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Functional groups in microalgal extracellular polymeric substances: A promising biopolymer for microplastic mitigation in marine ecosystems

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ABSTRACT

This study aims to characterise the amounts of EPS produced and the chemical functional groups of three different microbial phyla, namely Chlorophyta (*Dunaliella* sp.), Bacillariophyta (*Phaeodactylum* sp.), and Cyanobacteria (*Spirulina* sp.). Microalgae of *Dunaliella* sp., *Phaeodactylum* sp. and *Spirulina* sp. were grown in culture media with continuous aeration and lighting and controlled temperature. At the beginning of the stationary phase, the culture medium was centrifuged, ethanol precipitated, dialysed with deionised water and freeze-dried to produce white EPS biopolymer. The dry EPS weight of microalgae *Dunaliella* sp., *Phaeodactylum* sp. and *Spirulina* sp. were 0.356±0.01 gL^{-1}, 0.245±0.02 gL^{-1} and 0.477±0.02 gL^{-1}, respectively. In the present study we used micro scopic Fourier-Transform Infrared Spectroscopy (FTIR) to investigate functional group of EPS microalgae. The Fourier Transform Infrared (FTIR) spectra of all three exopolysaccharides (EPS) reveal key similarities, including O-H stretching vibrations around 3500 cm^{-1}, indicative of hydroxyl groups that enhance hydrophilicity, and C=O stretching vibrations between 1700–1600 cm^{-1}, suggesting the presence of carbonyl groups. These functional groups, along with C-H stretching vibrations around 2920–2850 cm^{-1} linked to aliphatic hydrocarbons, contribute to the structural integrity, solubility, and versatility of EPS in biological and industrial applications. The study concludes that EPS-producing microalgae hold significant potential for mitigating microplastic pollution through aggregation and possible biodegradation, especially in marine environments.

Keywords: extracellular polymeric substances, *Dunaliella* sp., *Phaeodactylum* sp., *Spirulina* sp., FTIR.

INTRODUCTION

Plastic has become a global environmental problem due to increased production, improper disposal and human activities. Plastic waste is spread throughout the world's oceans, mainly accumulating in five major oceans (Hale et al., 2020). Around 80% of the rubbish that accumulates on land, shorelines, sea surfaces and the seabed is made up of plastic. The most common types of plastic debris include plastic film, windblown plastic bags, discarded fishing gear, and food and beverage packaging. (Barnes et al., 2009). Plastic pollution is particularly acute in densely populated regions, especially near plastic processing facilities or locations with significant wastewater discharge. In 2010, around 2.7 million tonnes of plastic into the ocean, and this statistic persists in its upward trajectory (Jambeck et al., 2015). Microplastics, defined as plastic particles ranging from 1 µm to 5 mm in size, are one of the most detrimental forms of waste (Andrady, 2011).

Microplastics present a significant threat to marine ecosystems. Recent studies indicate that microalgae possess the capability to absorb and eliminate microplastics from the environment, potentially mitigating the effects of microplastic pollution. (Cheng and Wang, 2022; Chia et al*.,* 2020). Marine microalgae, including diatoms and cyanobacteria, that produce substantial quantities of extracellular polymeric substances (EPS), are integral to the marine ecology. EPS, a sophisticated polymer produced by microalgae, serves multiple ecological roles, such as biofilm development, nutrient cycling, and carbon sequestration (Laroche, 2022). Certain microalgae, including Chlorella vulgaris and Scenedesmus obliquus, synthesize extracellular polymeric substances (EPS) containing hydroxyl, carboxyl, and amide groups that facilitate microplastic aggregation via electrostatic interactions, hydrogen bonding, and van der Waals forces (Esmaeili Nasrabadi et al*.,* 2023; Su et al*.,* 2023).

Extracellular polymeric substances (EPS) are biodegradable macromolecules released by algae cells (Babiak and Krzemińska, 2021). EPS has garnered interest in recent years owing to its potential applications across various sectors, including cosmetics, food, aquaculture, and medicines. The primary constituents of microalgal extracellular polymeric substances (EPS) comprise polysaccharides, lipids, proteins, and nucleic acids. The polymer network established in EPS facilitates cellular connectivity and regulates adherence to the surface, all while preserving a stable matrix structure (Xiao and Zheng, 2016).

EPS produced by microalgae is a biogenic product that has significant potential for use in wastewater management due to its sorption characteristics. One application of EPS in sewage treatment is its ability to remove harmful heavy metals (Babiak and Krzemińska, 2021). EPS polysaccharides contain hydroxyl, carboxylate, and sulfate groups, due to the breakdown of these functional groups on the cell surface, microalgal cells are negatively charged. EPS is also involved in flocculation and the nature of EPS controls the rate of floc formation, sulphate in EPS is able to produce flocs in the presence of deoxy sugars. Sulphate also contributes to the hydrophilicity of EPS so that EPS has a gel-like consistency as it retains water molecules (Xiao and Zheng, 2016). The removal of microplastics by microalgae may occur by an adsorption mechanism through the formation of hetero-aggregates between microplastics and microalgae facilitated by the formation of extracellular polymeric substances (EPS). EPS secretion by microalgae in response to microplastics can facilitate the formation of heteroaggregates and colonisation of microplastics (Shiu et al., 2020) . Microalgae can produce extracellular polymeric substances (EPS) and microplastics can induce the production of EPS, which are polymers produced during the metabolic process of microbial cells(Song et al., 2020; Chentir et al*.,* 2017). The synthesis of EPS by microalgae may facilitate the formation of heteroaggregates with microplastics and potentially improve the biodegradation process (Cunha et al*.,* 2019).

The characterization of exopolysaccharides generated by microalgae is essential. EPS significantly contributes to microplastic aggregation by sticking to their surfaces, so creating a dense complex. The quantities of EPS and its functional groups are determinants that affect the aggregation process. Microalgae, including Chlorella vulgaris and Scenedesmus obliquus, synthesize extracellular polymeric substances (EPS) that contain hydroxyl, carboxyl, and amide groups, offering many binding sites for microplastics. The functional groups in EPS can engage with microplastics via mechanisms including electrostatic interactions, hydrogen bonding, and van der Waals forces (Esmaeili Nasrabadi et al*.*, 2023; Su et al*.,* 2023).

This study seeks to delineate the quantities of EPS generated and the chemical functional groups present in three distinct microbial phyla: Chlorophyta (*Dunaliella* sp.), Bacillariophyta (*Phaeodactylum* sp.), and Cyanobacteria (*Spirulina* sp.). *Dunaliella* sp. was selected due to its tolerance to diverse salinities and its demonstrated ability to decompose oxidized oxium and HDPE plastics (Hadiyanto et al., 2022). Phaeodactylum sp. was selected due to its extracellular polymeric substance (EPS) production and capacity to aggregate with microplastics (Cunha et al., 2019). Simultaneously, Spirulina sp. was selected for its function in the biodegradation of several plastic polymers (Hadiyanto et al., 2021).

The study tested several hypotheses, including the suspicion that the quantity and chemical composition of extracellular polymeric substances (EPS) produced by microalgae varied across different species. Additionally, it was hypothesized that the EPS of microalgae had different functional groups according to their species, which determined the differences in the potential of microalgae to form hetero-aggregates with microplastics through adsorption mechanisms.. The novelty of this research

lay in the findings related to the use of microalgae as bioremediation agents for microplastics through the production of EPS. These results provided new insights into the potential of microalgae-derived EPS as a key factor in addressing microplastic pollution, opening avenues for future research and practical applications in environmental management.

MATERIALS AND METHODS

Culture preparation of microalgae

Microalgae Dunaliella sp. and Phaeodactylum sp. were sourced from the Natural Feed Laboratory of the Situbondo Brackish Water Aquaculture Centre (BPBAP), whilst Spirulina sp. was procured from the Natural Feed Laboratory of the Jepara Brackish Water Aquaculture Centre (BBPBAP). The initial step in microalgae cultivation was sterilizing the instruments by immersing them in chlorine for 24 hours. Seawater media was prepared by mixing seawater with distilled water as needed. For the culture of *Dunaliella* sp, *Phaeodactylum* sp. and *Spirulina* sp. required water with a salinity of 28±2 ppt. Sterilisation was also carried out on the culture media by applying 60 ppm chlorine solution for at least 24 hours, aerated and neutralised using 0.01 N Na-Thiosulphate solution. A total of 900 mL of media water was filtered using a filter bag, then put into a 1 litre erlenmeyer and then sterilised the culture media with an autoclave process at 121 °C for 15 minutes. The inoculum was grown in a 1000 mL Erlenmeyer flask, with the combined volume of the medium and inoculum totalling 1000 mL. Culture of *Dunaliella* sp. was done by adding Walne fertiliser at a dose of 1 mL/L and vitamin at a dose of 1 mL L^{-1}. Similarly, *Spirulina* sp. was cultured by adding Walne fertiliser and vitamin at 1 mL L^{-1} each. While *Phaeodactylum* sp, was cultured by adding Guillard fertiliser, silicate and vitamin at a dose of 1 mL L^{-1} each. The microalgae inoculum grown was approximately 100,000 cells mL^{-1}. The cultures were kept under continuous light (24 hours of light with no dark cycle). The incubation settings included a temperature of 25±2 °C, light intensity of 1500 lux, and constant aeration over a period of eight days. Cultures of *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp. were performed in triplicate. After eight days, the microalgae biomass was collected, and the culture medium was used for EPS analysis.

Observations during microalgae culture

Microalgae population was counted daily for 8 days. Cell growth was tracked by counting the cells of *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp. using a Neubauer hemocytometer at daily intervals for 8 days. Before counting, cells were fixed with Lugol's iodine solution. Calculation of microalgae density was done using an Olympus CX23 binocular microscope, while observation of microalgae morphology was done using an Inverted Biological Microscope LIBM-A10. Physical chemical quality, especially pH, dissolved oxygen levels, salinity, and light intensity were kept stable. During the culture process, pH was measured using Lutron YK-2001PHA, dissolved oxygen (DO) was measured using Trans Instrument HD3030 DO meter, salinity was measured with Atago Master-S refractometer.

Extraction of EPS

Determination of microalgae EPS production using a modification of the method Cheng and Wang (2022) and (Silva et al*.,* 2020). Microalgae were harvested on the eighth day of culture, then 300 mL of culture medium was centrifuged at 4500 rpm for 30 minutes, at 4 °C. After the centrifugation process, the supernatant was collected and filtered using GF/C filter paper. The filtered filtrate was added with cold absolute ethanol gradually for EPS precipitation, with the ratio of supernatant and ethanol solution being 1:1 (v/v). The mixture was kept at 4 °C for 24 hours. The precipitate was separated from the solvent by centrifugation at 4500 rpm for 20 min, at 4 °C. The precipitate obtained was separated and added with 5 mL of distilled water. The mixture was then dialyzed with distilled water using a dialysis tube with a cut off between 12,000–14,000 daltons. The volume of distilled water used was at least 20 times the volume of the precipitate that was dialyzed for 24 hours. The dialyzed EPS precipitate was frozen at -20 ºC and dried with a freeze dryer (Martin Christ Alpha 1-2 LSCbasic). EPS yield was determined gravimetrically as grams of dry EPS per liter of media.

Analyses of carbohydrate and protein

Carbohydrate analysis was performed by the phenol-sulfuric acid method (Dubois et al., 1956). A total of 1 mg of dried EPS pellet was

added with 1 mL of aquades. The mixture was then added with 0,5 mL of 5% phenol solution, after which the mixture was added with 2.5 mL of concentrated sulfuric acid solution, shaken and allowed to stand for 30 minutes. Total carbohydrate content was measured using UV Vis spectroscopy at a wavelength of 490 nm. A calibration curve was prepared using glucose as a standard solution. Total carbohydrate content was calculated from the calibration curve of glucose standard solution.

Total protein was analyzed using the Lowry method (Lowry et al., 1951), as much as 0.5 mg of dry EPS sample was dissolved in 0.5 mL of distilled water, then added with 0.5 mL of 1 N NaOH solution, heated in a water bath at a temperature of 100 °C for 10 minutes. The mixture was then added with 2.5 mL of Lowry's reagent, and homogenized until evenly mixed with a vortex for 10 seconds. The mixture was then allowed to stand for 10 minutes, then added with 0.5 mL of Folin Ciocalteu reagent. Afterward, it was homogenized evenly using a vortex for 10 seconds and left for 30 minutes. Absorbance was measured at a wavelength of 750 nm. Calibration curves were obtained using bovine serum albumin (BSA) solution.

Analysis using ATR-FTIR (attenuated total reflection-fourier transform infrared spectroscopy)

Dried EPS samples from *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp. were examined via Fourier transform infrared (FTIR) spectroscopy in the attenuated total reflection (ATR) mode using the IRSpirit/ATR-S model (Serial Number A224158/Shimadzu). For ATR-FTIR analysis, 2 mg of dried EPS was placed in an ATR-FTIR holder, and wavelength spectra were recorded from 4000 to 400 cm^{-1}.

RESULTS AND DISCUSSIONS

Comparison of the morphological characterization and growth behavior of *Dunaliella* sp., *Phaeodactylum* sp. and *Spirulina* sp.

The morphological evaluations of *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp. were performed with the Inverted Biological Microscope LIBM-A10 with the × 40 objective (Figure 1).

Dunaliella sp. are oval or round, green in colour, and flagellated. The cells are motile due to the presence of two flagella of equal length, which aids in movement within the culture medium. *Dunaliella* sp. does not have a rigid cell wall, allowing it to survive in high salinity environments. *Phaeodactylum* sp., a diatom, shows a more elongated or fusiform structure with sharp tips. It has silica frustules (external shell) that provide rigidity. Its cells are usually rod-shaped with visible chloroplasts scattered throughout. *Spirulina* sp. is a filamentous cyanobacterium that forms spiral-shaped trichomes, often intertwined. Its cylindrical filaments are composed of several intertwined individual cells, and exhibit a helical ribbon-like structure.

The growth behaviour of the three microalgae species was assessed by conducting biological triplicate experiments in Erlenmeyer flasks for comparison. Figure 2 shows the growth graph between *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp. cultured under relatively similar conditions of temperature, light intensity and aeration. *Dunaliella* sp. and *Spirulina* sp. were both cultured on Walne's medium, while *Phaeodactylum* sp. was cultured on Guillard's medium with the addition of silicate, all three received the same vitamin supplements. *Spirulina* sp. experienced a sharp increase in growth, especially in the middle period, indicating high growth rates during the exponential phase before stabilising in the

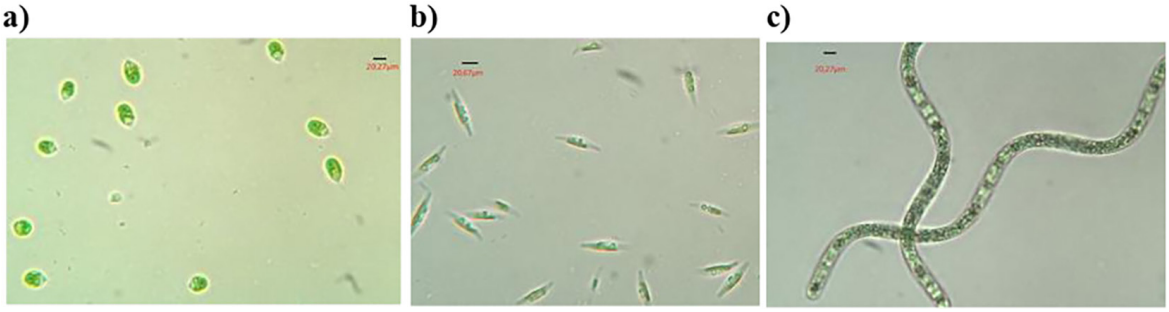

Figure 1. *Dunaliella* sp. (a), *Phaeodactylum* sp. (b), and *Spirulina* sp. (c) (Research Documentation, 2024)

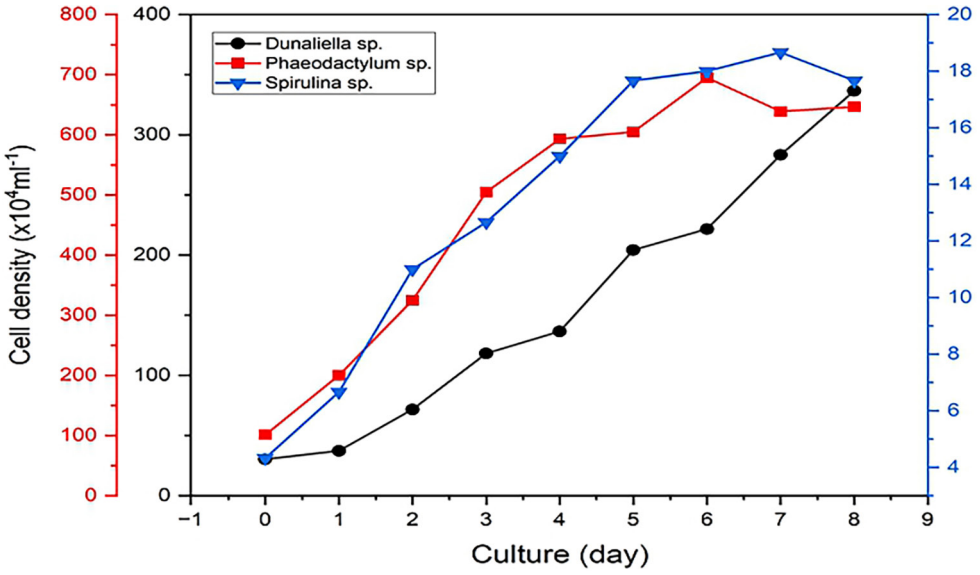

Figure 2. Cell density of *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp.

stationary phase. *Phaeodactylum* sp. also experienced rapid growth and slightly surpassed *Spirulina* at the beginning of the exponential phase. Although both had similar growth rates, *Phaeodactylum* sp. eventually grew slightly faster than *Spirulina* sp. Meanwhile, *Dunaliella* sp. showed slower growth than the other two species, especially in the early stages, and took longer to reach higher biomass. Both *Spirulina* sp. and *Phaeodactylum* sp. showed significant growth during the exponential phase and had similar final biomass, but *Phaeodactylum* sp. reached the stationary phase slightly faster. *Dunaliella* sp., on the other hand, grew more slowly and took longer to reach maximum biomass compared to *Spirulina* sp. and *Phaeodactylum* sp.

Isolation and characterization of EPS

Microalgae of *Dunaliella* sp., *Phaeodactylum* sp. and *Spirulina* sp. were grown in culture media with continuous aeration and lighting and controlled temperature. At the beginning of the stationary phase, the culture medium was centrifuged, ethanol precipitated, dialysed with deionised water and freeze-dried to produce white EPS biopolymer (Figure 3). The dry EPS weight of microalgae *Dunaliella* sp., *Phaeodactylum* sp. and *Spirulina* sp. were 0.356±0.01 gL^{-1}, 0.245±0.02 gL^{-1} and 0.477±0.02 gL^{-1}, respectively (Table 1). EPS production by various eukaryotic microalgae species has considerable variation and is strongly influenced by the culture media and growth conditions used (Delattre et al., 2016).

Mishra and Jha (2009), observed a significant rise in EPS production with rising salinity levels. The maximum EPS yield, 944 mgL^{-1}, was seen at 5 M NaCl, whilst the minimum production, 56 mgL^{-1}, was noted at 0.5 M NaCl. The results indicate that increased salinity significantly boosts EPS generation in *Dunaliella salina* under salt stress conditions. The EPS results in this study were higher when compared to the EPS produced by Dunaliella salina with a NaCl concentration of 0.5 M carried out by Mishra and Jha (2009). Research on EPS by *Phaeodactylum* sp. conducted by Halaj et al. (2019), yielded a concentration of 83 mg/L, with carbohydrate content at 1.0 wt% and protein content at 1.6 wt%. Research conducted by Nur et al. (2019) on *Phaeodactylum* sp. cultivated at 22.5 °C and 35 ppt salinity yielded a

Table 1. Biomass and extracellular polymeric substances (EPSs) generated by microalgae

Microalgae species	Total biomass (g/L)	Total EPS (g/L)
Dunaliella sp.	0.24±0.01	0.356±0.01
Phaeodactylum sp.	0.15±0.03	0.245±0.02
Spirulina sp.	0.16±0.05	0.477±0.02

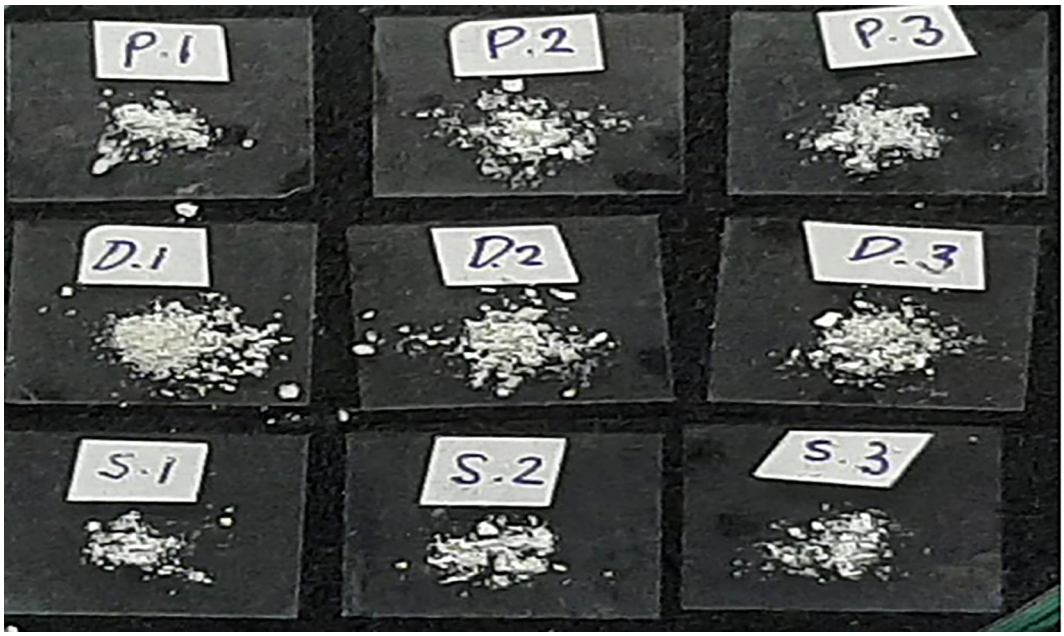

Figure 3. EPS extraction results from the culture media of *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp.

biomass of 0.12 ± 0.00 g/L and EPS polysaccharide of 87.33 ± 1.17 mg/L. The synthesis of extracellular polymeric substances (EPS) by *Spirulina platensis* is influenced by many culture factors, including nitrate concentrations and photon flux density (PFD).

Silva et al. (2020), demonstrated that in conditions of low nitrate levels (0.25 g/L $NaNO_3$) and low photon flux density (200 µE $m^{-2}s^{-1}$), the production of extracellular polymeric substances (EPS) peaks at roughly 111 mg g^{-1}. The maximum biomass output of 1.292 g/L is achieved at elevated nitrate concentrations (2 g/L NaNO3) and a moderate photon flux density (600 µE $m^{-2}s^{-1}$). The findings indicate that stress situations, such as nitrate, can augment EPS synthesis by promoting polysaccharide formation. Moreover, substantial EPS yields, approximately 100 mg/g, were recorded in low nitrate conditions coupled with high photon flux density (1000 µE $m^{-2}s^{-1}$), indicating that both light and nutrient availability are essential for optimizing EPS formation. Chentir et al. (2017) reported that Spirulina, the microalgae, yielded the highest quantities of EPS at 1.02 g g^{-1} of dry biomass weight. The elevated EPS production resulted from a culture with a low light intensity of 10 µmol photons $m^{-2}s^{-1}$ and a high NaCl content of 39 g L^{-1}. The salinity of the culture media influences EPS production by microalgae. Mishra and Jha (2009) reported the effect of increasing salinity on the EPS production of Dunalilla saline microalgae, the higher the salinity of the culture media used, the higher the EPS production.

Table 2 presented data on total carbohydrate and protein levels produced by three microalgal species: *Dunaliella sp.*, *Phaeodactylum sp.*, and *Spirulina sp.*. According to the table, *Dunaliella sp.* achieved the highest carbohydrate concentration (66.331 ± 1.73 mg/L), reflecting its well-known capacity to accumulate carbohydrates, often as glycerol, under stress conditions like high salinity. Its protein content was moderate at 9.381 ± 3.43 mg/L. In comparison, *Phaeodactylum sp.* showed slightly lower carbohydrate production (55.858 ± 0.94 mg/L) and the lowest protein yield (6.365 ± 1.92 mg/L), which aligned with other studies highlighting this genus' tendency to produce valuable pigments and fatty acids rather than large amounts of protein. *Spirulina sp.* stood out

Table 2. Total carbohydrate and protein produced by microalgae

Microalgae species	Total carbohydrate (mg/L)	Total protein (mg/L)
Dunaliella sp.	66,331±1,73	9,381±3,43
Phaeodactylum sp.	55,858±0,94	6,365±1,92
Spirulina sp.	32,025±1,44	18,746±2,97

for its significantly higher protein content (18.746 ± 2.97 mg/L), a result that corroborated previous literature describing *Spirulina*, often *Arthrospira platensis*, as a highly protein-rich microalga. Although its carbohydrate level (32.025 ± 1.44 mg/L) was comparatively low, *Spirulina's* overall nutritional profile, dominated by protein, made it a promising candidate for use as a dietary supplement. These findings were consistent with other research, indicating that environmental factors (such as light intensity, nutrient availability, and salinity) and genetic traits shaped the biochemical composition of different microalgal species, ultimately influencing their potential applications in various industries.

Araj-Shirvani (2024) highlighted that the microalgae species *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp. were extensively studied for their potential in producing valuable biochemical compounds, including carbohydrates and proteins (Senousy et al*.,* 2022). Among these, *Dunaliella* sp. was notable for its ability to accumulate high levels of carbohydrates, which could be utilized in various biofuel production processes. Specifically, *Dunaliella salina* exhibited significantly higher carbohydrate accumulation compared to other species within the genus and other microalgae. However, *Dunaliella sp.* was less frequently employed in carbohydrate accumulation and biofuel production due to its comparatively lower carbohydrate yields.

Dunaliella sp. also produced valuable bioactive metabolites, such as phenolic compounds, flavonoids, proteins, and carotenoids, which exhibited protective and antioxidant properties (Senousy et al*.,* 2022). Additionally, research explored the commercial potential of *Dunaliella sp.* in producing pigments like lutein (Joseph et al*.,* 2021). However, *Dunaliella salina* showed limitations for industrial lutein production due to its slow growth rate under high light intensities. In the context of protein production, *Dunaliella parva* demonstrated the ability to grow heterotrophically by utilizing glucose as an organic carbon source. Furthermore, while the sugars in cheese whey were insufficient for achieving high carbohydrate production in *Chlamydomonas* sp., *Dunaliella parva* effectively utilized the glucose present in cheese whey to support its growth (Mohammad et al*.,* 2022).

Phaeodactylum sp., a diatom species, was widely investigated for its capacity to produce valuable biochemical compounds. *Phaeodactylum tricornutum* was particularly recognized for its production of carbohydrates, proteins, unsaturated fatty acids, and pigments (Gammoudi et al., 2021; Zhao et al., 2022). Under nutrient starvation, *Phaeodactylum tricornutum* accumulated triacylglycerols (TAGs), positioning it as a promising candidate for renewable energy production (Tsuji, 2024). *Spirulina* sp. (also known as *Arthrospira*) was a cyanobacterium extensively studied for its potential to produce carbohydrates and proteins. For instance, the addition of carbohydrates (0.05 g/L) to the nutrient medium was shown to enhance the growth rate of *Spirulina platensis* biomass, resulting in carbohydrate accumulation of up to 41.1% (Andreeva et al*.,* 2021). *Dunaliella* sp. was recognized for its carbohydrate production, albeit with some limitations in biofuel applications. *Phaeodactylum* sp. excelled in the production of a diverse range of biochemical compounds, including TAGs under stress conditions, while *Spirulina sp.* demonstrated significant carbohydrate accumulation when supplemented with additional nutrients. Together, these microalgae represented promising candidates for the sustainable production of biochemicals and renewable energy (Babich et al*.,* 2022).

Fourier transform infrared (FTIR) spectroscopy analysis

In EPS production, important parameters that need to be considered are the quantity, quality and constituent components of EPS. It is generally known that the main components of EPS are carbohydrates and proteins (Boonchai et al., 2015). However, to ensure the components of EPS in microalgae *Dunaliella* sp., *Phaeodactylum* sp. and *Spirulina* sp. it is necessary to analyze the functional groups of EPS. Figure 4 shows the FTIR spectra of the three microalgae in question. The ATR-FTIR spectra of the analyzed polysaccharide fractions are presented in Figure 4.

The FTIR spectra presented illustrate the functional groups in the exopolysaccharides (EPS) produced by three microalgae species: *Spirulina* sp. (blue), *Phaeodactylum* sp. (red), and *Dunaliella* sp. (black). These spectra reveal both similarities and differences in their chemical composition, which are crucial for understanding their potential applications, particularly in biopolymer production and microplastic mitigation.

All three exopolysaccharides (EPS) exhibit notable similarities in their Fourier Transform

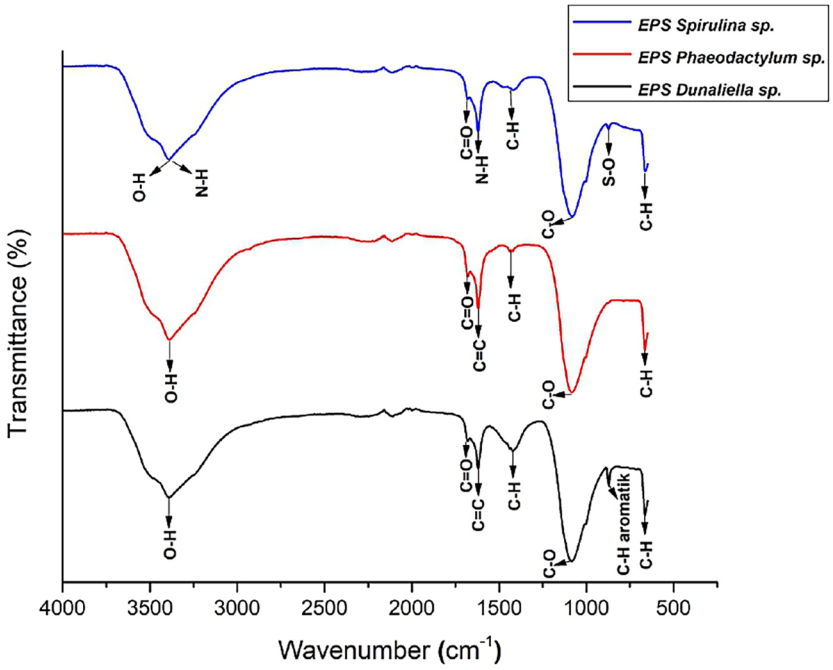

Figure 4. ATR-FTIR spectrum of EPS from *Dunaliella* sp., *Phaeodactylum* sp., and *Spirulina* sp.

Infrared (FTIR) spectra, particularly characterized by a broad absorption peak around 3500 cm^{-1}. This peak is indicative of O-H stretching vibrations, which are typical of hydroxyl groups present in polysaccharides (Sign et al., 2014). The presence of these hydroxyl groups suggests a high affinity for water, contributing to the hydrophilic nature of these compounds. Such hydrophilicity is crucial for their functionality in various biological and industrial applications, as it enhances their solubility and interaction with water. In addition to the O-H stretching vibrations, all three EPS spectra display peaks in the range of 1700–1600 cm^{-1} (Peng et al., 2021). These peaks correspond to C=O stretching vibrations from carbonyl groups, which are commonly found in carboxylic acids or esters associated with EPS. The presence of these functional groups is significant as they can influence the chemical reactivity and biological interactions of the EPS. Another common feature across the spectra is the C-H stretching vibration observed around 2920–2850 cm^{-1} (Isah et al., 2024). This range signifies the presence of aliphatic hydrocarbons, which are likely linked to lipid or protein components embedded within the EPS matrix. The incorporation of such components can enhance the structural integrity and functional properties of EPS, making them versatile materials in various applications, including food technology and pharmaceuticals. Despite the similarities observed in the FTIR spectra of the three exopolysaccharides (EPS), distinct differences highlight their unique chemical compositions. Spirulina sp. is characterized by unique peaks around 1230 cm^{-1}, which correspond to S-O stretching vibrations, indicating the presence of sulfated polysaccharides (Aflori et al., 2024). This feature is significant as sulfated polysaccharides are known for their biological activities, including anticoagulant and antioxidant properties, which have been well-documented in various studies. Additionally, peaks at 1650 cm^{-1} and approximately 1550 cm^{-1} confirm the presence of N-H bending and amide II bonds, suggesting a notable protein component within the Spirulina EPS. This protein content can contribute to the functional properties of the polysaccharide, enhancing its potential applications in food and pharmaceuticals. In contrast, Phaeodactylum sp. displays a prominent peak around 1640 cm^{-1} associated with C=C stretching, indicative of unsaturated bonds (Liu et al., 2022). This feature is less pronounced in the spectra of Spirulina and Dunaliella species, suggesting a different structural composition that may influence its reactivity and interaction with other compounds. Furthermore, strong absorption peaks between 1050–1000 cm^{-1} in *Phaeodactylum* sp. suggest a higher polysaccharide content, particularly from C-O stretching vibrations, which are critical for understanding its potential as a thickening agent or stabilizer in various applications (Naveen et al., 2023). Dunaliella sp. exhibits a distinct C-H aromatic bending peak

near 700 cm^{-1}, indicating the presence of aromatic compounds that are absent or minimal in the other spectra. This characteristic suggests that Dunaliella EPS may possess unique properties related to its aromatic content, potentially affecting its antioxidant capacity and stability in different environments (Xiao et al., 2016). Additionally, the broader spectra observed in *Dunaliella* sp. with fewer sulfate-related peaks reflect a different chemical composition compared to Spirulina and Phaeodactylum, which may influence its functional applications.

Sulfated polysaccharides particularly abundant in *Spirulina* sp., are noteworthy due to their strong negative charge (Rajasekar et al., 2019). This characteristic enhances their ability to bind with positively charged pollutants, including microplastics and heavy metals, making them valuable in bioremediation applications (Nagahawatta et al., 2023). Studies have shown that sulfated polysaccharides can effectively chelate heavy metals, thereby reducing their toxicity in aquatic environments and potentially aiding in detoxification processes within biological systems. The structural properties of these polysaccharides contribute to their effectiveness in binding and removing contaminants from water. The EPS produced by *Phaeodactylum* sp. is rich in polysaccharides, which provides a structural advantage for physical adsorption (Zhang et al., 2020). This property allows for effective removal of pollutants through mechanisms such as adsorption, where the EPS can capture and hold onto various contaminants, enhancing the microalga's role in maintaining water quality. The polysaccharide matrix not only aids in pollutant removal but also plays a crucial role in the stability of microbial communities in aquatic ecosystems. On the other hand, *Dunaliella* sp. contains unique aromatic components within its EPS, suggesting additional bioactivity that may be beneficial for specialized applications (Kilic et al., 2019). These aromatic compounds have been associated with various health benefits, including antioxidant properties, which can help mitigate oxidative stress in biological systems. The bioactive compounds derived from *Dunaliella* have been shown to exhibit antimicrobial and anti-inflammatory activities, making them promising candidates for use in pharmaceuticals and nutraceuticals.

The extracellular polymeric substances produced by microalgae represent a promising avenue for addressing environmental challenges, particularly in the context of microplastics and biodegradable polymers. These natural polymers can serve as effective alternatives to synthetic plastics, which are notorious for their persistence in the environment and contribution to pollution (Abdelfattah et al., 2023). EPS from microalgae, such as those derived from *Spirulina* sp., *Phaeodactylum* sp., and *Dunaliella* sp., contain functional groups like sulfates and hydroxyls that enhance their ability to adsorb microplastics and other contaminants in aquatic environments (Gopalakrishnan and Kashian, 2022). The negatively charged sulfate groups allow these biopolymers to interact with positively charged pollutants, facilitating their removal from water bodies. Studies have demonstrated that EPS can effectively aggregate microplastic particles, leading to their subsequent removal from water through sedimentation or filtration processes (Hasan et al., 2024).

Moreover, the biodegradability of EPS minimizes environmental pollution compared to traditional plastics. When EPS are released into the environment, they naturally degrade over time, reducing the accumulation of harmful materials. This characteristic makes them ideal candidates for eco-friendly biopolymer applications in various industries, including packaging, agriculture, and wastewater treatment. The use of algal-derived biopolymers not only addresses plastic pollution but also promotes sustainability by utilizing renewable resources. Additionally, microalgal EPS can contribute to the circular economy by providing a source of biodegradable materials that can be integrated into existing waste management systems. The production of these biopolymers from microalgae involves low energy inputs and can be achieved through autotrophic growth processes that utilize sunlight and carbon dioxide, further reducing greenhouse gas emissions associated with plastic production. In conclusion, the FTIR spectra reveal critical insights into the chemical diversity of EPS produced by *Spirulina*, *Phaeodactylum*, and *Dunaliella*. These differences in functional groups determine their bioactivity and potential for microplastic adsorption, highlighting their suitability as sustainable biopolymers to combat plastic pollution.

CONCLUSIONS

The study successfully demonstrated that extracellular polymeric substances produced by microalgae species *Dunaliella* sp., Phaeodactylum

sp., and *Spirulina* sp. played a significant role in microplastic aggregation through electrostatic interactions and bonding processes. It revealed novel insights into the functional composition of EPS, highlighting the presence of polysaccharides and proteins, which were identified through FTIR spectroscopy. The research filled a gap in understanding the specific mechanisms by which microalgae-derived EPS could mitigate microplastic pollution, an area that had not been fully explored by previous studies. These findings opened new prospects for utilizing microalgae-derived EPS in environmental management, particularly in wastewater treatment and microplastic pollution reduction. Furthermore, the study highlighted the potential of these biopolymers as eco-friendly alternatives to conventional microplastic removal techniques, offering sustainable solutions for tackling environmental contamination in the future.

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