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Microalgal-based biopolymer for nano- and microplastic removal: a possible biosolution for wastewater treatment[H]

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ABSTRACT

The increasing water pollution caused by the presence of nano- and microplastics has shown a need to pursue solutions to remediate this problem. In this work, an extracellular polymeric substance (EPS) producing freshwater *Cyanothece* sp. strain was exposed to nano- and microplastics. The bioflocculant capacity of the biopolymer produced was evaluated. The influence of different concentrations (1 and 10 mg L^{-1}) of polystyrene nano- and microplastics in the extracellular carbohydrates and in the EPS production was studied. The presence of nano- and microplastics induced a negative effect on the microalgal growth (of up to 47%). The results show that the EPS produced by *Cyanothece* sp. exhibits high bioflocculant activity in low concentrations. Also, the EPS displayed very favourable characteristics for aggregation, as the aggregates were confirmed to consist of microalga, EPS and both the nano- and microplastics. These results highlight the potential of the microalgal-based biopolymers to replace hazardous synthetic flocculants used in wastewater treatment, while aggregating and flocculating nano- and microplastics, demonstrating to be a multi-purposed, compelling, biocompatible solution to nano- and microplastic pollution.

1. Introduction

Plastic pollution has become a concern due to the increased plastic production every year, which tends to accumulate in the environment (Geyer et al., 2017). Plastic particles can come in a variety of sizes and types, one being microplastics commonly defined as plastic particles with sizes below 5 mm (Andrady, 2011). There is still no general consensus regarding the size of nanoplastics, but it is generally defined as particles below 0.1 μm in diameter (Mattsson et al., 2018). Microplastics can be divided by origin into two main categories: primary and secondary microplastics. Primary microplastics originate from the intentional production of commercialized products such as microbeads, which are used in personal care products. Secondary microplastics enter the environment via 'leakage' during manufacture, transportation or use (Andrady, 2017), as a result of the fragmentation of macroplastics, mainly caused by UV-induced degradation or physical abrasion (GESAMP, 2016). Thus, the process results in the release of increasingly smaller sized low-molecular-weight polymer fragments. It is therefore crucial to understand the interaction of nano- and microplastics with the aquatic microbiota. Given the nature and small dimensions, the ingestion of such products by marine organisms has had a subsequent impact on marine life, including the transfer of biological or chemical contaminants (Wright et al., 2013). This is especially true when considering transfer along the trophic chain and possible translocation, for which the hazards are less well understood (Chae and An, 2017; Paul-Pont et al., 2018).

While large plastics can be easily removed during water processing, the currently used technologies are unable to retain neither nano- nor microplastics (Mintenig et al., 2016), since these are too small and cannot be detected and eliminated cost-effectively (Andrady, 2017). Regarding microplastic pollution in wastewater treatment plants (WWTPs), a recent overview by Sun

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et al. (2019) has highlighted that microplastic concentrations in the effluents can be as high as 447 particles L^{-1}, with PS being one of the most detected polymers. Data from over 70 WWTPs has shown that although the inclusion of tertiary treatment processes (TTPs) highly reduces the average amount of microplastics in the effluents, this can still be as high as 51 particles L^{-1}, and only approximately 24% of WWTPs incorporate TTPs. The total discharge of microplastics is even higher and more worrisome; the same study reported that the median value of microplastic discharge in the WWTPs studied was 2×10^6 particles/day, which raises concerns with respect to WWTPs-derived microplastic pollution. On the other hand, concerns were raised regarding the urgency of developing microplastic-targeted treatment technologies to avoid this massive emission of microplastics.

For the past years, the attention given to extracellular polymeric substances (EPS) has increased due to their potential applications within different industries, such as the food, cosmetic, aquaculture and pharmaceutical ones. More recently, recognition of the potential of EPS producing microorganisms in bioremediation has led to a greater focus on EPS producing bacteria (Sheng et al., 2010; Wang et al., 2014), cyanobacteria (Philippis and Vincenzini, 1998; Pereira et al., 2011) and microalgae (Kaplan, 2013; Ge and Champagne, 2016; Xiong et al., 2018). EPS are biodegradable (Nouha et al., 2018) and range from gels to fully dissolved states (Decho, 2000), displaying viscous gel-like structures (Cunha et al., 2019) in which the polymer molecules are assembled to form tangled networks or covalently crosslinked networks (Decho, 2000). EPS are already used in industries mainly as gelling and thickening agents suspending or stabilizing the aqueous phase (Philippis and Vincenzini, 1998), further exhibiting an excellent property of gelation that can be exploited for nano- and microplastic removal. Also, due to their anionic properties, EPS have a large metal-binding potential owing to the abundance of negatively charged amino acids like aspartic and glutamic acid (Decho and Gutierrez, 2017). These complex molecules can also establish London forces, electrostatic interactions and hydrogen bonding in the adhesion and cohesion of suspended solids (Nouha et al., 2018), making them potential candidates for bioalternative solutions to hazardous bioflocculant salts and synthetic polymers used in wastewater treatment, which also constitute a source of environmental pollution (Deng et al., 2003). Bioflocculants are also regarded as safe and biodegradable, with less sludge generation and no secondary toxin production (Mohammed & Dagang, 2019).

The freshwater microalga *Cyanothece* sp. has been used in bioremediation processes of industrial effluents (Dubey et al., 2011), especially in phycoremediation linked to its nitrogen/metal removal capabilities (Reddy et al., 1993; Shah et al., 2000) and bioflocculation activity (Patil et al., 2010), due to its ability to secrete EPS (Ohki et al., 2014) mainly constituted by species-specific exo-heteropolysaccharides (Mota et al., 2013).

Thus, the present study evaluates the EPS production by *Cyanothece* sp. under nano- and micro-PS exposure conditions and describes the phenomenon of gel formation, which can have the potential to affect the bioavailability of nano- and microplastics. The influence of PS nano- and microplastics, at low and high concentrations (1 and 10 mg L^{-1}), on microalgal growth, extracellular carbohydrates, EPS and hetero-aggregate formation was also evaluated.

2. Materials and methods

2.1. Microalga selection and culture conditions

In the present study the freshwater microalga *Cyanothece* sp. (Class *Cyanophyceae*; 5–10 μm) was selected. *Cyanothece* sp. was obtained from the Spanish Bank of Algae (BEA) of the University of Las Palmas de Gran Canaria (Spain) and was grown in Waris-H + Si medium [0.1 g L^{-1} KNO_3; 0.02 g L^{-1} $MgSO_4.7H_2O$; 0.02 g L^{-1} $(NH_4)_2HPO_4$; 0.1 g L^{-1} $Ca(NO_3)_2.4H_2O$; 0.24 g L^{-1} HEPES; PII Metals (0.003 g L^{-1} Titriplex III; 0.001 g L^{-1} H_3BO_3; 0.00014 g L^{-1} $MnCl_2.4H_2O$; 0.00021 g L^{-1} $ZnSO_4.7H_2O$; 0.000004 g L^{-1}); Fe-EDTA (0.0052 g L^{-1} Tritiplex II; 0.0049 g L^{-1} $FeSO_4.7H_2O$; 0.054 mL L^{-1} KOH; 0.1421 g L^{-1} Silica; Vitamins (0.0002 mg L^{-1} Vitamin B12; 0.001 mg L^{-1} Biotin; 0.1 mg L^{-1} Thiamine-HCl; 0.0001 mg L^{-1} Niacinamide); 10 mL L^{-1} Soil Extract]. The initial selected cell concentration was 4.24×10^5 cells mL^{-1} to ensure that the culture could grow exponentially throughout the experimental period. The cultures were then maintained for 14 days (experimental period) at 25 ± 1 °C, under the irradiance of 1738 lux (HOBO® Pendant® MX Temp MX2201) supplied by a cool white Osram L 18W 840 Lumilux lamp, with a 14/10 h (light/dark) photoperiod (Aralab CP500 growth chamber). The culture conditions were not altered to enhance EPS production but to evaluate the microalgal EPS production in standard culturing conditions.

During the experimental period, the growth was monitored using a spectrophotometer at 730 nm (UV-6300PC Double Beam Spectrophotometer). A calibration curve ($R^2 = 0.993$) plotting cell density (cells mL^{-1}) against absorbance was used to determine cell density.

Microalgal cell abundance was used to determine the potential effects of nano- and microplastic exposure. The coefficient of variation was calculated for the plastic exposed conditions against the control, as:

$$\Delta cell = [(x - y)/y] \times 100$$

where x is the microalgal cell abundance in the plastic exposed conditions (cells mL^{-1}) and y is the microalgal cell abundance in the control (cells mL^{-1}).

2.2. Plastic particles

Two sizes of polystyrene (PS) plastic particles were used: *1*- pristine 0.1 μm PS nanoplastics (orange fluorescent, spherical, uncharged, density of 1.05 g mL^{-1}) solution in deionised water containing 0.1% Tween 20, obtained from Phosphorex; *2*- pristine 10 μm PS microplastics (green fluorescent, spherical, uncharged, density of 1.06 g mL^{-1}, solution in deionised water containing 0.1% Tween 20), obtained from Thermo Scientific.

Four stock solutions (nano- and microplastics) were prepared at two concentrations: 1 mg L^{-1} and 10 mg L^{-1}. Both PS nano- and microplastics solutions were vortexed before application, to guarantee homogeneity. The solutions were prepared in Waris-H + Si medium, in glass flasks in order to minimize losses of nano- and microplastics due to the possible establishment of electrostatic bonds to the flask walls.

2.3. Exposure conditions of microalga to plastic particles

Five experimental groups were made in triplicate: a control group, with the selected microalga grown in Waris-H + Si medium, and four other experimental groups in which the microalga was subjected to two distinct concentrations of PS nanoplastics: 1 mg L^{-1} (named NP1) and 10 mg L^{-1} (named NP10) and PS microplastics: 1 mg L^{-1} (named MP1) and 10 mg L^{-1} (named MP10).

Cultures were kept without medium renewal during the experimental period and were manually stirred 3 times a day. Nutrients (nitrate and phosphate) were verified every day to ensure that the culture provided enough nutrients.

2.4. Extracellular carbohydrates determination

Extracellular carbohydrates were determined using the phenol-sulfuric acid method, according to DuBois et al. (1956), with slight modifications: 0.5 mL of 5% (w/v) phenol aqueous solution was added to supernatant (1 mL) of the centrifuged culture (13400 rpm, 4 min). This was promptly followed by 2.5 mL of concentrated sulfuric acid and left for 10 min to react, before being vortexed and spending another 20 min at 21 ± 1 °C (room temperature) for colour development. Absorbance was measured at 490 nm in a UV-6300 PC Double Beam Spectrophotometer. A calibration curve was made with D-(+)-Glucose (99.5%, Sigma Aldrich®), using a freshly prepared stock solution (500 mg L^{-1}) diluted to 5, 10, 15, 20, 25 and 50 mg L^{-1} ($R^2 = 0.997$).

2.5. Viscosity determination

Dynamic viscosity measurements were performed using a BROOKFIELD RVDV-III Ultra Programmable Rheometer (Brookfield Engineering Laboratories) at 25 °C, spindle 1 and 200 rpm. During the experimental period, viscosity was monitored for the control and all the nano- and microplastic exposed conditions.

2.6. EPS determination

Mota et al. (2013) method with modifications were used to determine the microalgal EPS production across all treatments. After the 14 experimental days, the cultures were centrifuged at 6000 rpm for 30 min, at 21 ± 1 °C to remove microalgae and other debris. The supernatant was concentrated, and methanol was gradually added for the EPS precipitation, kept at 4 °C for 12 h. After, the precipitate was centrifuged at 6000 rpm for 20 min at room temperature, washed with ethanol and re-dissolved in Milli-Q water. The dissolved EPS were dialysed (MWCO: 12–14 kDa, Spectra/Por®, USA) against distilled water for 2 days to remove ions and salt. The dialysed EPS were frozen at -20 °C and freeze-dried. The EPS yield was determined gravimetrically as dry EPS mg per mL of medium.

2.7. Fluorescent microscopy analysis

Hetero-aggregates were collected from the bottom of the experimental flasks using a pipette. The different hetero-aggregates were individually analysed, under the microscopic I3 filter (excitation 450–490 nm, emission 515 nm). Observations were performed with a Leica DM2700P coupled with a CoolLED's $pE\text{-}300^{lite}$ LED fluorescent illumination system.

2.8. Bioflocculant activity determination

Two EPS solutions recovered from the control conditions were tested: 1- raw supernatant EPS, collected directly from the control culture medium treatment; 2- centrifugated EPS solution, obtained after culture centrifugation (microalga removal). The bioflocculant activity and bioflocculant rate (%) were determined using a modification of the Sanayei et al. (2010) method. Briefly, at 21 ± 1 °C and with pH 7.0, 10 mL of 4 g L^{-1} clay (<140 mesh) was used as the suspended solid; 1 mL of EPS solution (supernatant or centrifugated) and 1 mL of $CaCl_2$ 1% (w/v) solution were mixed. The mixture was gently shaken to ensure homogeneity. 1 mL of the mixture's upper layer was collected at 10, 20, 30 and 60 min and measured at 550 nm, using a UV-6300 PC Double Beam Spectrophotometer. The absorbance of a blank sample was also measured, replacing the EPS sample with distilled water. All assays were performed in triplicate. The bioflocculant activity and bioflocculant rate (%) were calculated using the following equations:

$$Bioflocculant = \frac{1}{OD_{550}} - \frac{1}{OD_{550x}}$$

$$Bioflocculant\ rate = [(OD_{550x} - OD_{550}) / OD_{550x}]*100$$

where OD_{550} and OD_{550_x} represent the absorbance of the EPS and the blank samples, respectively.

2.9. Statistics analysis

Statistical analysis was performed using IBM SPSS statistics software (V.25). Differences in growth rates, extracellular carbohydrate production, medium viscosity and bioflocculant activity between different experimental conditions were assessed by one-way analyses of variance (ANOVA), with a level of statistical significance of p-value < 0.05.

3. Results and discussion

3.1. Microalgal growth

The microalgal growth for the control and the polystyrene (PS) nano- and microplastic exposed conditions are shown in Fig. 1. Results of the daily cell abundance of the PS nano- and microplastics exposed conditions compared with the control group are presented in Table 1. The results show that after the adaptation phase of approximately 2 days, *Cyanothece* sp. grew exponentially through the entire experimental period in all conditions.

Still, Fig. 1 and Table 1 also reveal that cell abundance was significantly lower ($p< 0.05$) in all experimental groups compared to control group throughout the entire experimental period. Until day 4, however, statistically different cell abundance differences were not too pronounced, as all cultures were still starting the exponential phase. From day 4 onwards, as the control group grew faster, cell abundance variation was more evident (Table 1), with the steepest difference being observed at day 9 (46% in NP1 condition and 47% in MP10 condition). Literature reports are in agreement with the current results, showing that different types, sizes and concentrations of nano- and microplastics influence microalgal growth: nano- (0.05 μm) and micro-PS (0.5–400 μm) at

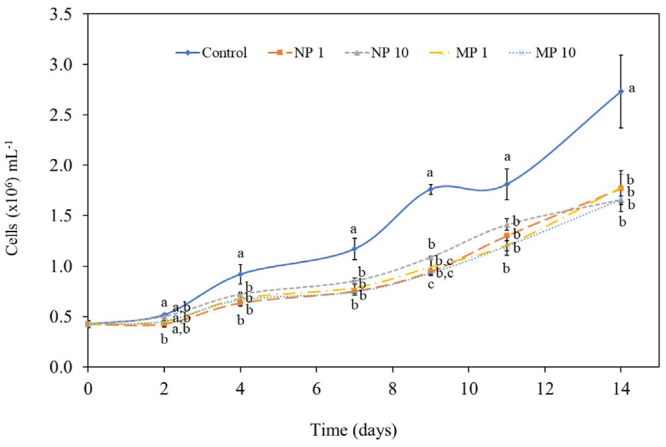

Fig. 1. Growth curves for the control and each of the PS nano- and microplastic exposed conditions. Initial *Cyanothece* sp. cell abundance was 4.24×10^5 cells mL^{-1}. Distinct letters represent significantly different means of the correspondent day ($p< 0.05$). NP1: 1 mg L^{-1} nanoplastics; NP10: 10 mg L^{-1} nanoplastics; MP1: 1 mg L^{-1} microplastics; MP10: 10 mg L^{-1} microplastics.

Table 1
Microalgal cell abundance variation (Δ_{cell}) for each experimental condition studied.

Day	Δ_{cell} (%)			
	NP1	NP10	MP1	MP10
2	−17.57 ± 1.64	−3.47 ± 2.46	−13.32 ± 2.05	−12.36 ± 1.16
4	−30.94 ± 3.15	−21.46 ± 1.82	−26.14 ± 4.14	−27.56 ± 1.16
7	−35.81 ± 1.79	−27.09 ± 2.68	−32.91 ± 0.18	−36.41 ± 1.41
9	−46.31 ± 1.10	−38.07 ± 0.34	−43.64 ± 4.86	−47.05 ± 0.86
11	−28.18 ± 0.85	−22.09 ± 3.16	−33.15 ± 5.56	−33.70 ± 2.61
14	−35.16 ± 4.89	−39.19 ± 1.15	−34.80 ± 6.13	−39.19 ± 4.40

The values are mean of three replicates ± SD. NP1: 1 mg L^{-1} nanoplastics; NP10: 10 mg L^{-1} nanoplastics; MP1: 1 mg L^{-1} microplastics; MP10: 10 mg L^{-1} microplastics.

0.004–1100 mg L^{-1}, micro-PMMA (<106–400 μm) at 12.5–125 mg L^{-1} and micro-PVC (1 μm) at 1–50 mg L^{-1} have been shown to affect the growth of microalgae like *Chaetoceros neogracile*, *Heterocapsa triquetra, Tisochrysis lutea, Scenedesmus obliquus*, *Dunaliella tertiolecta*, *Microcystis panniformis* and *Skeletonema costatum*, with tests ranging from 72 h to 27 days (Besseling et al., 2014; Sjollema et al., 2016; Long et al., 2017; Zhang et al., 2016; Cunha et al., 2019). The mechanisms of nano- and microplastic growth inhibition have been largely linked to: shading effects, actively reducing microalga light exposure (Sjollema et al., 2016); mobility reduction due to particle adsorption (Davarpanah & Guilhermino, 2015); inducing morphological changes, with damage to the pyrenoids, thylakoids, plasma and cell wall (Mao et al., 2018); interference in cell division, by attachment to the surface of zoospores (Chae et al., 2018); and even changes in genes related to the chloroplast and extracellular polymeric substance (EPS) biosynthesis (Lagarde et al., 2016).

The obtained results highlight the sensitivity of microalgae when exposed to nano- and microplastics in the lower range of concentrations usually tested.

3.2. Bioflocculant capacity

Flocculation of clay suspension is a widely applied assay to explore the flocculation capacity of biopolymers (Prasertsan et al., 2006; He et al., 2010; Xiong et al., 2010; Okaiyeto et al., 2013; Tang et al., 2014; Chen et al., 2017). Fig. 2 and Table 2 present the bioflocculant activity and rate measurements performed at 10, 20, and 30 min, at room temperature. At minute 10, the centrifugated EPS solution displayed a significantly higher bioflocculant capacity compared to the raw supernatant EPS, both displaying higher ($p< 0.05$) clay flocculant capacity when compared to the blank. By minute 20, no significant differences were found between the EPS samples (raw and centrifugated), but still displayed higher bioflocculant activity against the blank condition. After 30 min, only the centrifugated EPS solution displayed a significantly higher flocculation of the clay suspension. The raw supernatant EPS and the centrifugated EPS solution were used to compare the bioflocculant activity of the culture medium with and without microalga. This ensures the results show that the key player in the bioflocculant process is characteristics of the EPS and not the microalgae. The highest decrease (60%) in turbidity (flocculation) was achieved by the centrifugated EPS solution at 30 min (Table 2). The estimated concentration of the EPS in the supernatant is 0.122 mg mL^{-1} (Fig. 5), which is considerably lower than previously tested concentrations of EPS (0.5–0.7 mg mL^{-1}) by Patil et al. (2010).

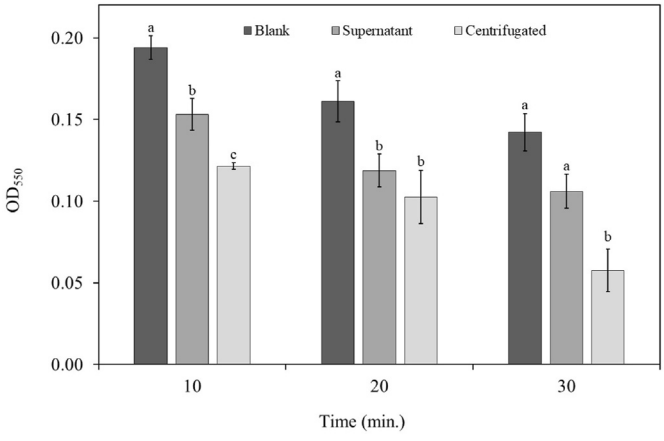

Fig. 2. Absorbance at 550 nm (OD_{550}) of the EPS solutions (supernatant and centrifugated) and the blank for 30 min. Distinct letters represent significantly different means of the correspondent time period ($p< 0.05$).

Table 2
Bioflocculant activity and bioflocculant rate of *Cyanothece* sp. raw supernatant EPS and centrifugated EPS solution, against the blank (distilled water).

	Raw supernatant EPS		Centrifugated EPS solution	
	Bioflocculant		Bioflocculant	
	activity	rate (%)	activity	rate (%)
10 min	1.58 ± 0.58	23.20 ± 7.29	3.08 ± 0.05	37.40 ± 1.26
20 min	2.55 ± 0.20	29.10 ± 0.02	3.65 ± 2.06	35.78 ± 15.12
30 min	2.07 ± 0.35	22.61 ± 1.58	10.80 ± 4.66	59.04 ± 12.57

The values are mean of three replicates ± SD.

To the best of our knowledge, these are the first set of results that assess both the bioflocculant and the nano/microplastic aggregation capacity (see section 3.4) of any microalgal-derived biopolymer.

The use of traditional flocculants in wastewater treatment plants, such as inorganic-based salts and organic synthetic polymers, raises a series of environmental and health concerns, leading to a ban in several countries (Xiong et al., 2010). It has been reported that these flocculants are toxic towards higher plants (Kuboi and Fuji, 1983) and aquatic biota (Albassam et al., 1987; Takigami et al., 1998; Buczek et al., 2017). Also, several authors have shown high toxicity and inflammatory activity of wastewater samples treated with organic flocculants (Makene et al., 2019) and the influence on the development of cancer and neuro-related diseases (Nwodo & Okoh, 2012). On the other hand, it has been suggested that public water supplies are likely to be the most affected by potential microplastic pollution, as suppliers use wastewater or surface water as direct and indirect raw water resources (Storck et al., 2015) for water production, which have been shown to be contaminated across the globe (Cole et al., 2011; Eerkes-Medrano et al., 2015; Wezel et al., 2015). This hypothesis has been recently confirmed as several studies have focused on understanding the magnitude of microplastic-related pollution in both water sources and raw/treated drinking water (Pivokonsky et al., 2018; Mintenig et al., 2019). Therefore, the search for a biocompatible solution for both the use of hazardous bioflocculants and microplastic pollution is imperative. A recent study has highlighted the potential of EPS-based polymers as cheap and eco-friendly alternatives to wastewater treatment (Lotti et al., 2019). Thus, after our results showed the high bioflocculant ability in relation to the low concentration of EPS used, the nano- and microplastic aggregation potential was studied (see section 3.4).

3.3. Extracellular carbohydrates production and medium viscosity

The production of extracellular carbohydrates was evaluated for the control and each of the nano- and microplastic exposed conditions, on the 1st, 7th and 14th day of the experiment. The results shown in Fig. 3 display a significantly higher production of extracellular carbohydrates, on day 7, of the NP10 condition when compared to the control. On day 14, the production of carbohydrates in the NP1, NP10, MP1 and MP10 conditions, in relation to the control, were significantly higher. It is noteworthy that the carbohydrate production increases dramatically upon exposure to nano- and microplastics, suggesting a self-defence mechanism as the EPS are produced to aggregate the microparticles, likely due to selective overexpression of genes involved in the EPS biosynthesis pathway (Lagarde et al., 2016).

The extracellular carbohydrate production results follow the culture viscosity trend displayed in Fig. 4, showing that in the nano- and microplastic exposed conditions there was a parallel between higher extracellular carbohydrate production and higher culture viscosity values. The correlation between higher viscosity values at higher EPS concentration solutions has been demonstrated by Tuinier et al. (1999), so the results displayed for the microalgae cultures fall in line with related field reports in the literature. Further, some major structure-function relationship between EPS composition and flocculation capacity have been established.

The importance of the carbohydrate and protein fractions of EPS regarding flocculation potential is not yet fully understood, but some reports claim that EPS with high carbohydrate fractions exhibit high flocculating abilities (Deng et al., 2005). Also, Kavita et al. (2014) showed that the decrease in monosaccharide percentage in the carbohydrate fraction resulted in a decrease in flocculation ability. Still, the flocculation ability of the EPS is also highly linked to the protein fraction, with the hydrogen bonding made possible by negatively charged amino acids being crucial to the EPS ability to agglomerate suspended particles (Nouha et al., 2018). Our results show that, in the control condition, the carbohydrate fraction accounted for 47% of the EPS extracted (see section 3.4.). In contrast, in the nano- and microplastic exposed conditions, the percentage of carbohydrates was, except for the NP10 condition, higher than the EPS extracted: 141% for the NP1 condition, 91% for the NP10 condition, 147% for the MP1 condition and 163% for the MP10 condition. These results are likely due to differences in the production ratios of tightly bound EPS and loosely bound EPS as a result of nano- and microplastic exposure, as it has been reported that genetic expression modulation of the EPS biosynthesis pathway in microalgae is affected upon microplastic exposure (Lagarde et al., 2016). Still, these differences are likely linked to limitations of the extraction method, since distinct EPS fractions require different extraction methods, which vary from physical to chemical ones (Aguilera et al., 2008; Nouha et al., 2018). The carbohydrate measurement is performed on the supernatant, so the losses from extraction are absent. Still, it is important to note that different extraction methods greatly vary the concentration and composition (carbohydrates/proteins ratio, humic acids and DNA content) of the EPS (Nouha et al., 2018). Therefore, further investigation is needed to fully understand the impact of the extraction method on the composition and, consequently on the bioflocculant and nano- and microplastic aggregation ability of the EPS.

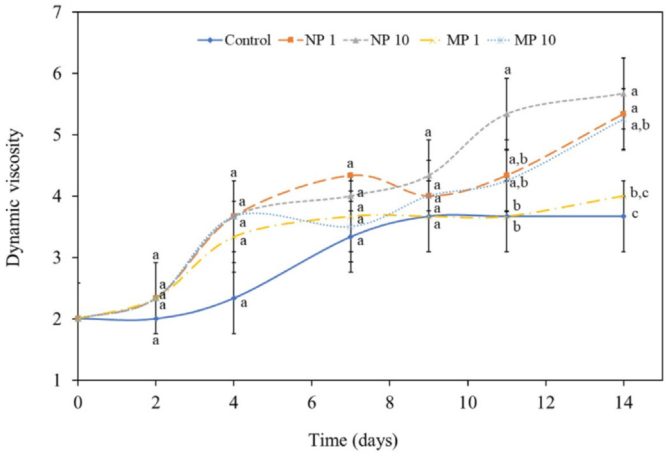

Fig. 4. Culture dynamic viscosity of the control and each of the PS nano- and microplastic exposed conditions, throughout the experimental period. Distinct letters represent significantly different means of the correspondent day ($p < 0.05$). NP1: 1 mg L^{-1} nanoplastics; NP10: 10 mg L^{-1} nanoplastics; MP1: 1 mg L^{-1} microplastics; MP10: 10 mg L^{-1} microplastics.

3.4. EPS and hetero-aggregates formation

The results regarding the EPS production of the control and each

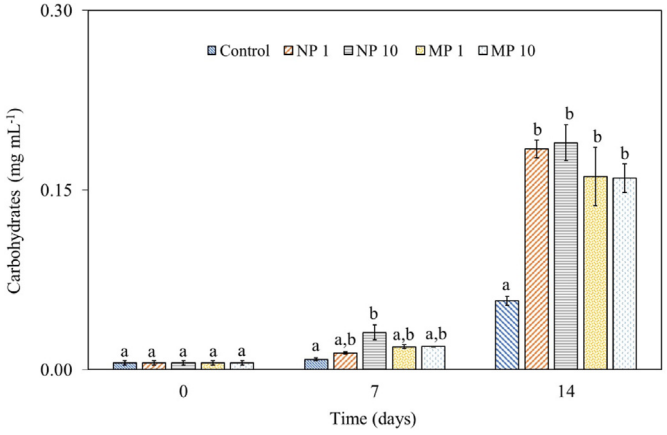

Fig. 3. Carbohydrates production (mg mL^{-1}) of the control and each of the PS nano- and microplastic exposed conditions, throughout the experimental period. Distinct letters represent significantly different means of the correspondent day ($p < 0.05$). NP1: 1 mg L^{-1} nanoplastics; NP10: 10 mg L^{-1} nanoplastics; MP1: 1 mg L^{-1} microplastics; MP10: 10 mg L^{-1} microplastics.

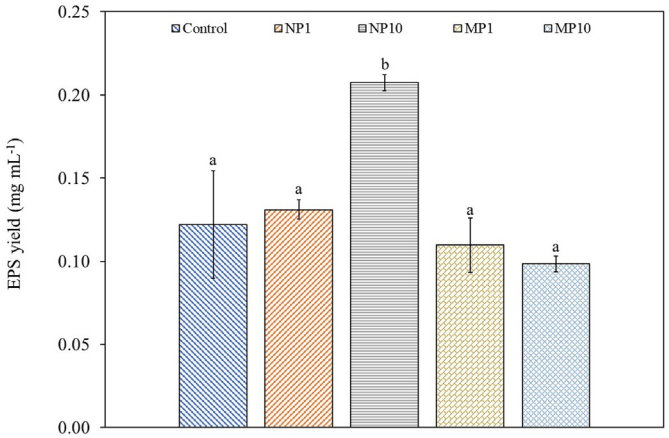

Fig. 5. EPS production (mg mL^{-1}) for the control and each of the PS nano- and microplastic exposed conditions. Distinct letters represent significantly different means of the correspondent day ($p < 0.05$). NP1: 1 mg L^{-1} nanoplastics; NP10: 10 mg L^{-1} nanoplastics; MP1: 1 mg L^{-1} microplastics; MP10: 10 mg L^{-1} microplastics.

of the nano- and microplastic exposed conditions are presented in Fig. 5. The freshwater *Cyanothece* sp. is known to be an EPS producing microalga (Ohki et al., 2014), and to the best of our knowledge, these are the first results regarding the specific EPS production from a freshwater strain of *Cyanothece*. Results from the current study showed that the microalgae in all experimental conditions produced EPS, though a significantly higher EPS production was found for the high nanoplastics concentration treatment (NP10). While handling the cultures, it was also possible to macroscopically infer that the EPS produced by *Cyanothece* sp. were visible, as well as stable and not easy to disaggregate. The EPS yield, size and stability has been shown to positively influence the aggregation capabilities of microalgae (Cunha et al., 2019). According to Mota et al. (2013), the EPS produced by a marine strain of *Cyanothece* has been shown to be composed of mannose, glucose, galactose, xylose, rhamnose, fucose, arabinose, galacturonic acid and glucuronic acid, with galactose and xylose accounting for over 25% of the total molar composition. However, possible strain-related variations in monosaccharidic composition cannot be ruled out. Polymer type-dependent large overexpression of genes involved in the synthesis of xylose and galactose when exposed to microplastics (Lagarde et al., 2016) might help to explain the considerably higher EPS production by the microalgae in study when exposed to high concentrations of PS nanoparticles. This phenomenon suggests a dependence on concentration when determining the variation in gene's expression involved in the EPS biosynthesis pathway. It has been shown that the ratios of sugar monomers play an important role in the cohesive properties of EPS (Zhoul et al., 1998), indicating that higher production of monosaccharides may enhance the ability of microalgae to form hetero-aggregates. Therefore, the higher expression and overproduction of these sugars might directly correlate with higher EPS production, resulting in a higher aggregation potential.

Fig. 6 exhibits the macroscopic photographs of the aggregates formed at the bottom of the culture flasks, throughout the experimental culture period. A comparison between cultures, on the same day, shows that *Cyanothece* sp. displayed similar formation of aggregates across all nano- and microplastic exposed conditions. The evolution of the aggregation process is visible within each condition. The aggregates formed at the bottom of the flasks were considerably large, but since the nano- and microplastics were too small to be detected macroscopically, and to confirm if the aggregates formed on the bottom of the flasks were homo-aggregates (microalga + EPS) or hetero-aggregates (microalga + EPS + PS nano/microplastics), they were further analysis by fluorescence microscopy was performed, with results being shown in Fig. 7.

This technique has also been used to confirm the hetero-aggregation of 2 μm PS microplastic particles by *Chaetoceros neogracile* (Long et al., 2017) and 106–250 μm of PS and poly(methyl methacrylate) (PMMA) microplastic particles by the freshwater microalgae *Microcystis panniformis* and *Scenedesmus* sp. as well as by the marine microalgae *Tetraselmis* sp. and *Gloeocapsa* sp. (Cunha et al., 2019). In the present study, fluorescence micrographs (Fig. 7) confirmed the hetero-nature (microalga + EPS + PS nano/microplastics) of the aggregates collected from the bottom of the culture flasks, revealing nano- and microplastic aggregation. Fig. 7a displays a bright field micrograph of the abundant hetero-aggregates in the NP1 condition, in which both the microalgae and the EPS are visible. Fig. 7b displays a magnified image of the aggregates, revealing the adhesion of the polystryene nanoplastic particles. Regarding the higher nano-PS exposure condition (NP10), Fig. 7c and d displays the bright field micrograph and the corresponding fluorescence micrograph, showing the abundance of aggregates. The micrographs of *Cyanothece* sp. exposed to the lowest concentration of micro-PS (MP1) are shown in Fig. 7e and f. Abundant and sizeable aggregates are found in Fig. 7e, with the exposure under fluorescent I3 filters, in Fig. 7f, confirming the aggregation of the micro-PS particles. In the higher micro-PS exposed conditions (MP10), the hetero-aggregation of the microplastic particles is shown to be vast and abundant, both in individual hetero-aggregates (Fig. 7f and h) and in larger hetero-aggregation networks (Fig. 7i and j).

These results confirm, for the first time, both the hetero-aggregation capabilities of nano- and microplastics and by the

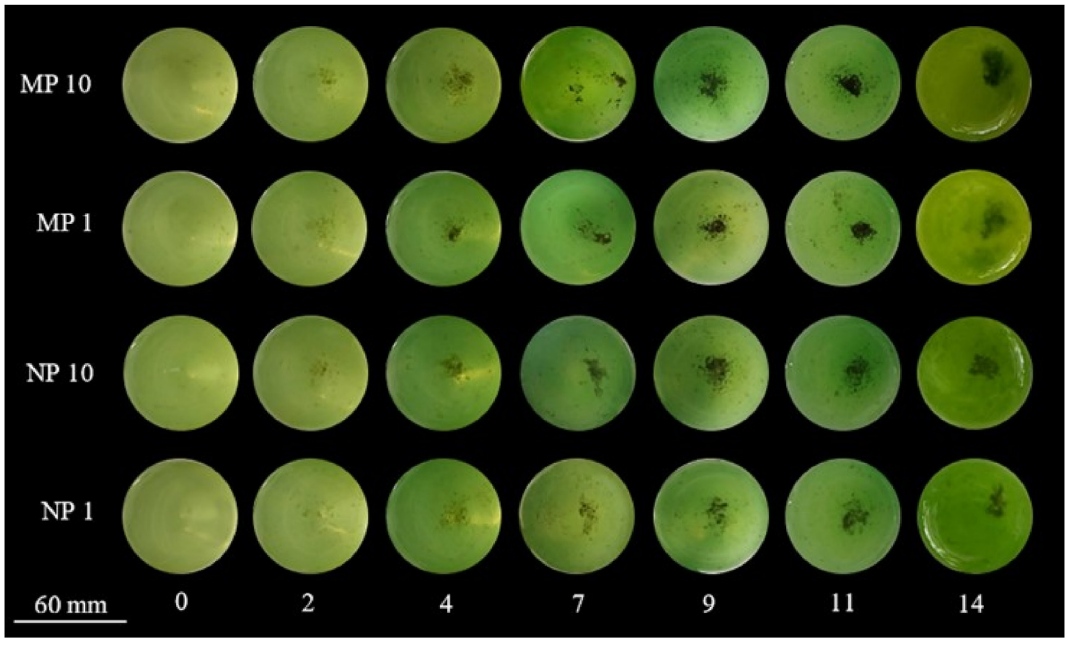

Fig. 6. Photographs of the different nano- and microplastic exposed *Cyanothece* sp. cultures, as observed from below the culture flasks, throughout the experimental period, using a digital camera. NP1: 1 mg L^{-1} nanoplastics; NP10: 10 mg L^{-1} nanoplastics; MP1: 1 mg L^{-1} microplastics; MP10: 10 mg L^{-1} microplastics.

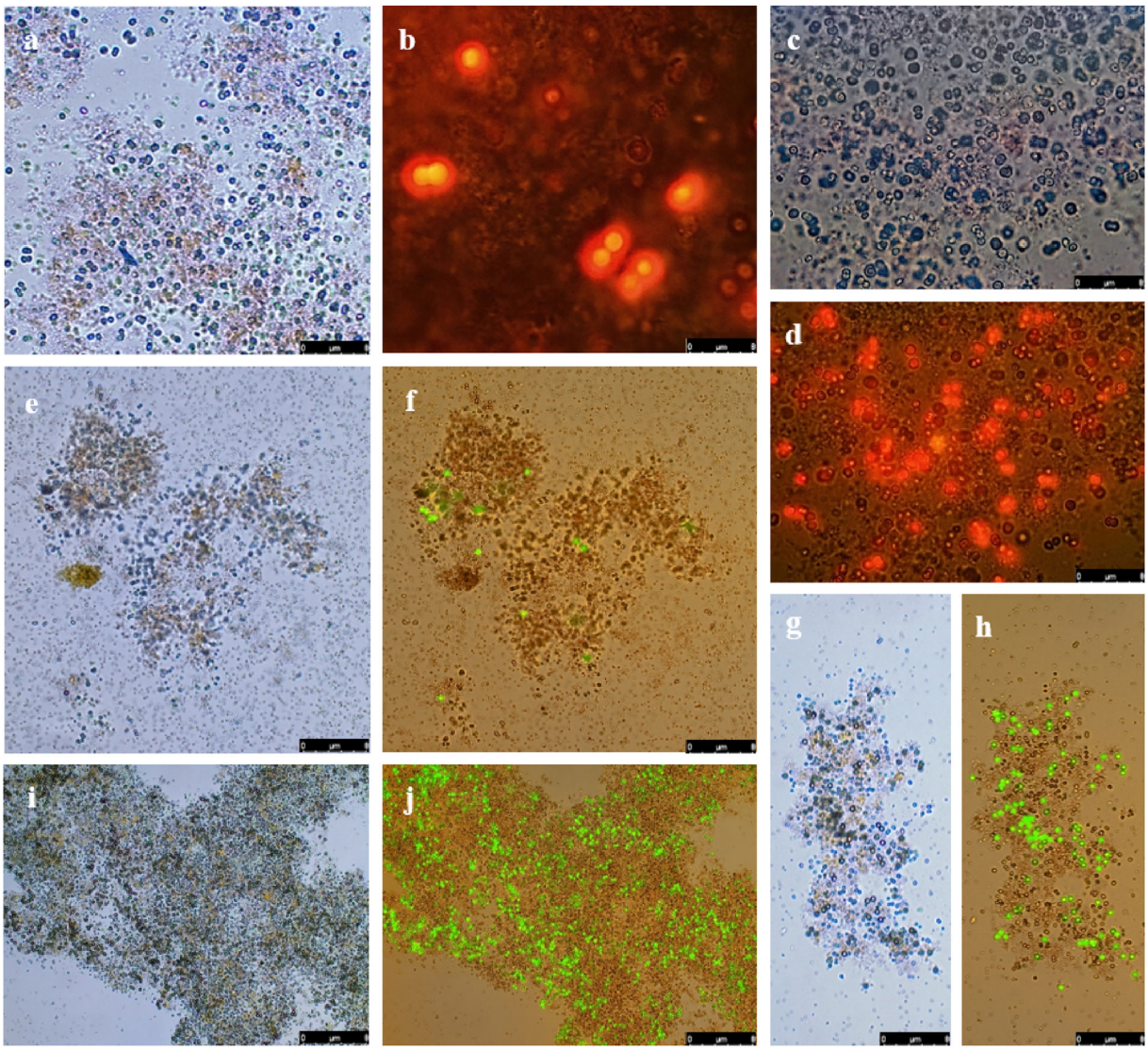

Fig. 7. (a) Bright field micrograph of the NP1 condition (low nanoplastics concentration: 1 mg L^{-1}) *Cyanothece* sp. hetero-aggregates (x20). (b) Amplified micrograph seen in (a), but under I3 filters (excitation 450–490 nm; emission 515 nm) (x100), making visible the presence of the orange fluorescent PS nanoplastics in the hetero-aggregates. (c) Bright field micrograph of the NP10 condition (high nanoplastics concentration: 10 mg L^{-1}), displaying aggregates of cells and EPS (x40). (d) Same micrograph observed in (c) but under I3 filters, showing the abundant nanoplastics aggregation (x40). (e) Bright field micrograph of the MP1 condition (low microplastics concentration: 1 mg L^{-1}) hetero-aggregates of *Cyanothece* sp. (x10). (f) Same micrograph taken in (e) under I3 filters, exhibiting aggregation of the PS microplastics (fluorescent green). (g) Bright field micrograph of an MP10 condition (high microplastics concentration: 10 mg L^{-1}) considerably sized hetero-aggregate (x10). (h) Same micrograph seen in (g), but under I3 filters, showing hetero-aggregates composed of microalga, EPS and PS microplastics (fluorescent green) (x10). (i) Bright field micrograph of *Cyanothece* sp. abundant aggregation (high microplastics concentration) (x10). (j) Micrograph seen in (i) under I3 filters, revealing vast hetero-aggregation of the PS microplastics. Scale 0–8 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

freshwater *Cyanothece* sp. EPS. Aside from the incorporation of nano- and microplastics in the aggregates, microalgae have also shown the potential to colonize, adsorb and adhere these particles (1–400 μm), which was confirmed using scanning electron microscopy (Zhang et al., 2016; Lagarde et al., 2016; Cunha et al., 2019). Fig. 6 shows that the EPS produced by *Cyanothece* sp. ranged from 40 to 80 μm, limiting the aggregate potential to particles under the size of the EPS produced. In terms of the issue regarding microplastic pollution in WWTPs addressed in the introduction, these results are remarkable since it has been suggested that conventional mechanical, chemical and biological processes in wastewater treatment plants are able to remove 99.9% of the microplastics >300 μm, but display low retention for microplastics <300 μm (Storck et al., 2015). Also, aggregate size is known to influence the stickiness of the EPS (Passow, 2002; Long et al., 2015), increasing the likelihood of collisions between microalgae/EPS and nano- and microplastics (Engel, 2000), consequently increasing the hetero-aggregation potential. The nano/microplastics aggregation quantification was not performed due to aggregate sampling that removes most of the particles from the culture, effectively altering it. Nonetheless, since it has been shown that EPS are able to aggregate morphologically irregular microplastics within a wide spectrum of sizes (Cunha et al., 2019), further studies should focus on confirming the EPS aggregation potential of fibers, since these are widely found when analysing microplastic profiles in WWTPs (Estahbanati & Fahrenfeld, 2016; Murphy et al., 2016; Lares et al., 2018) and, as a consequence, in marine environments (Cesa et al., 2017; Graca et al., 2017; Tsang et al., 2017).

3.5. Call for research

Regarding the growing environmental and health-related concerns due to microplastic pollution, it is expected that new data will emerge in upcoming years concerning the harmful effects of microplastic exposure on humans. As the population evolves to have an ever more demanding need for biological alternatives to fossil fuels and harmful synthetic products, microalgae represent a very versatile and viable alternative to many of the problems we face today. The microalgal-based EPS are biodegradable, heavy metal adsorbents and sources of carbon substrate. Also, they display excellent bioflocculant properties along with nano- and microplastic aggregation capabilities, these being key properties for biopolymer application. All these characteristics make for a possible solution to many of the wastewater treatment steps, giving EPS the potential to play various roles in the process. Further research should focus on investigating more microalgal-based biopolymers. There is a need to assess the most optimized set of methodologies to obtain the EPS with the carbohydrate/protein ratio that would improve the bioflocculant activity, while maintaining all its anionic characteristics. Also, the surface charges of both the EPS and the particles being flocculated play an important role in the aggregation and consequent flocculation, as it has been reported by Bezawada et al. (2013). Since the surface charges were not evaluated here, further studies would be needed to understand the importance of charge neutralization as a possible mechanism of nano- and microplastic aggregation. The study of hydrophobic and hydrophilic interactions should also be taken into account when designing further studies. Another crucial aspect is the need to optimize culture conditions and possible symbiosis with other microorganisms towards enhancing biopolymer cost-production efficiency for future industrial use, with the main focus being its bioflocculant capacity and nano- and microplastic aggregation potential. Finally, and based on the results of this pilot study, further well-structured and critical studies should aim to report the EPS interactions with the complex matrices of wastewater samples, with a clear emphasis on stratifying and characterizing the hetero-aggregates based on polymer type and morphology.

4. Conclusions

The aim of the current study was to evaluate the ability of the extracellular polymeric substances (EPS) produced by *Cyanothece* sp. to aggregate nano- and microplastics, as well as the influence of the exposure to these plastic particles on the production of extracellular carbohydrates and EPS. The results showed that polystyrene nano- and microplastics (at 1 and 10 mg L^{-1}) had a significant negative impact on the growth of this freshwater microalga. The production of extracellular carbohydrates was shown to be largely enhanced in the presence of nano- and microplastics. Also, EPS production was significantly higher when exposed to 10 mg L^{-1} of PS nanoplastics, likely acting as a self-protecting mechanism, due to selective overexpression of genes involved in the EPS biosynthesis pathway. *Cyanothece* sp. produces EPS with high bioflocculant activity in relation to the low concentration tested, which is suitable for nano- and microplastics aggregation, displaying hetero-aggregation potential at 1 and 10 mg L^{-1} of both nano- and microplastics. The results of this investigation highlight the promising potential for microalgal-based biopolymers to replace the hazardous bioflocculants used in wastewater treatment, in addition to the ability to aggregate the <300 μm microplastics fraction that conventional removal methods in wastewater treatment are unable to remove. Thus, the results in this work show the potential for microalgal-based biopolymers to be further explored as a possible bio-compelling and -compatible solution to nano- and microplastic pollution in wastewater treatment plants.

Declaration of competing interest

The authors have no conflict of interest to declare.

CRediT authorship contribution statement

César Cunha: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Laura Silva:** Data curation. **Jorge Paulo:** Data curation. **Marisa Faria:** Validation, Writing - review & editing. **Natacha Nogueira:** Validation, Writing - review & editing. **Nereida Cordeiro:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing - review & editing.

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References

Aguilera, A., Egipsy, V., Úriz, P., Amils, R., 2008. Extraction of extracellular polymeric substances from extreme acidic microbial biofilms. Appl. Microbiol. Biotechnol. 78 (6), 1079–1088. https://doi.org/10.1177/030098588702400107.

Albassam, M., Moore, J., Sharma, A., 1987. Ultrastructural and clinicopathological studies on the toxicity of cationic acrylamide-based flocculant to rainbow trout. Veterinary Pathol. 24 (1), 34–43. https://doi.org/10.1177/030098588702400107.

Andrady, A., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Andrady, A., 2017. The plastic in microplastics: a review. Mar. Pollut. Bull. 119 (1), 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082.

Besseling, E., Wang, B., Lürling, M., Koelmans, A., 2014. Nanoplastic affects growth of S. Obliquus and reproduction of *D. Magna*. Environ. Sci. Technol. 48 (20), 12336–12343. https://doi.org/10.1021/es503001d.

Bezawada, J., Hoang, N., More, T., Yan, S., Tyagi, N., Surampalli, R., 2013. Production of extracellular polymeric substances (EPS) by *Serratia* sp.1 using wastewater sludge as raw material and flocculation activity of the EPS produced. J. Environ. Manag. 128, 83–91. https://doi.org/10.1016/j.jenvman.2013.04.039.

Buczek, S., Cope, W., MacLaughlin, R., Kwak, T., 2017. Acute toxicity of polyacrylamide flocculants to early life stages of freshwater mussels. Environ. Toxicol. Chem. 36 (10), 2715–2721. https://doi.org/10.1002/etc.3821.

Cesa, F., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. Sci. Total Environ. 598, 1116–1129. https://doi.org/10.1016/j.scitotenv.2017.04.172.

Chae, Y., An, Y., 2017. Effects of micro- and nanoplastics on aquatic ecosystems: current research trends and perspectives. Mar. Pollut. Bull. 124 (2), 624–632. https://doi.org/10.1016/j.marpolbul.2017.01.070.

Chae, Y., Kim, D., Kim, S., An, Y., 2018. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. Sci. Rep. 284 (8), 1–11. https://doi.org/10.1038/s41598-017-18849-y.

Chen, Z., Li, Z., Liu, P., Liu, Y., Wang, Y., Li, Q., He, N., 2017. Characterization of a novel bioflocculant from a marine bacterium and its application in dye wastewater treatment. BMC Biotechnol. 17 (1), 84. https://doi.org/10.1186/s12896-017-0404-z.

Cole, M., Lindeque, P., Halsband, C., Galloway, T., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Cunha, C., Faria, M., Nogueira, N., Ferreira, A., Cordeiro, N., 2019. Marine vs freshwater microalgae extracellular polymeric substance as biosolutions to microplastics pollution. Environ. Pollut. 249, 372–380. https://doi.org/10.1016/j.envpol.2019.03.046, 0.

Davarpanah, E., Guilhermino, L., 2015. Single and combined effects of microplastics and copper on the population growth of the marine microalgae *Tetraselmis chuii*. Estuar. Coast Shelf Sci. 167 (A), 269–275. https://doi.org/10.1016/j.ecss.2015.07.023.

Decho, A.W., Gutierrez, T., 2017. Microbial extracellular polymeric substances (EPSs) in ocean systems. Front. Microbiol. 8, 922. https://doi.org/10.3389/

fmicb.2017.00922.
Decho, A., 2000. Microbial biofilms in intertidal systems: an overview. Continent. Shelf Res. 20 (10–11), 1257–1273. https://doi.org/10.1016/S0278-4343(00)00022-4.
Deng, S., Bai, R., Hu, X., Luo, Q., 2003. Characteristics of a bioflocculant produced by Bacillus mucilaginosus and its use in starch wastewater treatment. Appl. Microbiol. Biotechnol. 60 (5), 588–593. https://doi.org/10.1007/s00253-002-1159-5.
Deng, S., Yu, G., Ting, Y., 2005. Production of a bioflocculant by *Aspergillus parasiticus* and its application in dye removal. Colloids Surf. B Biointerfaces 44 (4), 179–186. https://doi.org/10.1016/j.colsurfb.2005.06.011.
Dubey, S., Mehra, S., Tiwari, P., Bishwas, A., 2011. Potential use of cyanobacterial species in bioremediation of industrial effluents. Afr. J. Biotechnol. 10 (7), 1125–1132. https://doi.org/10.5897/AJB10.908.
DuBois, M., Gilles, K., Hamilton, J., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 (3), 350–365. https://doi.org/10.1021/ac60111a017.
Eerkes-Medrano, D., Thompson, R., Aldridge, D., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritization of research needs. Water Res. 75, 63–82. https://doi.org/10.1016/j.watres.2015.02.012, 0.
Engel, A., 2000. The role of transparent exopolymer particles (TEP) in the increase in apparent particle stickiness (α) during the decline of a diatom bloom. J. Plankton Res. 22 (3), 485–497. https://doi.org/10.1093/plankt/22.3.485.
Estahbanati, S., Fahrenfeld, N., 2016. Influence of wastewater treatment plant discharges on microplastic concentrations in surface water. Chemosphere 162, 277–284. https://doi.org/10.1016/j.chemosphere.2016.07.083.
Ge, S., Champagne, P., 2016. Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. Water Res. 88, 604–612. https://doi.org/10.1016/j.watres.2015.10.054.
GESAMP, 2016. In: Rochman, C.M. (Ed.), Sources, Fate and Effects of Microplastics in the Marine Environment: Part Two of a Global Assessment, vol. 93.
Geyer, R., Jambeck, J., Law, K., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3 (7), e1700782 https://doi.org/10.1126/sciadv.1700782.
Graca, B., Szewc, K., Zakrzewska, D., Dolega, A., Szczerbowska-Boruchowska, M., 2017. Sources and fate of microplastics in marine and beach sediments of the Southern Baltic Sea - a preliminary study. Environ. Sci. Pollut. Control Ser. 24 (8), 7650–7661. https://doi.org/10.1007/s11356-017-8419-5.
He, J., Zou, J., Shao, Z., Zhang, J., Liu, Z., Yu, Z., 2010. Characteristics and flocculating mechanism of a novel bioflocculant HBF-3 produced by deep-sea bacterium mutant Halomonas sp. V3a'. World J. Microbiol. Biotechnol. 26 (6), 1135–1141. https://doi.org/10.1007/s11274-009-0281-2.
Kaplan, D., 2013. Absorption and adsorption of heavy metals by microalgae. Handb. Microalgal Cult. 602–611. https://doi.org/10.1002/9781118567166.ch32.
Kavita, K., Singh, V., Mishra, A., Jha, B., 2014. Characterisation and anti-biofilm activity of extracellular polymeric substances from *Oceanobacillus iheyensis*. Carbohydr. Polym. 101, 29–35. https://doi.org/10.1016/j.carbpol.2013.08.099, 0.
Kuboi, T., Fuji, K., 1983. Toxicity of cationic polymer flocculants to higher plants. Soil Sci. Plant Nutr. 30 (3), 311–320. https://doi.org/10.1080/00380768.1984.10434697.
Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., Caruso, A., 2016. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. 215, 331–339. https://doi.org/10.1016/j.envpol.2016.05.006, 0.
Lares, M., Ncibi, M., Sillanpää, M., 2018. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res. 133, 236–246. https://doi.org/10.1016/j.watres.2018.01.049.
Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. 175 (20), 39–46. https://doi.org/10.1016/j.marchem.2015.04.003.
Long, M., Paul-Pont, I., Hégaret, H., Moriceau, B., Lamber, C., Huvet, A., Soudant, P., 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. 228, 454–463. https://doi.org/10.1016/j.envpol.2017.05.047, 0.
Lotti, T., Carretti, E., Berti, D., Montis, C., Buffa, S., Lubello, C., Feng, C., Malpei, F., 2019. Hydrogels formed by anammox extracellular polymeric substances: structural and mechanical insights. Sci. Rep. 9, 11633. https://doi.org/10.1038/s41598-019-47987-8.
Makene, V., Tijano, J., Massima, E., Petrik, L., Pool, E., 2019. Toxicity study of a textile effluent treated with electrohydraulic discharge and coagulant/flocculants. Water, air, Soil Pollut. 230, 167. https://doi.org/10.1007/s11270-019-4212-z.
Mao, Y., Ai, H., Chen, Y., Zhang, Z., Zeng, P., Kang, L., Li, W., Gu, W., He, Q., Li, H., 2018. Phytoplankton response to polystyrene microplastics: perspective from an entire growth period. Chemosphere 208, 59–68. https://doi.org/10.1016/j.chemosphere.2018.05.170.
Mattsson, K., Jocic, S., Doverbratt, I., Hansson, L., 2018. Nanoplastics in the aquatic environment. Microplastic Contam. Aquat. Environ. 13, 377–399. https://doi.org/10.1016/B978-0-12-813747-5.00013-8, 0.
Mintenig, S., Int-Veen, I., Löder, M., Primpke, S., Gerdts, G., 2016. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res. 108, 365–372. https://doi.org/10.1016/j.watres.2016.11.015, 0.
Mintenig, S., Löder, M., Primpke, S., 2019. Low numbers of microplastics detected in drinking water from ground water sources. Sci. Total Environ. 648, 631–635. https://doi.org/10.1016/j.scitotenv.2018.08.178, 0.
Mohammed, J., Dagang, W., 2019. Role of cationization in bioflocculant efficiency: a review. Environ. Process. 6 (2), 355–376. https://doi.org/10.1007/s40710-019-00372-z.
Mota, R., Guimarães, R., Büttel, Z., Rossi, F., Colica, G., Silva, C., Santos, C., Gales, L., Zille, A., Philippis, R., Pereira, S., Tamagnini, P., 2013. Production and characterization of extracellular carbohydrate polymer from *Cyanothece* sp. CCY 0110. Carbohydr. Polym. 92 (2), 1408–1415. https://doi.org/10.1016/j.carbpol.2012.10.070.
Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50 (11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416.
Nouha, K., Kumar, R., Balasubramanian, S., Tyagi, R., 2018. Critical review of EPS production, synthesis and composition for sludge flocculation. J. Environ. Sci. (China) 66, 225–245. https://doi.org/10.1016/j.jes.2017.05.020.
Nwodo, U., Okoh, A., 2012. Characterization and flocculation properties of biopolymeric flocculant (glycosaminoglycan) produced by *Cellulomonas* sp. Okoh. J. Appl. Microbiol. 114 (5), 1325–1337. https://doi.org/10.1111/jam.12095.
Ohki, K., Le, N., Yoshikawa, S., Kanesaki, Y., Okajima, M., Kaneko, T., Thi, T., 2014. Extracellular polymeric substance production by a unicellular freshwater cyanobacterium *Cyanothece* sp. isolated from a rice field in Vietnam. J. Appl. Phycol. 26 (1), 265–272. https://doi.org/10.1007/s10811-013-0094-4.v.
Okaiyeto, K., Nwodo, U., Mabinya, L., Okoh, A., 2013. Characterization of a bioflocculant produced by a consortium of *Halomonas* sp. Okoh and *Micrococcus* sp. Leo. Int. J. Environ. Res. Publ. Health 10 (10), 5097–5110. https://doi.org/10.3390/ijerph10105097.
Passow, U., 2002. Transparent exopolymer particles (TEP) in aquatic environments. Prog. Oceanogr. 55 (3–4), 287–333. https://doi.org/10.1016/S0079-6611(02)00138-6.
Patil, S., Salunkhe, R., Patil, C., Patil, D., Salunke, B., 2010. Bioflocculant extracellular polymeric substance production by *Azotobacter indicus* using flower extract of *Madhuca latifolia* L. Appl. Biochem. Biotechnol. 162 (4), 1095–1098. https://doi.org/10.1007/s12010-009-8820-8.
Paul-Pont, I., Tallec, K., Gonzalez-Fernandez, C., Lambert, C., Vincent, D., Mazurais, D., Zambonino-Infante, J., Brotons, G., Lagarde, F., Fabioux, C., Soudant, F., Huvet, A., 2018. Constraints and priorities for conducting experimental exposures of marine organisms to microplastics. Front. Mar. Sci. 5, 1–22. https://doi.org/10.3389/fmars.2018.00252.
Pereira, S., Micheletti, E., Zille, A., Santos, A., Ferreira, P., Tamagnini, P., Philippis, R., 2011. Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell? Microbiology 157, 451–458. https://doi.org/10.1099/mic.0.041038-0.
Philippis, R., Vincenzini, M., 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 22 (3), 151–175. https://doi.org/10.1016/S0168-6445(98)00012-6.
Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T., Janda, V., 2018. Occurrence of microplastics in raw and treated drinking water. Sci. Total Environ. 643, 1644–1651. https://doi.org/10.1016/j.scitotenv.2018.08.102.
Prasertsan, P., Dermlim, W., Doelle, H., Kennedy, J., 2006. Screening, characterization and flocculating property of carbohydrate polymer from newly isolated *Enterobacter cloacae* WD7. Carbohydr. Polym. 66 (3), 289–297. https://doi.org/10.1016/j.carbpol.2006.03.011.
Reddy, K., Haskell, B., Sherman, D., Sherman, L., 1993. Unicellular, aerobic nitrogen-fixing cyanobacteria of the genus *Cyanothece*. J. Bacteriol. 175 (5), 1284–1292. https://doi.org/10.1128/jb.175.5.1284-1292.1993.
Sanayei, Y., Ismail, N., Teng, T.T., Morad, N., 2010. Studies on flocculating activity of bioflocculant from closed drainage. Int. J. Chem. 2, 168–173. https://doi.org/10.5539/ijc.v2n1p168.
Shah, V., Ray, A., Garg, N., Madamwar, D., 2000. Characterization of the extracellular polysaccharide produced by a marine cyanobacterium, *Cyanothece* sp. ATCC 51142, and its exploitation toward metal removal from Solutions. Curr. Microbiol. 40 (4), 274–278. https://doi.org/10.1007/s002849910054.
Sheng, G., Yu, H., Li, X., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol. Adv. 28 (6), 882–894. https://doi.org/10.1016/j.biotechadv.2010.08.001.
Sjollema, S., Redondo-Hasselerharm, P., Leslie, H., Kraak, M., Vethaak, A., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquat. Toxicol. 170, 259–261. https://doi.org/10.1016/j.aquatox.2015.12.002.
Storck, F., Kools, S., Rinck-Pfeiffer, S., 2015. Microplastics in fresh water resources. Sci. Brief Glob. Water Res. Coalition. http://www.globalwaterresearchcoalition.net/reports/?q=7qQNYQHoLKpSCi7elXaDJSox8MC27CBI&c=10.
Sun, J., Dai, X., Wang, Q., Loosdrecht, M., Ni, B., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37. https://doi.org/10.1016/j.watres.2018.12.050.
Takigami, H., Taniguchi, N., Shimizu, Y., Matsui, S., 1998. Toxicity assays and their evaluation on organic polymer flocculants used for municipal sludge dewatering. Water Sci. Technol. 38 (7), 207–215. https://doi.org/10.1016/S0273-1223(98)00622-2.
Tang, W., Song, L., Li, D., Qiao, J., Zhao, T., Zhao, H., 2014. Production, characterization, and flocculation mechanism of cation independent, pH tolerant, and thermally stable bioflocculant from Enterobacter sp. ETH-2. PloS One 9 (12), e114591. https://doi.org/10.1371/journal.pone.0114591.
Tsang, Y., Mak, C., Liebich, C., Lam, S., Sze, E., Chan, K., 2017. Microplastic pollution in

the marine waters and sediments of Hong Kong. Mar. Pollut. Bull. 115 (1–2), 20–28. https://doi.org/10.1016/j.marpolbul.2016.11.003.
Tuinier, R., Zoon, P., Stuart, M., Fleer, G., Kruif, C., 1999. Transparent exopolymer particles (TEP) in aquatic environments. Prog. Oceanogr. 55 (3–4), 287–333. https://doi.org/10.1016/S0079-6611(02)00138-6.
Wang, J., Li, Q., Li, M., Chen, T., Zhou, Y., Yue, Z., 2014. Competitive adsorption of heavy metal by extracellular polymeric substances (EPS) extracted from sulfate reducing bacteria. Bioresour. Technol. 163, 374–376. https://doi.org/10.1016/j.biortech.2014.04.073.
Wezel, A., Caris, I., Kools, S., 2015. Release of primary microplastics from consumer products to wastewater in The Netherlands. Environ. Toxicol. Chem. 35 (7), 1627–1631. https://doi.org/10.1002/etc.3316.
Wright, S., Thompson, R., Galloway, T., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.
Xiong, J., Kurade, M., Jeon, B., 2018. Can microalgae remove pharmaceutical contaminants from water? Trends Biotechnol. 36 (1), 30–44. https://doi.org/10.1016/j.tibtech.2017.09.003.
Xiong, Y., Wang, Y., Yu, Y., Li, Q., Wang, H., Chen, R., He, N., 2010. Production and characterization of a novel bioflocculant from Bacillus licheniformis. Appl. Environ. Microbiol. 76 (9), 2778–2782. https://doi.org/10.1128/AEM.02558-09.
Zhang, C., Chen, X., Wang, J., Tan, L., 2016. Toxic effects of microplastic on marine microalgae Skeletonema costatum: interactions between microplastic and algae. Environ. Pollut. 220 (B), 1282–1288. https://doi.org/10.1016/j.envpol.2016.11.005.
Zhoul, J., Mopper, K., Passow, U., 1998. The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. Limnol. Oceanogr. 43 (8), 1860–1871. https://doi.org/10.4319/lo.1998.43.8.1860.