



Transparent exopolymer particles as critical agents in aquatic biofilm formation: implications for desalination and water treatment

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ABSTRACT

Transparent exopolymer particles (TEP), planktonic microgel particles that are ubiquitous and numerous in all waters, have been recently recognized as being involved in the formation of aquatic biofilm. Studies in several different environments (small scale experimental membrane arrays, pilot scale and full-sized, operational, sea water reverse osmosis, and water treatment plants) indicate that the extent of biofouling and clogging of filtration membranes is usually significantly correlated to the levels of TEP in the feedwater. Other studies have revealed that current pretreatment technology such as rapid sand filtration and microfiltration are only moderately effective in reducing the amounts of TEP in feedwater reaching reverse osmosis membranes. A revised paradigm has been proposed that takes into consideration the role of TEP microgels as important accelerators of aquatic biofilm formation. This model has applied implications for the desalination and water treatment industries. With the recognition of TEP as a critical “player” in aquatic biofilm formation, important aims for water industry R&D should be the design of improved pretreatment technologies to minimize the amounts of feedwater TEP reaching sensitive surfaces and the development of membranes that either impede surface adhesion or cause disintegration of microgels upon contact.

Keywords: TEP; Biofilm formation; Membrane clogging; Pretreatment

1. Introduction

At the European Desalination Society Conference in Barcelona (April 2012) a special session was the first at an international meeting to examine various aspects of transparent exopolymer particles (TEP) in desalination and water treatment. Although TEP were first described some 19 years ago [1] and have been studied in detail by oceanographers and limnologists, the sug-

gestion that TEP particles might be involved in aquatic biofilm formation on sensitive surfaces such as Ultrafiltration (UF) and reverse osmosis membranes is relatively recent [2]. Nevertheless, investigations by several research groups have now begun to clarify the role of TEP in biofilm formation in desalination and wastewater treatment facilities. Recognition of TEP involvement in biofilm formation has broad implications for a better understanding of the complexities of

the fouling process in aquatic environments. Understanding the properties of these microgel particles can contribute to the considerable efforts being made in the global water industry to mitigate the harmful effects of biofouling in water treatment and desalination plants. This short review is intended to provide an overview of current knowledge about TEP as critical agents in the process of aquatic biofilm development.

2. What are TEP?

TEP are microscopic, deformable, gel-like organic particles that are numerous and ubiquitous in all kinds of source waters. In 1993, Alldredge et al. [1] reported a high abundance of previously undetected, transparent, gel-like particles in seawater that were visualized by staining with Alcian Blue, a dye specific for acid mucopolysaccharides. Extensive research has shown that TEP play extremely important roles in the ecosystem functioning of both marine and freshwater environments, especially in respect to the cycling and transportation of organic carbon [3–6]. TEP range in size from >0.4 to 200–300 μm and appear in many forms; amorphous blobs, clouds, sheets, filaments, or clumps (Fig. 1). TEP may be considered a planktonic subgroup of exopolymeric substances (EPS) [7,8]; recently Barzeev et al. [9] proposed the term “protobiofilm” for large TEP that were heavily colonized by bacteria and other microorganisms. The presence of highly surface active polysaccharides in TEP explains the strong tendency of these particles to form metal ion bridges and hydrogen bonds [10]. As a result, TEP are usually extremely sticky, about 2–4 orders of magnitude more sticky than phytoplankton or mineral particles, with a high probability of attachment upon collision [3,11,12]. TEP are essential for the aggregation of particles in the open water, and for coating natural surfaces [13].

These particles may derive from numerous sources. In some aquatic environments, TEP form abiotically from dissolved organic exudation products by processes of coagulation and gelation [14,15] or by bubble adsorption [16]. Considerable amounts of TEP are also produced from the gelatinous envelopes surrounding diatoms and other algae [4,17] and from bacterial mucous [18]. TEP are also released under stress conditions or at senescence by algae and cyanobacteria [19] or from the breakdown of marine or lake “snow”. TEP constitute a significant portion of the gel phase that forms an intermediate stage in the dissolved organic matter to particulate organic matter continuum in seawater and freshwaters [13].

In oceans and lakes, TEP are often colonized by bacteria and other microorganisms [3,17,20] and may

serve as the matrix for “hot spots”, sites of intense microbial and chemical activity within the water mass [9,21]. TEP may aggregate with each other or with other detrital fragments to form marine or lake “snow” [22,23]. In addition to surface active acidic polysaccharides [10], many other substances, including proteins [24], nucleic acids [25] and trace elements can be associated with these gel-like particles. They adsorb trace metals [26] and dissolved organic materials, thus providing favorable and specialized sites (e. g. low oxygen or anaerobic environments) for bacterial development. Additionally these particles, together with their associated flora and fauna, can serve as “food packages” for protists [27], microzooplankton [28] and even larval fish [23].

3. TEP and the process of biofouling development

Based on the known characteristics of TEP, in particular their extreme stickiness and the observation that numerous bacteria were frequently observed on or in many of these particles, Berman and Hølenberg [2] first suggested that TEP should be involved in the biofouling process. However, it was only in subsequent experimental studies that TEP were actually shown to be actively involved in aquatic biofilm formation.

Initial studies by Bar-Zeev et al. [29] using glass slides suspended in seawater over several days showed early adhesion (within several hours) of Alcian Blue stained material to surfaces and indicated that these organic patches were not due to EPS proliferated by bacteria attached to the surface but derived from TEP originally in the feed water. These initial indications were confirmed by a more detailed study [9] in which coastal seawater was passed through custom-designed flow cells that enabled direct observation of biofilm development on immersed surfaces inside the flow cells. Within minutes of exposure to seawater, patches of Alcian Blue staining material were seen adhering to these surfaces. By 30 min, confocal laser-scanning microscopy (CLSM) revealed numerous structures of polysaccharide material (TEP/EPS), 28–210 μm thick, with associated bacteria covering the surfaces. Atomic force microscopy also showed details of a much thinner (\sim 10–150 nm thick), highly sticky, organic layer between these thicker patches, corresponding to the “classical” conditioning film formed from organic colloidal material. With time, increasing areas of the surfaces became covered with Alcian Blue staining material identified as EPS, which could only have come from TEP in the seawater feed.

To evaluate the impact of TEP on early biofilm development, flow cell experiments were made with either untreated seawater or seawater filtered through

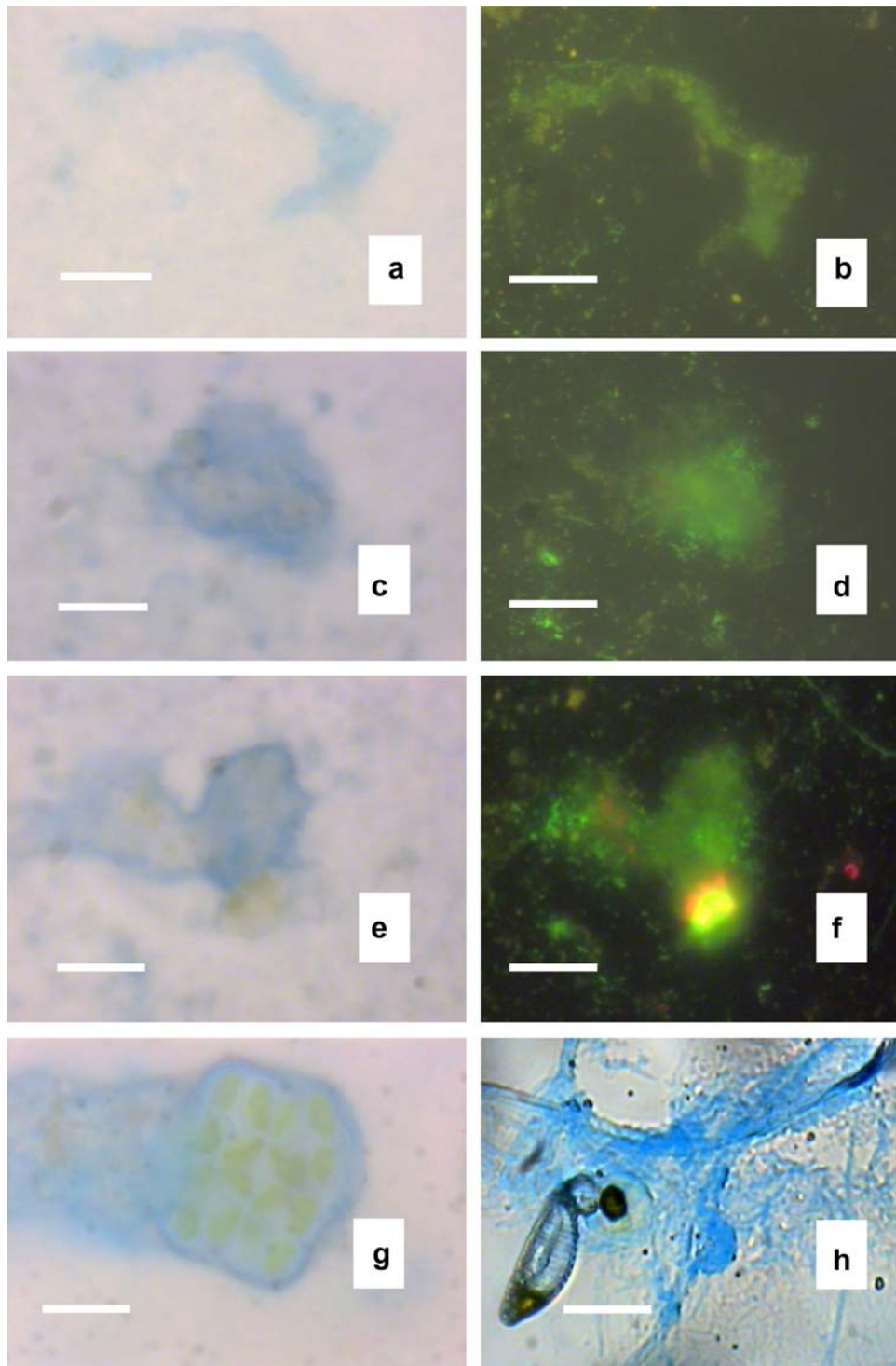


Fig. 1. Some examples of TEP. When stained with Alcian Blue, TEP are colored blue under regular microscope illumination, (a), (c), (e), (g), and (h). When the same fields are viewed under ultra-violet epifluorescent illumination, bacteria staining with SYBR Green (b), (d) and (f) appear as green dots or rods. The hazy green staining of some TEP is probably due to nucleic acids adsorbed to these particles. Yellow-green or red staining objects are chlorophyll containing algal cells. (a)–(f): TEP in Mediterranean coastal water; (g) and (h) TEP being released from a *Merismopedia* colony and from *Amphora ovalis* diatoms in Lake Kinneret. For other images of TEP in various waters see [25]. Scale bar = 20 μm .

GF/F filters to reduce the concentrations of large microgels in the feedwater. Bright-field and epifluorescence microscopy and CLSM showed that biofilm development (observed until 24 h) was profoundly inhibited in flow cells with seawater prefiltered to remove most large TEP and protobiofilm (i.e. TEP heavily colonized by bacteria). In filtered seawater, the slow development of biofilm occurred mainly by the permanent attachment of bacteria to the surface and subsequent bacterial outgrowth and EPS proliferation. By contrast, in flow cells with untreated seawater feed, biofilm development was accelerated by the continuous adhesion of large (>20 μm) TEP, with and without bacterial colonization (see below).

Although the above studies were made with seawater, it seems reasonable to assume that TEP microgels function similarly in biofilm formation with freshwater.

4. TEP and membrane clogging

As noted, TEP have been observed in a multitude of water sources. De la Torre et al. [30] reported the occurrence of TEP in the activated sludge of three membrane bioreactor units in Germany, and suggested using this parameter as an indicator for potential fouling of membranes. In a recent study, Villacorte et al. [31] confirmed the presence of TEP in six different fresh and salt water sources in the Netherlands and Belgium, with higher concentrations in the seawater (Fig. 2). These investigators also found that the majority of Alcian Blue staining particles was always detected in the 0.05–0.4 μm fraction in these waters. Another important observation in these studies was that high rates of UF membrane fouling at a seawater UF-RO desalination plant coincided with periods when there were high TEP levels in the feedwater [32]. Thus, the increased amounts of TEP in coastal seawater that occur when there are blooms of Harmful Algae should be a factor to consider in the operation of sea water reverse osmosis (SWRO) plants.

A direct relationship between feedwater TEP concentrations and rate of clogging of membranes was shown by Berman et al. [33] who used an experimental cross-flow membrane array (CFMA) to investigate the relationship between the rate of membrane clogging and levels of TEP and other water quality variables in a lake water source. In three experimental series with coastal lake water run under conditions of laminar, intermediate or turbulent flow conditions, feedwater TEP concentrations correlated significantly with the membrane fouling rate. Subsequent experiments with this experimental set-up but

under different environmental conditions have further confirmed the direct relationship between feedwater TEP concentrations and the rate of membrane clogging.

To check whether feedwater TEP could be a source for EPS of early biofilm, CFMA experiments were run with either untreated (active bacteria) or chlorinated (inactivated bacteria) lakewater. Confocal scanning laser microscopy and image analysis of biofilm showed similar amounts of EPS in the biofilm that had formed on membranes after 50 h, irrespective of whether the bacteria in the feedwater were ~98% inactivated or fully active. This would confirm that most of the EPS appearing at early stages of biofilm on membranes originated from TEP in the feedwater rather than from metabolizing bacteria adhering to the surface (see above, [9,29]). Taken together, these experiments supported the premise that TEP in source waters play a significant role in the early stages of aquatic biofilm formation and are an important causative factor in membrane fouling.

5. Does existing pretreatment technology remove TEP adequately from source waters?

Increasing awareness of the potential of TEP to cause biofouling has prompted several studies to evaluate how effective current pretreatment methods are in lowering TEP levels in feedwater. Bar-Zeev et al. [29] monitored the efficiency of pretreatment (coagulation, rapid sand filtration and (RSF), followed by cartridge filtration) in decreasing the amounts of TEP reaching the RO membranes at a large operational SWRO plant in Israel. This study revealed that although pretreatment effectively lowered the levels of some water quality parameters (e.g. chlorophyll and silt density index) reaching the RO membranes by ~90% relative to input, TEP concentrations were usually decreased by only ~30%. TEP levels did not decrease further subsequent to the RSF and, in fact, tended to rise after passing through the cartridge filter prior to reaching the RO membranes. Similar increases in TEP concentrations downstream from cartridge filters have been recorded in more recent long-term monitoring at this SWRO plant (A. Levy, pers. comm.). The reason for this consistent increase was unclear; it may have been due to EPS/TEP being flushed from biofilm that forms within the cartridge medium and/or may have been caused by TEP formation due to turbulence in the water stream passing through the cartridge filters [29]. A detailed study of a full-sized RSF at another Israeli SWRO plant showed

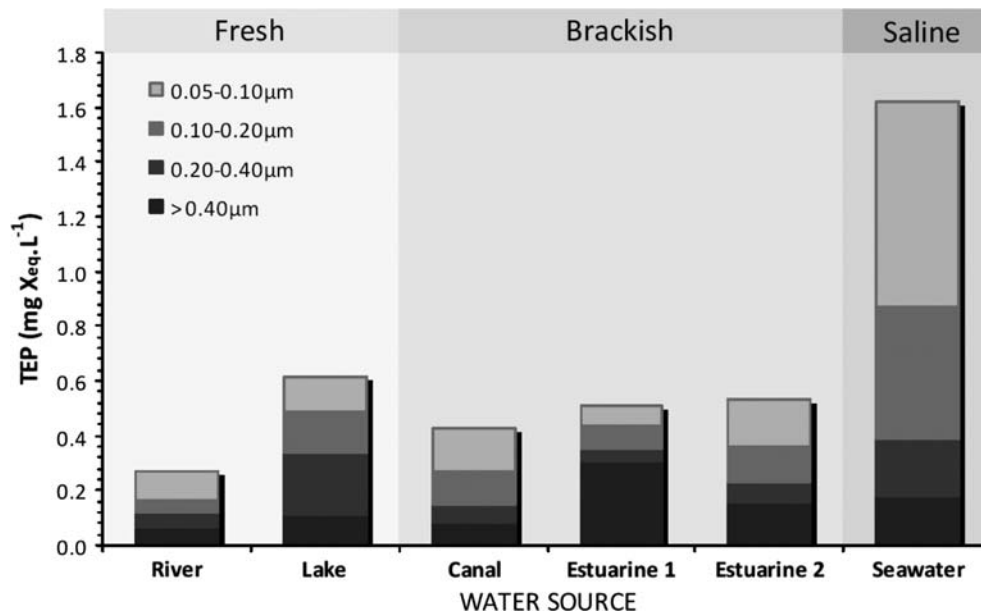


Fig. 2. Concentrations of different size fractions of TEP measured in six water sources. From left to right: River Meuse in Limburg, Netherlands (July 2008); Lake IJssel near Andijk, Netherlands (June 2008); Gent-Terneuzen canal, Belgium (July 2008); River Schelde estuary, Belgium (July 2008); River IJ estuary, Netherlands (June 2008) and Wadden Sea near Eemshaven coast, Netherlands (June 2008).

Source: Figure reproduced by permission from Elsevier from Villacorte et al. [31].

that, over a maturation period of about 3 months, the efficiency of TEP removal varied widely (from 19 to 91%) with an overall average of ~50% [34].

Villacorte et al. [31] monitored TEP in the feed-water and pretreatment stages of one pilot and five full-scale Integrated Membrane Systems plants in The Netherlands and Belgium. The six plants (one supplying drinking and the others industrial water) were treating different water sources with various types of pretreatment. UF pretreatment that was applied in four plants totally removed large (>0.4 µm) TEP. Microfiltration (in two plants) or coagulation followed by sedimentation and RSF (in three plants) only partially removed this TEP fraction. None of the pretreatment systems investigated totally removed colloidal (0.05–0.4 µm) TEP.

Although relatively few data are available, the general impression is that present pretreatment technologies are not particularly effective in dealing with TEP given the gel-like characteristics of these particles and that with the recognition of their potential harmful impact, more effective pretreatment technology can be designed [34]. Some commercial companies (Ahlstrom, Amiad Water Systems) have already begun to test the effectiveness of their products in removing TEP and Sumitomo Electric Co. (Japan) has advertised a membrane which is claimed to effectively trap TEP and also to enable backwashing of the trapped material.

6. A revised paradigm for biofilm formation: facilitation by TEP microgels

Recognition of the potential role of TEP in aquatic biofilm formation implied that the classic descriptions of this process [35,36] were incomplete. In a recent paper Bar-Zeev et al. [9] have proposed a revised paradigm for aquatic biofilm formation that takes into account the previously unrecognized role of microgel particles such as TEPs and protobiofilm (i.e. heavily colonized, large [$>20\mu\text{m}$] TEP) in facilitating and accelerating this process. The new model posits the following stages of early biofilm development (Fig. 3):

- (1) Conditioning of a pristine surface begins immediately upon contact with water. A patchy, thin (<250 nm) conditioning layer is formed by organic polymers and colloids (Fig. 3(a) and (b)). In addition, occasional thicker (>100 µm thick) clumps of TEP and protobiofilm adhere to the surface (Fig. 3(c) and (d)). These highly adhesive, microgel particles alter the physical and chemical properties of the surface, thus providing a favorable substrate for the further attachment of bacteria and additional microgels. During this initial phase, single planktonic bacteria also make reversible contact with clean surface areas, but, tend to attach permanently to areas of conditioning film (Fig. 3(e)).

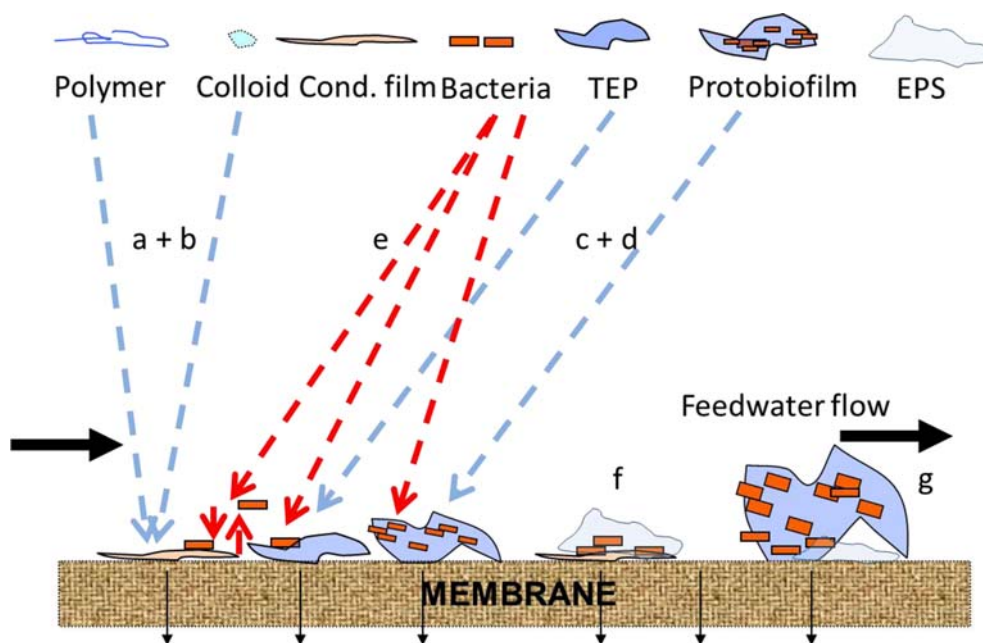


Fig. 3. Cartoon illustrating the revised paradigm for aquatic biofilm formation showing involvement of TEP and protobiofilm [9]. Immediately on exposure of a pristine surface to water, organic polymers (a) and colloids (b) in the feedwater begin to form patches of “conditioning film”. Microgels such as TEP (c) and Protobiofilm (d) also begin to adhere to the surface, effectively “jump-starting” biofilm formation. Planktonic bacteria at first make contact reversibly with the clean surface and then stick irreversibly to areas with conditioning film (e). With time (hours to days), these bacteria multiply, proliferate EPS and the biofilm develops (f). However, very much faster development and more rapid maturation of the biofilm occur in areas where TEP and Protobiofilm adhered (g). See text for details.

- (2) During the first ~30–60 min of seawater/surface interaction, further TEP and protobiofilm particles adhere firmly to the surface. Attached protobiofilm, with its complement of fully functioning microbial communities, provides a jump-start for the early development of biofilm.
- (3) Under favorable environmental conditions, a widespread 3D network of early mature biofilm, derived mainly from TEP and protobiofilm, becomes established within a few hours. Bacterial populations associated with the attached microgels and also single bacteria adhering to the surface begin to grow out and proliferate EPS. Nevertheless, biofilm development is greatly accelerated in untreated feedwater (i.e. with TEP and Protobiofilm; Fig. 3(g)) in comparison with that in filtered feedwater in which most large TEP and Protobiofilm have been removed; Fig. 3(f).

harmful algae, have been correlated with increased accumulation of biofilm and membrane clogging [32,33]. The recent model proposed by Bar-Zeev et al. [9], has clarified details of the role played by TEP and Protobiofilm in early biofilm formation and shows how this process can be accelerated many-fold by these microgel particles.

The practical implications of this model are obvious; the less TEP that reaches surfaces such as UF or RO membranes, the less is the potential for biofilm formation. However, as yet, most applied research effort in biofilm mitigation has concentrated on either removing or killing bacteria in feedwater or inhibiting bacterial multiplication and growth within the biofilm. With the recognition of TEP as a critical “player” in aquatic biofilm formation, important aims for water industry R&D should be the design of improved pretreatment technologies to minimize the amounts of feedwater TEP reaching sensitive surfaces and the development of membranes that either impede surface adhesion or cause disintegration of microgels upon contact. Successful strategies for alleviating the problems caused by microgels such as TEP and Protobiofilm in feedwater will require better understanding of the physical, chemical and microbiological characteristics of these particles in many different source waters.

7. Conclusions

Over the past few years, it has become very evident that TEP are intimately involved in aquatic biofilm formation. Increased levels of TEP in feedwater, such as occur with the incidence of dense blooms of

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References

- [1] A.L. Alldredge, U. Passow, B.E. Logan, The abundance and significance of a class of large, transparent organic particles in the ocean, *Deep Sea Res.* 40 (1993) 1131–1140.
- [2] T. Berman, M. Hohenberg, Don't fall foul of biofilm through high TEP levels, *Filtration Sep.* 42(4) (2005) 30–32.
- [3] U. Passow, Transparent exopolymer particles (TEP) in aquatic environments, *Prog. Oceanogr.* 55 (2002) 287–333.
- [4] T. Berman, Y. Viner-Mozzini, Abundance and characteristics of polysaccharide and proteinaceous particles in Lake Kinneret, *Aq. Microbial Ecol.* 24 (2001) 255–264.
- [5] E. Bar Zeev, I. Berman-Frank, N. Stambler, E. Vázquez Domínguez, T. Zohary, E. Capuzzo, E.D.J. Meeder, D. Suggett, D. Iluz, G. Dishon, T. Berman, Transparent exopolymer particles (TEP), a link between phytoplankton and bacterial productivity in the Gulf of Aqaba, *Aq. Microbial Ecol.* 56 (2009) 217–225.
- [6] E. Bar-Zeev, T. Berman, E. Rahav, G. Dishon, B. Herut, N. Kress, I. Berman-Frank, Transparent exopolymer particles (TEP) dynamics in the eastern Mediterranean Sea, *Mar. Ecol. Prog. Ser.* 431 (2011) 107–118.
- [7] A.W. Decho, Microbial exopolymer secretions in ocean environments: Their role(s) in food web and marine processes, *Oceanogr. Marine Biol.: An Annual Rev.* 28 (1990) 73–153.
- [8] K. Hoagland, J. Rosowski, Diatom extracellular polymeric substances: Function, fine structure, chemistry, and physiology, *J. Phycol.* 29 (1993) 537–566.
- [9] E. Bar-Zeev, I. Berman-Frank, O. Girshevitz, T. Berman, Revised paradigm of aquatic biofilm formation facilitated by microgel transparent exopolymer particles. *Proc. Natl. Acad. Sci.* (2012) 1–6. doi/10.1073/pnas.1203708109.
- [10] K. Mopper, J. Zhou, K.S. Ramana, U. Passow, H.G. Dam, D.T. Drapeau, The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm, *Deep Sea Res. Part II* 42 (1995) 47–73.
- [11] A. Engel, 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 (2000) 485–497.
- [12] X. Mari, H.G. Dam, Production, concentration and isolation of transparent exopolymer particles using paramagnetic functionalized microspheres, *Limnol. Oceanogr.: Meth.* 2 (2004) 13–24.
- [13] P. Verdugo, A.L. Alldredge, F. Azam, D. Kirchman, U. Passow, P. Santschi, The oceanic gel phase: A bridge in the DOM/POM continuum, *Marine Chem.* 92 (2004) 67–85.
- [14] W.C. Chin, M.V. Orellana, P. Verdugo, Spontaneous assembly of marine dissolved organic matter into polymer gels, *Nature* 391 (1998) 568–572.
- [15] U. Passow, Formation of transparent exopolymer particles, TEP, from dissolved precursor material, *Mar. Ecol. Prog. Ser.* 192 (2000) 1–11.
- [16] J. Zhou, K. Mopper, U. Passow, The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater, *Limnol. Oceanogr.* 43 (8) (1998) 1860–1871.
- [17] U. Passow, A.L. Alldredge, Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean, *Mar. Ecol. Prog. Ser.* 113 (1994) 185–198.
- [18] K.E. Stoderegger, G.J. Herndl, Production of exopolymer particles by marine bacterioplankton under contrasting turbulence conditions, *Mar. Ecol. Prog. Ser.* 189 (1999) 9–16.
- [19] I. Berman-Frank, G. Rosenberg, O. Levitan, L. Haramaty, X. Mari, Coupling between autocatalytic cell death and transparent exopolymeric particle production in the marine cyanobacterium *Trichodesmium*, *Environ. Microbiol.* 9 (2007) 1415–1422.
- [20] X. Mari, T. Kiørboe, Abundance, size distribution and bacterial colonization of transparent exopolymer particles (TEP) in the Kattegat, *J. Plankton Res.* 18 (1996) 969–986.
- [21] F. Azam, Microbial control of oceanic carbon flux: The plot thickens, *Science* 280 (1998) 694–696.
- [22] B.E. Logan, U. Passow, A.L. Alldredge, H.-P. Grossart, M. Simon, Rapid formation and sedimentation of large aggregates is predictable from coagulation rates (half-lives) of transparent exopolymer particles (TEP), *Deep Sea Res.* 42 (1995) 203–214.
- [23] H.-P. Grossart, T. Berman, M. Simon, P. Pohlmann, Occurrence and microbial dynamics of macroscopic organic aggregates (lake snow) in Lake Kinneret, Israel in fall, *Aq. Microbial Ecol.* 14 (1998) 59–67.
- [24] R.A. Long, F. Azam, Abundant protein-containing particles in the sea, *Aq. Microbial Ecol.* 10 (1996) 213–221.
- [25] T. Berman, R. Parparova, Visualization of transparent exopolymer particles (TEP) in various source waters, *Desalin. Water Treat.* 21 (2010) 382–389.
- [26] S.E.H. Niven, P.E. Kepkay, J.B.C. Bugden, The role of TEP in 234th scavenging during a coastal bloom, *Radioprotection-Colloques* 32 (1997) 213–218.
- [27] X. Mari, F. Rassoulzadegan, Role of TEP in the microbial food web structure: Grazing behavior of a bacterivorous pelagic ciliate, *Mar. Ecol. Prog. Ser.* 279 (2004) 13–22.
- [28] U. Passow, A.L. Alldredge, Do transparent exopolymer particles (TEP) inhibit grazing by the euphasid *Euphausia pacifica*? *J. Plankton Res.* 21 (1999) 2203–2217.
- [29] E. Bar-Zeev, I. Berman-Frank, B. Liberman, E. Rahav, U. Passow, T. Berman, Transparent exopolymer particles: Potential agents for organic fouling and biofilm formation in desalination and water treatment plants, *Desalin. Water Treat.* 3 (2009) 136–142.
- [30] T. de la Torre, B. Lesjean, A. Drews, M. Kraume, Monitoring of transparent exopolymer particles (TEP) in a membrane bioreactor (MBR) and correlation with other fouling indicators, *Water Sci. Technol.* 58 (2008) 1903–1909.
- [31] L.O. Villacorte, M.D. Kennedy, G.L. Amy, J.C. Schippers, The fate of transparent exopolymer particles (TEP) in integrated membrane systems: Removal through pretreatment processes and deposition on reverse osmosis membranes, *Water Res.* 43 (2009) 5039–5052.
- [32] L.O. Villacorte, R. Schurer, M.D. Kennedy, G.L. Amy, J.C. Schippers, Removal and deposition of transparent exopolymer particles in a seawater UF-RO system, *IDA J. First Quarter* (2010) 45–55.
- [33] T. Berman, R. Mizrahi, C.G. Dozoretz, TEP: A critical factor in aquatic biofilm formation and fouling on filtration membranes, *Desalination* 276 (2011) 184–190.
- [34] E. Bar-Zeev, N. Belkin, B. Liberman, T. Berman, I. Berman-Frank, Rapid sand filtration pretreatment for SWRO: Microbial maturation dynamics and filtration efficiency of organic matter, *Desalination* 286 (2012) 120–130.
- [35] P. Stoodley, K. Sauer, D.G. Davies, J.W. Costerton, Biofilms as complex differentiated communities, *Ann. Rev. Microbiol.* 56 (2002) 187–209.
- [36] H.-C. Flemming, J. Wingender, The biofilm matrix, *Nat. Rev. Microbiol.* 8 (2010) 623–633.