerts TMM, Kyomugasho C, Van Looveren N, Gheysen L, Foubert I, Hendrickx ME, et al. Molecular and rheological characterization of different cell wall fractions of Porphyridium cruentum. Carbohydr Polym. 2018;195:542–50.
53. Fimbres-Olivarría D, López-Elías JA, Carvajal-Millán E, Márquez-Escalante JA, Martínez-Córdova LR, Miranda-Baeza A, et al. Navicula sp. sulfated polysaccharide gels induced by Fe(III): rheology and microstructure. Int J Mol Sci. 2016. https://doi.org/10.3390/ijms17081238.
54. Ahmed Z, Wang Y, Anjum N, Ahmad A, Khan ST. Characterization of exopolysaccharide produced by Lactobacillus kefiranofaciens ZW3 isolated from Tibet kefir–Part II. Food Hydrocoll. 2013;30(1):343–50.
55. Cristofoli NL, Lima AR, Rosa da Costa AM, Evtyugin D, Silva C, Varela J, et al. Structural characterization of exopolysaccharides obtained from Porphyridium cruentum exhausted culture medium. Food Bioprod Process. 2023;138:162–71.
56. Moreira JB, Kuntzler SG, Bezerra PQM, Cassuriaga APA, Zaparoli M, da Silva JLV, et al. Recent advances of microalgae exopolysaccharides for application as bioflocculants. Polysaccharides. 2022;3(1):264–76.
57. Wang H, Qi B, Jiang X, Jiang Y, Yang H, Xiao Y, et al. Microalgal interstrains differences in algal-bacterial biofloc formation during liquid digestate treatment. Bioresour Technol. 2019;289:121741.
58. Yang L, Zhang H, Cheng S, Zhang W, Zhang X. Enhanced microalgal harvesting using microalgae-derived extracellular polymeric substance as flocculation aid. ACS Sustain Chem Eng. 2020;8(10):4069–75. https://doi.org/10.1021/acssuschemeng.9b06156.
59. Cunha C, Silva L, Paulo J, Faria M, Nogueira N, Cordeiro N. Microalgal-based biopolymer for nano- and microplastic removal: a possible biosolution for wastewater treatment. Environ Pollut. 2020;263: 114385.
60. Ajao V, Bruning H, Rijnaarts H, Temmink H. Natural flocculants from fresh and saline wastewater: Comparative properties and flocculation performances. Chem Eng J. 2018;349:622–32.
61. Nehal F, Sahnoun M, Smaoui S, Jaouadi B, Bejar S, Mohammed S. Characterization, high production and antimicrobial activity of exopolysaccharides from Lactococcus lactis F-mou. Microb Pathog. 2019;132:10–9.
62. Ayyash M, Abu-Jdayil B, Itsaranuwat P, Galiwango E, Tamiello-Rosa C, Abdullah H, et al. Characterization, bioactivities, and rheological properties of exopolysaccharide produced by novel probiotic Lactobacillus plantarum C70 isolated from camel milk. Int J Biol Macromol. 2020;144:938–46.
63. Biliavska L, Pankivska Y, Povnitsa O, Zagorodnya S. Antiviral activity of exopolysaccharides produced by lactic acid bacteria of the genera pediococcus, leuconostoc and lactobacillus against human adenovirus type 5. Medicina (Kaunas). 2019;55:9.
64. Mailänder-Sánchez D, Braunsdorf C, Grumaz C, Müller C, Lorenz S, Stevens P, et al. Antifungal defense of probiotic Lactobacillus rhamnosus GG is mediated by blocking adhesion and nutrient depletion. PLoS ONE. 2017;12(10): e0184438.
65. Riaz Rajoka MS, Wu Y, Mehwish HM, Bansal M, Zhao L. Lactobacillus exopolysaccharides New perspectives on engineering strategies, physiochemical functions, and immunomodulatory effects on host health. Trends Food Sci Technol. 2020;103:36–48.
66. Gongi W, Cordeiro N, Pinchetti JLG, Ben OH. Production of exopolymer substances from the thermophilic chlorophyte Graesiella: industrial and ecological applications. J Appl Phycol. 2021;33:343–56.
67. Netanel Liberman G, Ochbaum G, Malis Arad S, Bitton R. The sulfated polysaccharide from a marine red microalga as a platform for the incorporation of zinc ions. Carbohydr Polym. 2016;152:658–64.
68. Radonić A, Thulke S, Achenbach J, Kurth A, Vreemann A, König T, et al. Anionic Polysaccharides From Phototrophic Microorganisms Exhibit Antiviral Activities to Vaccinia Virus. Robert Koch-Institut, Biologische Sicherheit; 2011.
69. Hamdi AM, Fida M, Deml SM, Abu Saleh OM, Wengenack NL. Retrospective analysis of antimicrobial susceptibility profiles of Nocardia species from a tertiary hospital and reference laboratory 2011 to 2017. Antimicrob Agents Chemother. 2020;64(3):10–1128.
70. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31(3):426–8.
71. Kielkopf CL, Bauer W, Urbatsch IL. Bradford assay for determining protein concentration. Cold Spring Harb Protoc. 2020. https://doi.org/10.1101/pdb.prot102269.
72. Kostas E, Stuart W, Daniel W, David C. Optimization of a total acid hydrolysis based protocol for the quantification of carbohydrate in macroalgae. J Algal Biomass Util. 2016;7(1):21–36.
73. de Castro JPL, Costa LEC, Pinheiro MP, dos FranciscoVasconcelos TS, Funari LM, et al. Polysaccharides of red alga Gracilaria intermedia structure, antioxidant activity and rheological behavior. Polímeros. 2018;28:178–86.
74. Vázquez-Martínez J, Ramírez-Chávez E, Gutierrez-Villagomez J, Molina-Torres J. Extracellular polysaccharides and biomass production in cyanobacteria isolated from stone monuments in dry zones of Mexico. J Chem Biol Phys Sci. 2014;30(4):33–43.
75. Alves A, Caridade SG, Mano JF, Sousa RA, Reis RL. Extraction and physicochemical characterization of a versatile biodegradable polysaccharide obtained from green algae. Carbohydr Res. 2010;345(15):2194–200.
76. Martínez-Cruz O, Paredes-Lopez O. Phytochemical profile and nutraceutical potential of chia seeds (Salvia hispanica L.) by ultra high performance liquid chromatography. J Chromatogr A. 2014;1346:43–8.

Publisher's Note