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Interaction of Cyanobacteria with Nanometer and Micron Sized Polystyrene Particles in Marine and Fresh Water

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ABSTRACT: Microplastics and nanoplastics are emerging pollutants, widespread both in marine and in freshwater environments. Cyanobacteria are also ubiquitous in water and play a vital role in natural ecosystems, using photosynthesis to produce oxygen. Using photography, fluorescence microscopy and cryogenic and scanning electron microscopy (cryo-SEM, SEM) we investigated the physicochemical response of one of the most predominant seawater cyanobacteria (*Synechococcus elongatus*, PCC 7002) and freshwater cyanobacteria (*S. elongatus* Nageli PCC 7942) when exposed to 10 μm diameter polystyrene (microPS) and 100 nm diameter polystyrene (nanoPS) particles. Marine and freshwater cyanobacteria formed aggregates with the nanoPS, bound together by extracellular polymeric substances (EPS), and these aggregates sedimented. The aggregates were larger, and the sedimentation was more rapid for the marine system. Aggregate morphologies were qualitatively different for the microPS samples, with the bacteria linking up a small number of particles, all held together by EPS. There was no sedimentation in these samples. The cyanobacteria remained alive after exposure to the particles. The particle size- and salt concentration-dependent response of cyanobacteria to these anthropogenic stressors is an important factor to consider for a proper understanding of the fate of the particles as well as the bacteria.
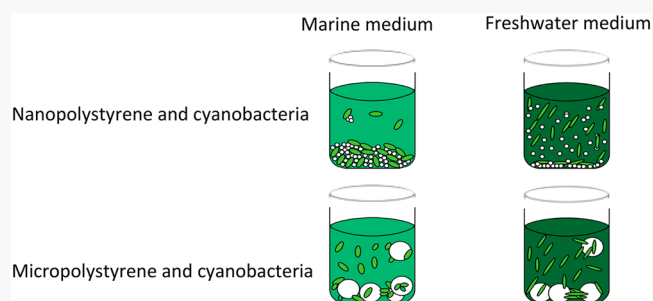
INTRODUCTION

About 200 million tons of plastic are in the world's oceans currently,[1] and around 8 million additional tons are added each year.[2] Land-based sources that bring plastic pieces to both ocean and fresh waters include stormwater runoff, visitors to beaches and lakes, industrial sources, construction activities, personal care products, and illegal dumping.[3−5] The fishing industry and aquaculture are one of the main ocean-based sources of plastic.[6] Ultraviolet (UV) radiation and wind action eventually break down large pieces into either microplastic (size between 0.1 μm to 5 mm) or nanoplastic (size less than 0.1 μm) particles.[4,7] Degradation of most plastics is extremely slow,[8] and deleterious effects caused by ingestion of these particles by ocean species are now well documented.[9,10]

The interaction of microplastics and microorganisms remains an open area of study. Particularly, there is a lot of interest in understanding the interaction of microplastics and cyanobacteria (CB), also known as blue-green algae, due to its importance to the earth's primary production of oxygen.[11−13] Oberbeckmann et al.[14] and Kaiser et al.[15] found that cyanobacteria represent the majority of the attached microbial communities on a variety of plastics types in the ocean. These surfaces seem to offer stability and protection to prokaryotes,[14] making it an attractive place for microorganisms to colonize.

Poly(methyl methacrylate) and polystyrene microparticles in sizes varying from 106 μm to 250 μm and from 106 μm to lower were exposed to two freshwater (*Microcystis panniformis* and *Scenedesmus sp.*) and two marine (*Tetraselmis sp.* and *Gleocapsa sp.*) cyanobacteria by Cunha et al.[16] They showed that aggregation relied on the production of extracellular polymeric substances (EPS), and therefore the interaction between cyanobacteria and microplastics was species specific. Bacterial EPS are macromolecules excreted in response to physiological changes because of environmental factors;[17,18] these EPS have a variety of functions[19,20] such as cell adhesion to surfaces,[19,21,22] cell aggregation,[18] and cell protection.[19]

In addition, Frère et al.[23] showed by collecting and analyzing microplastics from the Bay of Brest that the bacterial assemblages vary with polymer type. They found that polystyrene presents distinct bacterial communities compared to polypropylene and polyethylene samples. Lagarde et al.[24]
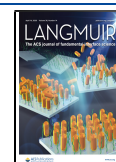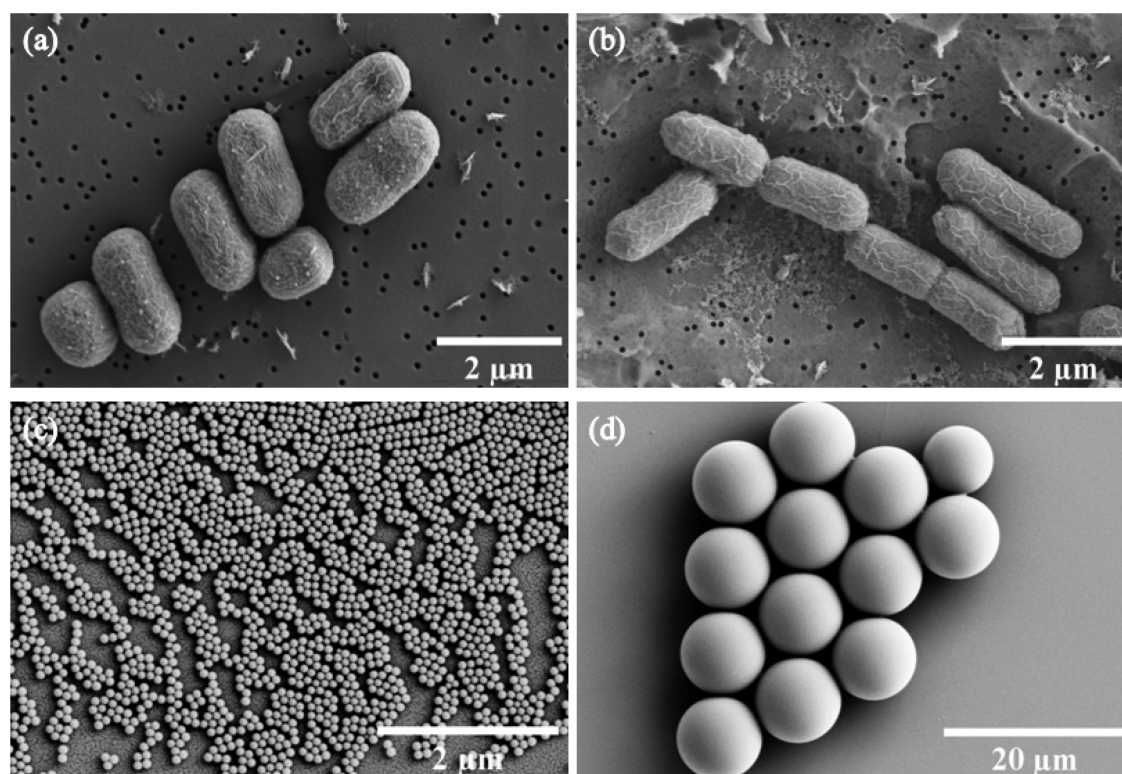
Figure 1. SEM images of (a) marine CB, (b) freshwater CB, (c) nanoPS, and (d) microPS.

also found differences between two polymers (polypropylene and polyethylene) regarding freshwater cyanobacteria colonization (*Chlamydomas reinhardtii*). Consequently, the interaction between cyanobacteria and microplastics depends on the bacteria and microplastic type.

The marine *Synechoccocus* cyanobacterium is one of the most predominant bacterial genus in the ocean environment.[13] Similarly, freshwater *Synechoccocus* is one of the most common genus in lakes around the world.[12,25] Given the ubiquitous presence and importance of these cyanobacteria, it is important to understand the impact of the micro and nanoplastics on these algae.

The focus of this work is the physicochemical aspects of cyanobacteria interacting with micro- and nanopolystyrene particles, an important first step in the response of these microorganisms to the presence of these particles. Polystyrene is chosen because it is one of the most commonly found, and refractory, polymers in ocean and fresh water.[26−29] We use photography, fluorescence microscopy, and cryogenic and scanning electron microscopy (cryo-SEM, SEM) to follow the formation of CB−particle aggregates and their sedimentation.

■ MATERIALS AND METHODS

Materials. *Synechococcus elongatus* Nageli PCC 7942 cyanobacteria (freshwater CB) and *Synechococcus sp.* PCC 7002 cyanobacteria (marine CB) were obtained from ATCC (American Type Culture Collection). BG-11 and A+ media were obtained from UTEX Culture Collection of algae. A 2.5 wt % suspension of monodispersed polystyrene spheres with diameters of 0.1 μm (nanoPS) and 10 μm (microPS) were obtained from Sigma-Aldrich. Glutaraldehyde (3% v/v) and 0.1 M phosphate buffer were purchased from Electron Microscope Sciences. Hexamethyldisilazane (HDMS), and Sytox Green Nucleic Acid Stain (5 mM solution in DMSO) were obtained from ThermoFisher. Bioreactors (500 mL) were obtained from

Grofizz. Deionized (DI) water was obtained from a Millipore Milli Q system.

Sample Preparation. NanoPS and microPS suspensions were centrifuged at 18 500 g for 45 min (microPS) and 4 h (nanoPS), the supernatant was removed, and the resulting pellet resuspended in DI water. This process was repeated twice. Centrifugation and washing removed the bactericide sodium azide and other preservatives from the stock suspensions.

Freshwater CB and marine CB were grown for 3 weeks in 100 mL of BG-11 and A+ medium, respectively, inside bioreactors with air bubbling. Both strains were cultured at 30 °C and exposed to 12 h/12 h light/dark periods in a Controlled Low Temperature Illuminated Incubator (Model 818, Fisher Scientific). Prior to exposure to polystyrene particles, the viability of the CB was verified live/dead assay using the Sytox probe and fluorescence microscopy.[30] A range of particle loadings, from 0.0004 to 0.005 g/mL, has been reported for natural water.[31,32] The CB samples in our experiments were exposed to a particle loading of 0.005 g/mL. The control experiments consisted of monitoring CB without exposure to particles and monitoring nanoPS and microPS in growth media without any CB. After addition of the CB to the particle suspension, the samples were placed on a Scilogex SK-O180-E Orbital shaker rotating at 100 rpm at 30 °C for the duration of exposure.

■ SAMPLE CHARACTERIZATION

Settling. A 2.5 cm long quartz cuvette was loaded with CB, particles, or mixtures of CB with particles in both fresh and marine water. The transient optical density (OD) was monitored for 6.5 h, with readings taken every 15 min (Jasco, Tokyo, Japan). The beam slit was 1 cm high, and its midpoint was located 1 cm from the bottom of the cuvette. Optical densities were normalized by the values at the beginning of each experiment. Sample vials were imaged at the end of 6.5 h.

Scanning Electron Microscopy. The SEM and cryo-SEM analysis were performed on a Zeiss Sigma VP field emission SEM equipped with a cryogenic chamber and cold stage (Gatan Alto 2500). Regular SEM was used to observe CB morphology and study
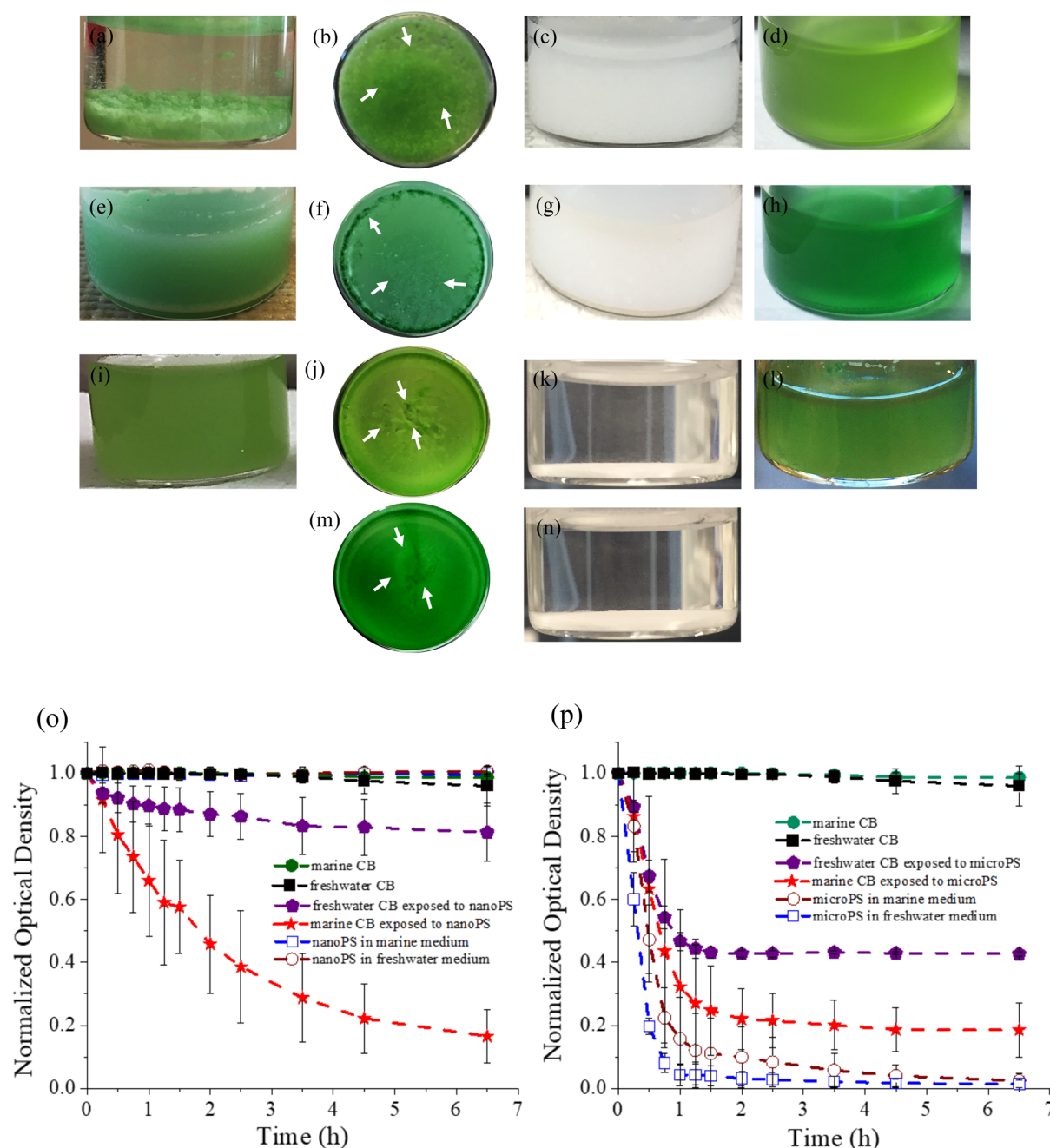
Figure 2. Images of vials after 6.5 h of exposure, and optical density (OD) data. The vials have a diameter of 2.8 cm: (a) nanoPS exposed to marine CB; (b) bottom of the vial showing aggregates of marine CB nanoPS; (c) nanoPS in marine medium; (d) marine CB in marine medium; (e) nanoPS exposed to freshwater CB; (f) bottom of the vial showing aggregates of freshwater CB nanoPS; (g) nanoPS in freshwater medium; (h) freshwater CB in a freshwater medium; (i) microPS exposed to marine CB; (j) bottom of the vial showing aggregates of marine CB microPS (marked by arrows); (k) microPS in marine medium; (l) microPS exposed to freshwater CB; (m) bottom of the vial showing aggregates of freshwater CB microPS (marked by arrows); (n) microPS in freshwater medium; (o) normalized OD versus time for nanoPS samples; (p) normalized OD versus time for microPS samples.

the marine CB-microplastic aggregates. A 300 μL aliquot of the CB suspension (with or without microplastics) was filtered through a 0.08 μm filter (Whatman, Nucleopore Hydrophilic Membrane). The cells on the filter were fixed with 3% v/v glutaraldehyde for 2 h at 4 °C. The filter specimen was washed twice with a 0.1 M phosphate buffer solution. The specimen was then dehydrated for 15 min during each step through a graded ethanol series of 50% v/v, 70% v/v, 90% v/v, and twice with 100% v/v ethanol. The filter was submerged in HDMS and air-dried.[33−35] The specimen was then sputtered with gold on a Cressington 105 sputter coater and imaged on the SEM at room temperature.

Cryo-SEM was used to observe the freshwater CB−microplastic aggregates in their native, hydrated state. Three microliters of each suspension were pipetted on a cryo-SEM stub and frozen in a liquid nitrogen slush. The frozen specimens were transferred under vacuum to the prechamber at −130 °C, where the samples were fractured using a cold flat-edge knife and sublimed for 3 min at −100 °C to enhance surface topology. After the temperature was lowered back to −130 °C, the specimens were sputtered for 60 s with gold. The specimens were kept at −130 °C and imaged at 3 kV acceleration voltage using an Everhart−Thornley secondary electron detector.

Fluorescence Microscopy. Fluorescence microscopy was used to test CB viability after exposure to particles. At several time points during CB incubation with particles, 100 μL of the sample was collected and stained with Sytox Green Nucleic Acid Stain, for a final concentration of 1 μM.[36] Dark field and fluorescence images were acquired by using a Cytoviva microscope equipped with a Dual Mode Fluorescent Module. CB with intact cell membranes emit red due to chlorophyll autofluorescence, whereas those with damaged cell membranes emit green or yellow-orange, easily allowing the differentiation between live and dead CB. A rough estimate of aggregate sizes is obtained using image analysis. These sizes represent the diameters of circles with the same area as the aggregates.

■ RESULTS AND DISCUSSION

Scanning Electron Microscopy Results. SEM images of both marine and freshwater CB are shown in Figure 1a,b. Marine CB is a coccoid bacterium about 1.5 μm in length, and 1.0 μm in diameter.[38] Fresh water CB is a rod-shaped bacterium, about 2 μm in length and ∼0.75 μm in diameter.[39] The polystyrene particles are shown in Figure 1c,d. The nanoPS particles are approximately 10 times smaller than CB, and the microPS particles are approximately 10 times larger than CB.

Particle Size and Water Salinity Effects. Figure 2 shows images of sample vials taken after 6.5 h of CB incubation with nanoPS and microPS. These samples are in 0.6 M NaCl. The most striking observation is the formation of large nanoPS–marine CB aggregates with sizes varying between 50 and 100 μm, which sediment within several hours, leaving a clear water layer on top (Figure 2a). Aggregates can also be seen at the bottom of the vial (Figure 2b). The nanoPS suspension in this medium is turbid (Figure 2c), implying some aggregation. However, these aggregates are easily dispersed with gentle shaking, and they do not sediment. Figure 2d shows that the CB remained stably suspended and showed no evidence of settling.

The freshwater results for nanoPS are shown in Figure 2e–h. The freshwater CB exposed to nanoPS shows sedimentation within 6.5 h of exposure (Figure 2e), much less dramatic than the marine CB–nanoPS system. NanoPS and some aggregates are visible at the bottom of the vial (Figure 2f). The nanoPS in the freshwater culture medium (Figure 2g) showed no evidence of sedimentation. The freshwater CB by itself did not show any settling over this 6.5 h period (Figure 2h).

The microPS-containing samples behaved differently from those with nanoPS. Figure 2i shows microPS exposed to marine CB. The sample remained turbid within the 6.5 h of exposure, and sedimented microPS particles could be seen at the bottom of the vial (Figure 2j). The microPS particles in the marine medium sedimented within a few hours, as shown in Figure 2l. When microPS particles were exposed to freshwater CB, the sample remained turbid (Figure 2m), but sedimented particles were easily detected (Figure 2n). The microPS particles by themselves settled within a few hours when exposed to a freshwater medium (Figure 2o).

The key observations from the transient OD results shown in Figure 2p,q are (i) that the marine and freshwater CB remain stably suspended in their respective media, (ii) nanoPS in freshwater and in marine water remains fairly well dispersed, while microPS particles in both freshwater and marine water settle rapidly, (iii) nanoPS-CB aggregates in a marine medium settle quickly, but they do not settle as rapidly in a freshwater medium, and (iv) microPS particles settle rapidly and leave most of the CB suspended stably in the vial.

The settling of semidilute suspensions in a finite container involves complex interactions between the downward moving particles and the upward flow caused by the displaced fluid. The transient optical density plotted in Figure 2o,p reflects the concentrations of aggregates averaged over the 1 cm height of the excitation beam. For a single spherical particle of diameter d_p and density ρ_p suspended in a fluid of density ρ_f, and viscosity μ, the particle settling velocity u can be estimated using well-known Stokes velocity $u = \frac{d_p^2(\rho_p - \rho_f)g}{18\mu}$, where g is the acceleration due to gravity. Using the polystyrene density $\rho_p = 1.06 \times 10^3$ kg/m^3, the marine water (0.6 M NaCl in water) density $\rho_{mw} = 1.03 \times 10^3$ kg/m^3, and the freshwater density as $\rho_{fw} = 1.00 \times 10^3$ kg/m^3, a nanoPS particle settles about 3.8 μm in marine water and 7.6 μm in freshwater in 6.5 h. This distance is reduced further because the settling of the particles in a concentrated solution induces a compensating backflow of solvent that reduces the average sedimentation velocity. The sedimentation front does not pass through the beam at a location 1.5 cm from the top. However, a microPS particle settles 3.8 cm in marine and 7.6 cm in fresh water over 6.5 h. Both these dimensions are larger than the depth of the vial. For microPS particles by themselves in both marine and fresh water, the sedimentation front moves past the excitation beam and the OD drops. This is reflected in Figure 2p. If a particle suspension is allowed to equilibrate under the influences of Brownian motion and gravity only, the particle number concentration will decay exponentially with position, with a decay length $kT/\{v_p(\rho_p - \rho_f)\}g$. Here v_p is the volume of a particle and ρ_f is the density of the surrounding fluid. The decay lengths for the nanoPS particle concentrations in freshwater and marine media at 30 °C are 1.3 and 2.6 cm, respectively. These lengths are comparable to the depth of the suspensions in the vials. For microPS particles the decay lengths in freshwater and marine media are 13.6 and 27.2 nm respectively, well below the depth of the suspensions in the vials. These length scales are consistent with the observations that nanoPS particles remain suspended in the vial, while microPS particles sediment. Using a particle loading of 0.005 g/mL, the average interparticle distance between nanoPS and microPS particles can be estimated to be ∼500 nm and ∼50 μm, respectively. Interparticle interactions between the PS beads can therefore be neglected.

Using this analysis and the experimental observations, we hypothesize the following: (a) NanoPS particles aggregate, and marine bacteria excrete exopolymeric substances (EPS) after exposure to nanoPS particles to create aggregates[40] that are 50 μm to 100 μm in diameter, causing them to sink. Indeed, biofouling is known to be a crucial component in the sinking of microplastics.[15,16,24] Both particle–particle and particle–CB aggregation are required, as such large aggregates are not formed when nanoPS particles are exposed to CB in fresh water. Particle–particle aggregation is induced by the presence of salt in the marine medium. (b) MicroPS particles sediment quickly in both marine and freshwater media. The time scales involved in EPS secretion and then aggregation of microPS and CB are longer than the sedimentation time scale. Thus, the microPS particles sediment and leave behind a stable suspension of the CB. These CB were responsible for the turbid appearance and the nonzero optical density of the sample after 6.5 h.

Fluorescence and dark field microscopy were used to analyze the aggregates formed by the microPS and nanoPS with CB
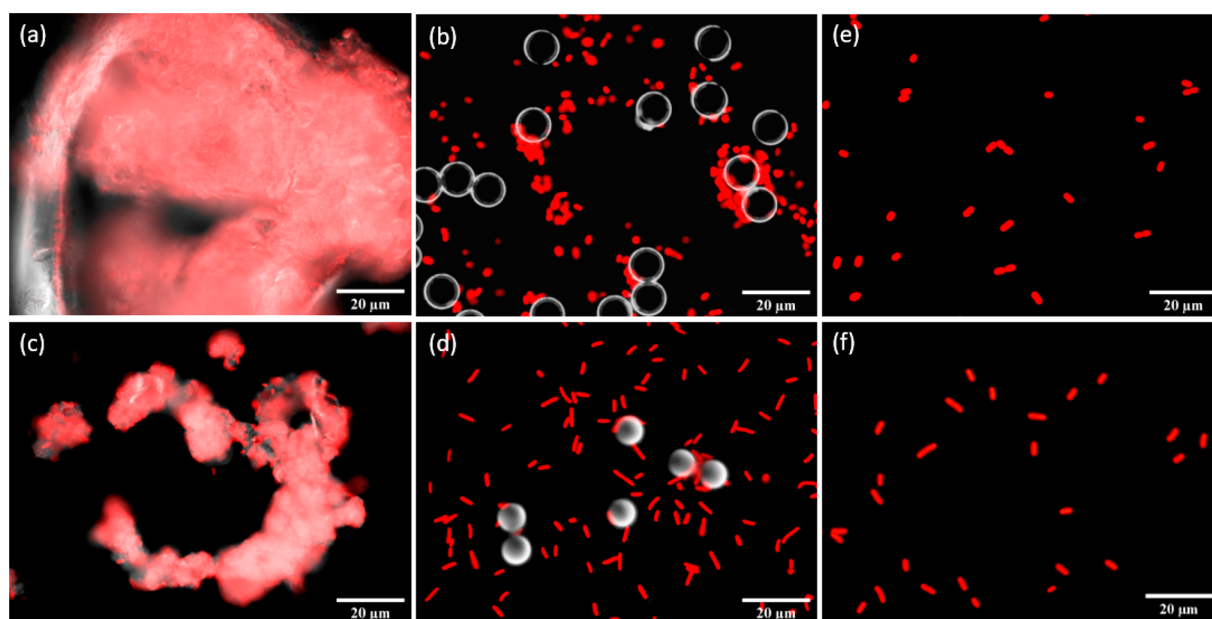
Figure 3. Fluorescence overlaid with dark field images of the nanoPS and microPS−CB aggregates: (a) marine CB exposed to nanoPS (large aggregates are formed); (b) marine CB exposed to microPS; (c) freshwater CB exposed to nanoPS; (d) freshwater CB exposed to microPS; (e) marine CB in marine medium; (f) freshwater CB in a freshwater medium.
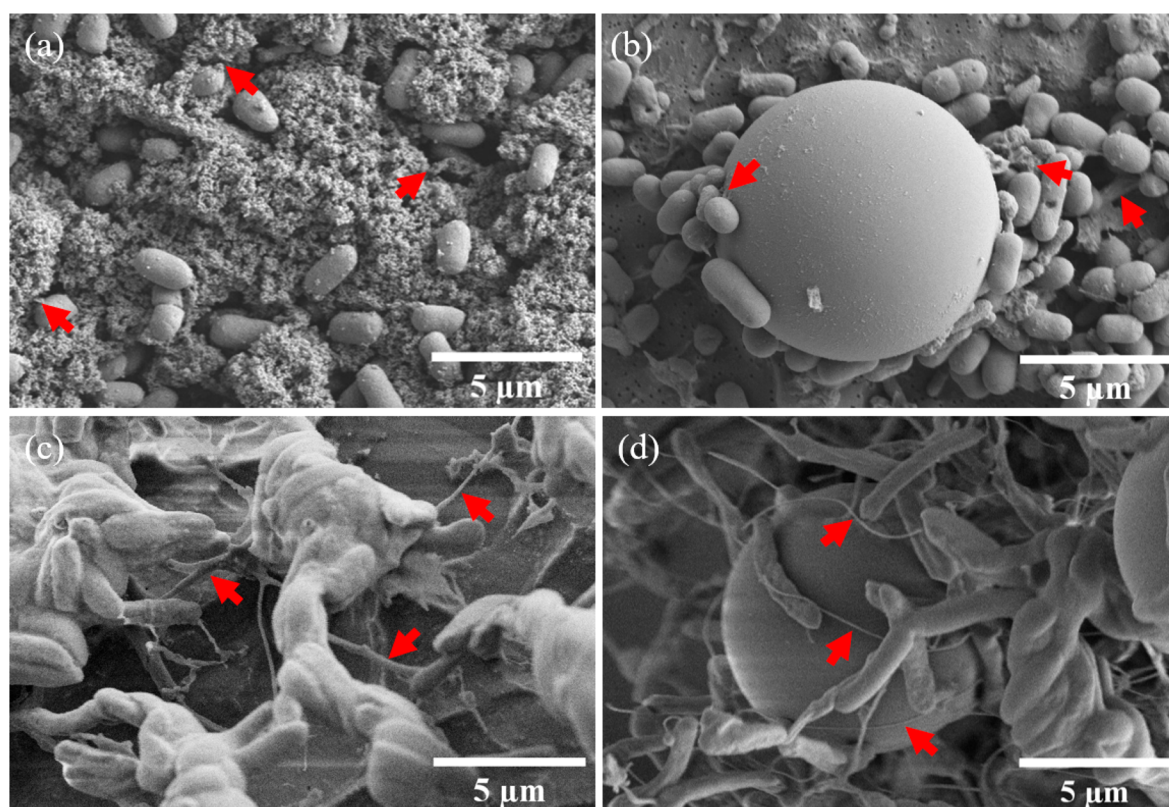

Figure 4. (a) SEM image of marine CB and nanoPS aggregates: (b) SEM image of marine CB and microPS aggregates; (c) cryo-SEM image of freshwater CB and nanoPS aggregates; (d) cryo-SEM image of freshwater CB and microPS aggregates. The red arrows point to EPS strands.

and to evaluate cell viability after 3 days of exposure (Figure 3). A key observation is that the cells remained viable after exposure to and interaction with the particles (red fluorescence). Figure 3a shows nanoPS exposed to marine CB. The aggregates are greater than 100 μm in effective dimensions and contain both marine CB nanoPS. Marine CB

attached to the microPS, as seen in Figure 3b. The size of the aggregates was comparable to the size of the particles, approximately 10 μm.

NanoPS−freshwater CB aggregates are shown in Figure 3c. The aggregates appear similar to the nanoPS−marine CB aggregates, although smaller (40−50 μm) in size. Figure 3d

shows microPS—freshwater CB aggregates. Little attachment of CB is observed around the microPS particles. When observed at this scale, particle—CB aggregation varies with particle size, the type of CB, and the medium. Parts e and f of Figure 3 show the CB in marine and freshwater media with no evidence of any aggregates in either case.

A more detailed picture of the attachment of the CB to the particles emerges from SEM and cryo-SEM images, taken after 3 days of exposure, and is shown in Figure 4. Figure 4a shows nanoPS exposed to marine CB. The nanoPS particles are highly aggregated and the CB are distributed within the aggregates. EPS strands are located around the cells and bind the particles. Examples of EPS filaments are highlighted in Figure 4. The difficulty in redispersing these aggregates indicates that large numbers of these nanoPS particles are being held together by EPS.[18,41] Figure 4b shows the attachment of marine CB to microPS. Several CB are visible around the particle, and large numbers of CB are accumulated below this particle. Strands of EPS are visible around the particle and between the cells, indicating biofilm formation on the microPS particles.[37]

NanoPS exposed to freshwater CB shows aggregation (Figure 4c). Strands of CB presumably containing nanoPS particles form a connected network. This morphology is not observed in the nanoPS marine CB samples. MicroPS freshwater CB aggregates show CB attached to the particles, creating fibrous structures (Figure 4d).[19] EPS secretion and CB—PS particle aggregation is a function of the relative size of CB to the particles, the type of CB, and the medium. These electron microscopy results are consistent with our observations from photographs as well as fluorescence microscopy.

CONCLUSIONS

We have examined the response of marine and freshwater cyanobacteria (CB) to polystyrene nano- and microparticles. These ubiquitous CB respond actively to the presence of the particles, and they remained viable in all cases. CB were able to promote the sedimentation of nanoPS within 6.5 h of exposure. Sedimentation was faster for the marine CB than for the freshwater CB. CB produced exopolymeric substances and formed aggregates with the PS particles. The detailed response of the CB to PS particles depends on the size of the particles, the type of CB and the medium. These findings have implications for microPS and nanoPS transport and fate in waters that have CB. They can promote nanoPS sinking and therefore subestimation of nanoPS concentrations in natural water ecosystems if sampling is performed on the surface. The physicochemical component evaluated in this work is the first step in the complete response of CB to nano and microPS particles.

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

REFERENCES

(1) van Sebille, E.; Wilcox, C.; Lebreton, L.; Maximenko, N.; Hardesty, B. D.; van Franeker, J. A; Eriksen, M.; Siegel, D.; Galgani, F.; Law, K. L. A global inventory of small floating plastic debris. *Environ. Res. Lett.* **2015**, *10*, 124006.

(2) Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768−770.

(3) Yousif, E.; Haddad, R. Photodegradation and photostabilization of polymers, especially polystyrene: review. *SpringerPlus* **2013**, *2*, 1−32.

(4) Nurmukhametov, R. N.; Volkova, L. V.; Kabanov, S. P. Fluorescence and Adsorption of Polystyrene Exposed to UV Laser Radiation. *J. Appl. Spectrosc.* **2006**, *73*, 55−60.

(5) Jang, Y. C.; Lee, J.; Hong, S.; Lee, J. S.; Shim, W. J.; Song, Y. K. Sources of Plastic Marine Debris on Beaches of Korea: More from the Ocean than the Land. *Ocean Sci. J.* **2014**, *49*, 151−162.

(6) Schoof, R. A.; DeNike, J. Microplastics in the context of regulation of commercial shellfish aquaculture operations. *Integr. Environ. Assess. Manage.* **2017**, *13*, 522−527.

(7) Galloway, T. S.; Cole, M.; Lewis, C. Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecology and Evolution* **2017**, *1*, 0116.

(8) Ward, C. P.; Armstrong, C. J.; Walsh, A. N.; Jackson, J. H.; Reddy, C. M. Sunlight Converts Polystyrene to Carbon Dioxide and Dissolved Organic Carbon. *Environ. Sci. Technol. Lett.* **2019**, *6*, 669−674.

(9) Guzzetti, E.; Sureda, A.; Tejada, S.; Faggio, C. Microplastic in marine organism: Environmental and toxicological effects. *Environ. Toxicol. Pharmacol.* **2018**, *64*, 164−171.

(10) Wright, S. L.; Thompson, R. C.; Galloway, T. S. The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* **2013**, *178*, 483−492.

(11) Flombaum, P.; Gallegos, J. L.; Gordillo, R. A.; Rincon, J.; Zabala, L. L.; Jiao, N.; Karl, D. M.; Li, W. K. W.; Lomas, M. W.;

Veneziano, D.; Vera, C. S.; Vrugt, J. A.; Martiny, A. C. Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 9824−9829.

(12) Ruber, J.; Bauer, F. R.; Millard, A. D.; Raeder, U.; Geist, J.; Zwirglmaier, K. Synechococcus diversity along a trophic gradient in the Osterseen Lake District, Bavaria. *Microbiology* **2016**, *162*, 2053−2063.

(13) Tai, V.; Poon, A. F. Y.; Paulsen, I. T.; Palenik, B. Selection in Coastal Synechococcus (Cyanobacteria) Populations Evaluated from Environmental Metagenomes. *PLoS One* **2011**, *6*, No. e24249.

(14) Oberbeckmann, S.; Loeder, M. G. J.; Gerdts, G.; Osborn, A. M. Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. *FEMS Microbiol. Ecol.* **2014**, *90*, 478−492.

(15) Kaiser, D.; Kowalski, N.; Waniek, J. J. Effects of biofouling on the sinking behavior of microplastics. *Environ. Res. Lett.* **2017**, *12*, 124003.

(16) Cunha, C.; Faria, M.; Nogueira, N.; Ferreira, A.; Cordeiro, N. Marine vs freshwater microalgae exopolymers as biosolutions to microplastics pollution. *Environ. Pollut.* **2019**, *249*, 372−380.

(17) Marvasi, M.; Visscher, P. T.; Casillas Martinez, L. Exopolymeric substances (EPS) from Bacillus subtilis: Polymers and genes encoding their synthesis. *FEMS Microbiol. Lett.* **2010**, *313*, 1−9.

(18) Eboigbodin, K. E.; Biggs, C. A. Characterization of the Extracellular Polymeric Substances Produced by Escherichia coli Using Infrared Spectroscopic, Proteomic, and Aggregation Studies. *Biomacromolecules* **2008**, *9*, 686−695.

(19) Costa, O. Y. A.; Raaijmakers, J. M.; Kuramae, E. E. Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. *Front. Microbiol.* **2018**, *9*, 16−36.

(20) Wotton, R. S. The ubiquity and many roles of exopolymers (EPS) in aquatic systems. *Sci. Mar.* **2004**, *68*, 13−21.

(21) Vardharajula, S.; Sk Z, A. Exopolysaccharide Production by Drought Tolerant Bacillus spp. and Effect on Soil Aggregation Under Drought Stress. *J. Microbiol., Biotechnol. Food Sci.* **2014**, *4*, 51−57.

(22) Summers, S.; Henry, T.; Gutierrez, T. Agglomeration of nano- and microplastic particles in seawater by autochthonous and de novo-produced sources of exopolymeric substances. *Mar. Pollut. Bull.* **2018**, *130*, 258−267.

(23) Frère, L.; Maignien, L.; Chalopin, M.; Huvet, A.; Rinnert, E.; Morrison, H.; Kerninon, S.; Cassone, A.; Lambert, C.; Reveillaud, J.; Paul-Pont, I. Microplastic bacterial communities in the Bay of Brest: Influence of polymer type and size. *Environ. Pollut.* **2018**, *242*, 614−625.

(24) Lagarde, F.; Olivier, O.; Zanella, M.; Daniel, P.; Hiard, S.; Caruso, A. Microplastic interactions with freshwater microalgae: aggregation and changes in plastic density appear strongly dependent on polymer type. *Environ. Pollut.* **2016**, *215*, 331−339.

(25) Cabello-Yeves, P. J.; Haro-Moreno, J. M.; Martin-Cuadrado, A. B.; Ghai, R.; Picazo, A.; Camacho, A.; Rodriguez-Valera, F. Novel Synechococcus genomes reconstructed from freshwater reservoirs. *Front. Microbiol.* **2017**, *8*, 1151.

(26) Ryan, P. G.; Moore, C. J.; van Franeker, J. A.; Moloney, C. L. Monitoring the abundance of plastic debris in the marine environment. *Philos. Trans. R. Soc., B* **2009**, *364*, 1999−2012.

(27) Andrady, A. L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596−1605.

(28) Carpenter, E. J.; Anderson, S. J.; Harvey, G. R.; Miklas, H. P.; Peck, B. B. Polystyrene spherules in coastal waters. *Science* **1972**, *178*, 749−750.

(29) Browne, M. A.; Galloway, T. S.; Thompson, R. C. Spatial patterns of plastic debris along estuarine shorelines. *Environ. Sci. Technol.* **2010**, *44*, 3404−3409.

(30) Lebaron, P.; Catala, P.; Parthuisot, N. Effectiveness of SYTOX Green stain for bacterial viability assessment. *Appl. Environ. Microbiol.* **1998**, *64*, 2697−700.

(31) Fadare, O. O.; Wan, B.; Guo, L. H.; Xin, Y.; Qin, W.; Yang, Y. Humic acid alleviates the toxicity of polystyrene nanoplastic particles to: Daphnia magna. *Environ. Sci.: Nano* **2019**, *6*, 1466−1477.

(32) Choy, C. A.; Robison, B. H.; Gagne, T. O.; Erwin, B.; Firl, E.; Halden, R. U.; Hamilton, J. A.; Katija, K.; Lisin, S. E.; Rolsky, C.; Houtan, K. S. V. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. *Sci. Rep.* **2019**, *9*, 7843.

(33) Abbasi, A.; Bothun, G. D.; Bose, A. Attachment of *Alcanivorax borkumensis* to Hexadecane-In-Artificial Sea Water Emulsion Droplets. *Langmuir* **2018**, *34*, 5352−5357.

(34) Hazrin-Chong, N. H.; Manefield, M. An alternative SEM drying method using hexamethyldisilazane (HMDS) for microbial cell attachment studies on sub-bituminous coal. *J. Microbiol. Methods* **2012**, *90*, 96−99.

(35) Braet, F.; de Zanger, R.; Wisse, E. Drying cells for SEM, AFM and TEM by hexamethyldisilazane: a study on hepatic endothelial cells. *J. Microsc.* **1997**, *186*, 84−87.

(36) Sato, M.; Murata, Y.; Mizusawa, M.; Iwahashi, H.; Shu-ichi, O. A Simple and Rapid Dual-fluorescence Viability Assay for Microalgae. *Microbiology and Culture Collections* **2004**, *20*, 53−59.

(37) Palmer, J.; Flint, S.; Brooks, J. Bacterial cell attachment, the beginning of a biofilm. *J. Ind. Microbiol. Biotechnol.* **2007**, *34*, 577−588.

(38) Johnson, P. W.; Sieburth, J. McN. Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. *Limnol. Oceanogr.* **1979**, *24*, 928−935.

(39) Goclaw-Binder, H.; Sendersky, E.; Shimoni, E.; Kiss, V.; Reich, Z.; Perelman, A.; Schwarz, R. Nutrient-associated elongation and asymmetric division of the cyanobacterium Synechococcus PCC 7942. *Environ. Microbiol.* **2012**, *14*, 680−690.

(40) Long, M.; Paul-Pont, I.; Hégaret, H.; Moriceau, B.; Lambert, C.; Huvet, A.; Soudant, P. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific aggregation. *Environ. Pollut.* **2017**, *228*, 454−463.

(41) Dorken, G.; Ferguson, G. P.; French, C. E.; Poon, W. C. K. Aggregation by depletion attraction in cultures of bacteria producing exopolysaccharide. *J. R. Soc., Interface* **2012**, *9*, 3490−3502.