Microbial extracellular polymeric substances in marine biogeochemical processes

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Microbial extracellular polymeric substances (EPS) are widely distributed in marine environments and are found in free dissolved form, colloids, discreet particles like TEP and/or associated with particulate matter, including cell aggregates, detritus, biofilms, microbial mats, etc. The chemical composition of EPS is influenced by various factors, including nutrients, temperature, pH, physiology and age of the culture. Microbial EPS perform various functions, and are involved in diverse marine processes. The unique gelling properties of microbial EPS are considered important in the transport and transformation of organic matter, complexation of dissolved metals and biogeochemical cycling of elements. EPS are rich in organic carbon and therefore are an important source of carbon for different organisms in the food chain. The production of dissolved EPS during the bacterial growth, production of particulate organic carbon from the dissolved EPS and enzymatic breakdown and conversion of particulate EPS into dissolved organic matter within the microbial loop, form an important alternate route of organic carbon cycling in the marine trophic web. In this article, recent findings on EPS and their significance in biogeochemical processes are discussed with respect to their role in marine microbial ecology, marine food-web and other areas of future research.

M ICROBIAL extracellular polymeric substances (EPS) produced by both prokaryotes (eubacteria and archaebacteria) and eukaryotes (phytoplankton, fungi, and algae) have been a topic of current research interest. The importance of microbial EPS is highlighted by recent publications covering the chemistry, structure and function of EPS[1,2], their role in microbial ecology[3,4], medicine[5], dairy industry[6], biofilms and corrosion[7,8], and their applications in the field of biotechnology[9,10]. However, these reviews are limited to microorganisms isolated from terrestrial environment and little is known about the role of these microbial EPS in marine environments. The marine environment is a dynamic one, wherein microorganisms experience changes in salinity, pressure, nutrient levels, etc. which can influence EPS production in microorganisms. Although marine microbial EPS have been studied in recent times for their various industrial applications, there have been only a few reviews

highlighting their importance in marine environments[3,4,11]. Over the last decade, substantial work has been carried out, increasing our insight in the properties and distribution of EPS and mechanisms by which they regulate various processes, including particle formation, sedimentation, organic carbon mineralization, microbial loop, cycling of dissolved metals and in sediment ecology and biogeochemistry. In this review, we attempt to highlight these recent findings and critically evaluate the significance of microbial EPS in marine environments, with special emphasis on their role in marine biogeochemistry.

Among all the marine microorganisms, bacteria[12] and phytoplankton, including diatoms[13], cyanobacteria[14] and dinoflagellates[15] are considered to be the major sources of EPS in marine waters. Hence, various aspects of EPS discussed hereafter indicate bacterial and/or phytoplankton origin unless specified.

Characteristics of EPS

Microorganisms grow in free planktonic state[16,17] or are attached to surfaces (natural and man-made structures)[4,18]. During their life cycle, bacteria, and to a lesser extent phytoplankton, produce EPS and the maximal EPS production generally occurs at the end of the growth phase of many bacteria and phytoplankton species[19-21]. In both natural environments and the laboratory, microorganisms tend to produce more EPS under nitrogen-limiting conditions. For example, an increase in dissolved and particulate carbohydrate concentrations has been reported at the end of phytoplankton growth in the oceanic environments[22], mesocosm studies[23] and laboratory studies[24,25], coinciding with depletion of nutrients like nitrate and phosphate. Other factors that influence the growth of organisms like pH, salinity, aeration and temperature may also influence the production of EPS[1,3].

EPS produced by the microorganisms exist as tightly bound (capsular), loosely adhered (slime type) to cells (Figure 1) or as free dissolved matter (component of dissolved organic matter; DOM). The capsule has a more organized polymeric structure, densely packed, less diffusible and tightly bound to the cell than slimes. Capsular EPS are held to the cell wall either by linkages between the carboxyl groups of EPS and hydroxyl groups of lipopolysaccharides (LPS)[26] or by a covalent bonding through phospholipids[27]

and glycoproteins[28]. The physico-chemical properties of EPS are attributed to their diverse and complex chemistry that changes with species diversity, age and, growth conditions, etc.

EPS chemistry

Chemically, EPS are rich in high molecular weight polysaccharides (10 to 30 kDa) and generally have heteropolymeric composition (Figure 2). The polysaccharide chain might be unbranched or branched with side chains of other compounds attached to the polymeric chain[26]. Generally, the polysaccharides are made of monosaccharides – with hexoses and pentoses forming the bulk of EPS. However, the contribution of different monomers to the total polysaccharide varies with the source and such variations in the polysaccharide chain composition can alter its physico-chemical properties. For example, bacterial EPS are generally rich in hexoses like glucose and galactose[8], whereas phytoplankton EPS have relatively higher content of sugars like rhamnose, xylose and mannose[1]. Such variations in monomer composition can alter the properties of EPS. The presence of sugars like arabinose in EPS helps in cell aggregation in bacteria[29], whereas deoxy sugars like fucos and rhamnose found in diatom EPS help in foaming and flocculation[30]. Apart from polysaccharides, EPS also contain fair amounts of proteins, non-sugar moieties like uronic acid, pyruvates, hexosamines, acetates, sulphate esters[31,32] and generally small amounts of lipids and nucleic acid. Although these non-sugar components are present in relatively smaller quantities, they are generally attached to the sugar residues[33] and are important in imparting unique characteristics to the EPS[3].

The composition of EPS varies considerably between phytoplankton and bacteria (Table 1), which in turn reflects on its properties and fate. For example, one of the major components of bacterial EPS is uronic acid, constituting up to 20–50% of the polysaccharide fraction[31]. On the other hand, phytoplankton EPS are relatively poor in uronic acid concentrations (<5%)[1]. The higher yield of

uronic acid and other moieties, including pyruvates and acyl groups gives an overall negative charge to the polymer, thereby imparting binding and adsorptive properties to the polymer[3]. Furthermore, the presence of pyruvates and acyl groups prevents the epimerization of uronic acid, thereby ensuring higher content of uronic acid in the polymer[3]. EPS are also involved in flocculation and the rate of floc formation is controlled by nature of the EPS. For example, EPS rich in sulphates have the ability to generate flocs in the presence of deoxy sugars[30]. Sulphates present in EPS also have the capacity to hold water molecules, which play an important role in imparting gel-like consistency to the EPS[2]. Moreover, EPS rich in acidic moieties are refractile and hence take longer time for bacterial decomposition and digestion by feeders[3]. Apart from organic non-sugar moieties, cations like Ca^{2+} and Mg^{2+} have also been widely reported

Table 1. Chemical characteristics of bacterial and phytoplankton EPS. All values are expressed as % (w/w)

| Culture	Carbo-hydrates	Pro-teins	Uronic acid	Sul-phates	Ref-erence
Bacteria					
Marinobacter sp.	16.9	43.9	17.6	2.7	98
Vibrio sp.	51.1	1.8	14.9	–	31
Halebacter eurihalina	36.3	10.2	1.2	–	99
Acetobacter infernos	57	4	42	8.8	100
A. macleodii	42	4	38	5	101
Phytoplankton					
Amphora costrata	22	7	23	10	102
Rhodotorula acheniorum	85.6	5.6	–	–	103
Navicula salinarum	80.8	4.9	4.8	–	104
N. subinflata	74.7	1.9	8.6	9.6	26
Amphora coffeaformis	41.2	5.9	17.9	7.9	25
Chaetoceros affinis	80	1.5	–	9	1
Coscinodscus nobilis	63	–	9.3	17	1
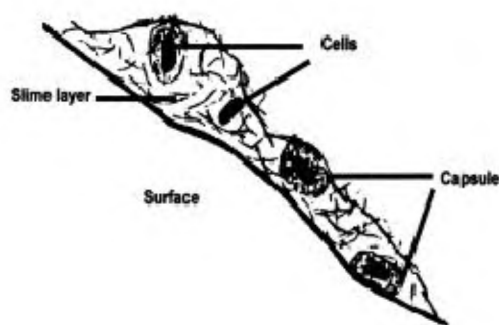
Figure 1. Schematic representation of biofilm showing cells enveloped with a capsule and embedded in a matrix of slime EPS. (Reproduced from Decho[3].)
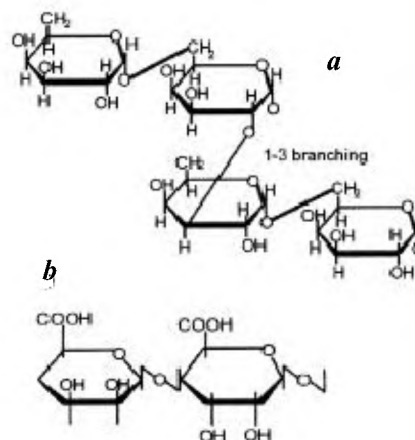

Figure 2. Chemical structure of dextran, a homopolysaccharide made of α-1-6 glucan monomers with 1–3 branched linkages (*a*) and bacterial alginate, a heteropolysaccharide having a combination of D-mannuronic acid and L-guluronic acid linked by β-1,6 linkage (*b*).

in both bacterial and diatom EPS[34,35]. The presence of divalent cations helps in multiple cross-linkages between the sugar molecules of different polysaccharide chains, thereby imparting a high degree of consistency and stability to the EPS[36]. Such differences in EPS chemistry therefore play a significant role in determining the fate of organic matter in marine environments.

EPS in marine environment

Constituent of dissolved organic matter

The fixing of CO_2 in the presence of sunlight and inorganic nutrients like ammonia, nitrate and phosphates and its conversion to different organic biomolecules (both cellular and extracellular) is defined as primary production (PP)[37]. A major fraction of the PP is released into the aquatic environments as EPS that contribute to the oceanic DOM pool[38]. The fraction of PP released as EPS, however, varies to a large extent with the method employed for estimation, species used and growth conditions. Generally, EPS may constitute <3% to more than 90% of the PP[39]. Since much of the EPS is released in the dissolved form, it is an important constituent (up to 50%) of the semi-labile DOM in oceanic waters[40]. Recent microscopic (transmission electron and atomic force microscopy) and NMR studies reveal that fibrillar polysaccharides formed the bulk of oceanic DOM[41] and there was close similarity between the oceanic DOM composition and the EPS released by certain phytoplankton species[42].

Unlike phytoplankton, bacterioplankton populations range between 10^6 and 10^9 ml^{-1} in the marine waters[43,44], are ubiquitously distributed (geographically and spatially), form the largest living surface in the oceanic environment[45] and have been isolated from practically all depths and extreme environments, including hydrothermal vents[46], deep-sea sediments[47] and cold seeps[48]. Moreover, under oligotrophic conditions, the bacterial biomass may exceed the phytoplankton biomass even in euphotic zones[49]. Most metabolically active bacteria produce EPS continuously and hence are an uninterrupted source of DOM[50,51], which in comparison to phytoplankton EPS, are a relatively less labile organic carbon source and tend to accumulate in marine environments[52,53]. However, unlike phytoplankton EPS, the significance of bacterial EPS in marine environments is not clearly understood since not much is known about the contribution of bacterial EPS to the DOM.

EPS in particulate form

In marine environments, microbial EPS exist as particles in the size range of few nm to few μm in length to form colloids[11] and account for up to 40% of the total organic carbon pool[54]. In the particulate form, EPS exists either as free discreet particles ranging from few μm to few hundred μm in length or in association with other particles-living and dead and other macroaggregates[54,55]. The particulate form of EPS plays an important role in the production, fluxes of particulate organic matter within the ocean environments and its transfer over various trophic levels. Some of these different forms of particulate EPS have been discussed to highlight their importance in marine environments.

EPS as transparent exopolymer particles

Transport exopolymer particles (TEP) are one type of EPS that are produced by both diatoms and bacteria during their growth (Figure 3). Alldredge et al.[56] first reported these particles during a phytoplankton bloom and since then, have been reported from various marine environments[57–59]. However, recent studies have shown that TEP can be generated abiotically from EPS of bacterial[45] and phytoplankton[60] origins. Apart from phytoplankton and bacteria, TEP is also produced from other sources like mucus produced by bivalves[61] and benthic macroalgae[62], which may have significance in coastal waters. TEP abundance and size range from 1 to 10^4 ml^{-1} and <2 μm to >500 μm respectively[11]. Chemically, these particles are made of acid polysaccharides and are stained by acid-polysaccharide-specific anionic stains like alcian blue[56] (pH 2.5). The concentrations of TEP, however, vary with source of the EPS, age of the bloom and presence of TEP-utilizing bacterial heterotrophs[11,63].

The biotic production of TEP occurs during the growth of phytoplankton and to some extent of bacteria. On the other hand, some of the abiotic modes of TEP production from DOM include bubble adsorption, surface coagulation, turbulent shear and laminar shear[11]. Once released into the surrounding waters either actively (as EPS) or passively (cell-lysis), TEP precursors of sub-micron dimensions coagulate to form larger particles. Stickiness of TEP or α', collision frequency of the precursors, presence of nuclei like bacterial/phytoplankton cells and divalent cation concentrations influence the production and stability of TEP[11].
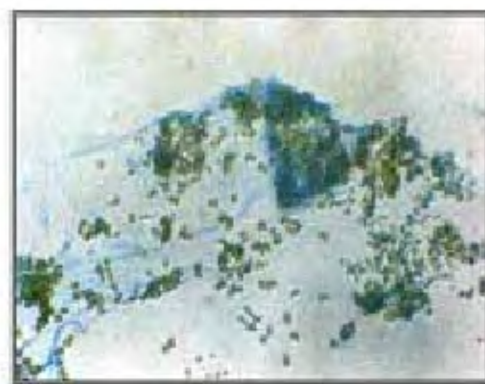

Figure 3. Photograph showing presence of transparent exopolymer particles in natural sample. Particles are stained blue with alcian blue (pH 2.5) and are associated with cells of *Phaeocystis* sp.

Biofilms

One of the more important functions of EPS involves adhesion of cells to natural and artificial surfaces–animate or inanimate. During adhesion, microorganisms first come closer to the surface where weak van der Waal forces of attraction hold them and the organisms can be easily removed from the surface by moderate shear forces[2]. This is defined as 'reversible sorption', wherein factors like electrostatic forces, surface charges, hydrophobicity of the surface and availability of cations play an important role. Once the cells are held closer to the surface, they produce EPS that enable the cells to attach irreversibly to the surface and grow, thereby forming biofilms[64].

The formation of microbial biofilms results in the conditioning of the surface, serving as a cue for other larger organisms to colonize the surfaces[65,66]. The colonization of surfaces by larger organisms is called fouling and is commonly encountered in all submerged structures, affecting their strength and performance. Bacterial biofilms on metal surfaces are also involved in regulation of corrosion. Although EPS may not be directly involved in inducing or inhibiting corrosion of metals, the presence of EPS in biofilms helps in creating an ideal environment that influences corrosion processes[67].

The adhering properties of EPS also play an important role in cell aggregation and floc formation. EPS produced by bacteria have been considered essential for the formation and maintenance of microcolonies and oxygen-free conditions within the flocs that influence the spatial distribution of different micro-organisms[68]. Such niches in the micro-colonies become the hotspots for microbial-aided organic transformation and elemental cycling. The formation of aggregates also helps in sequestering nutrients, trace metals and other essential elements, thereby increasing their accessibility to the microorganisms[3].

Apart from adhesion, EPS in biofilms are also implicated for gliding motility in bacteria and diatoms[4]. In sediments, motile diatoms use EPS to migrate within the sediment layers in response to changing light and aeration conditions[1]. This is most commonly observed in tidal mudflats and inter-tidal zones, which get periodic exposure to sunlight. The microbial EPS are highly hydrated structures (~99% water) that provide protection to the cells against desiccation. Such properties enhance the survival of micro-organisms in the inter-tidal zones and mudflats that remain exposed during low tides[4]. The presence of EPS in microbial mats helps in binding the sediment together, thereby providing stability to surface sediments and reducing erosion[4]. The hydrated nature helps EPS to behave like a sorptive sponge that enables them to sequester essential nutrients, DOM and dissolved metals[3]. EPS-coated microbial cells in microbial mats are frequently ingested by deposit feeders while feeding on sediment biofilms. However, such cells have been found to escape digestion and frequently get excreted. It has been argued that the complex structure

and chemistry of EPS tightly bound to cells play an important role in resistance to enzymes in the gut of animals[69].

EPS in marine biogeochemical processes

The distribution of EPS over a range of size classes, their overall contribution to the total organic carbon pool and their unique properties make them an integral component of marine biogeochemical processes, including cycling of elements (C, N, S) and metals and the marine food web (Figure 4). The entire biogeochemical process in Figure 4 can be segregated into biotic and abiotic controlled processes. The photosynthetic fixation of atmospheric carbon by phytoplankton, its aggregation and formation of marine snow, sedimentation and remineralization by microbial heterotrophs and other particle-feeders along with the production of DOM and nutrients form a major component of the biotic process. During the biotic process, organic matter is coupled between dissolved and particulate forms, thereby rerouting important elements like carbon and nitrogen through different trophic levels before reaching the sediments. In the abiotically controlled marine process, EPS as DOM and particulates like TEP may coagulate or
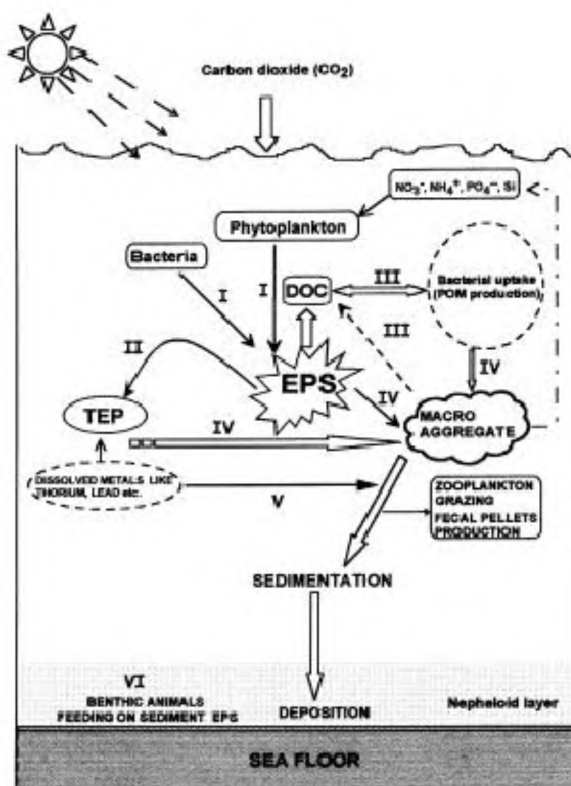

Figure 4. Schematic representation of the various roles of EPS in marine environment. Numbering indicates the different processes involving EPS. I, Production of EPS by bacteria and phytoplankton; II, Production of TEP from EPS; III, Microbial loop; IV, Formation of particles; V, Chelation of dissolved metals and VI, EPS as a carbon source for benthic community.

scavenge other particulate matter to form larger particles. Similarly, it may also either adsorb or bind dissolved metals and other charged species of toxic compounds and remove them from the water column. Upon reaching the sediment, the bound metals may further undergo biotic or abiotic transformation before getting buried into the sediment.

EPS in aggregation process

In simple terms, aggregation is defined as a physical process wherein micro particles collide and remain stuck to each other to form clumps of particles called aggregates. These aggregates are fractal in nature, highly porous, fragile and of varying size range. Aggregation is a common but unique process in marine environments that occurs generally in the euphotic waters, but may also take place in the pelagic waters. The process of aggregation is influenced by particle density, collision frequency and binding properties of the EPS[70,71]. Aggregation in the natural environment involves both biotic and abiotic processes. In the biotic process, live cells of phytoplankton and bacteria stick to each other either due to the presence of EPS that serve as biological glue or by cell–cell attachment. Aggregation has been reported from both laboratory and field observations. For example, the microbial degradation of dissolved organic matter leads to the production of particulate matter. These particles were clumps of bacterial cells glued together by EPS[72]. On the other hand, both field and laboratory observations of phytoplankton, including species of *Skeletonema, Chaetoceros* and dinoflagellates indicate increased aggregation in the presence of TEP released during their growth[11]. In most cases, production of TEP and TEP-influenced formation of large particles occurred during senescence of phytoplankton blooms. Such production of particles during a bloom might be due to higher particle abundance, increased concentration of EPS/TEP in the water column and EPS chemistry[73,74]. EPS production by phytoplankton may be in response to nutrient depletion, cell physiology, changes in growth conditions and/or bacterial-phytoplankton interactions[63,75].

In contrast to the biotic process, the production of TEP from dissolved EPS under abiotic conditions is another important mechanism of aggregation. In this process, particles like TEP are produced due to surface adsorption processes, self-coagulation and shear[11]. The production of TEP, its sticky nature and ability to coagulate make it important in flocculation of diatoms[11] and serves as a link between DOM and particulate organic matter (POM). Moreover, EPS in general and TEP in particular, either directly scavenge other suspended particles or trap them in mesh-like structures. TEP/EPS may also be coated out the particles, changing the stickiness of the sinking particles and thereby influencing their coagulation. Such EPS/TEP matrix having various forms of suspended particles, including live cells, cell debris, detrital matter, faecal pellets, clay minerals, etc. collectively form aggregates called marine snow[76]. The production

of marine snow is a major source of POM to the benthos and its production and fate are unique features of marine biogeochemical processes[68].

EPS in particle sedimentation and carbon flux

The sinking of large sized (>50 μm) particulate matter from the surface to the deeper waters is called sedimentation. This process generally coincides with increase in concentration of EPS at the termination of phytoplankton blooms[73]. In natural environments, microbial cells are buoyant in nature and do not sink by themselves. In the presence of EPS, microbial cells tend to coagulate along with other particulate matter to form macroaggregates as described earlier, attain a critical mass and start sinking into the deeper waters. Sedimentation of particles has ecological, climatological and biogeochemical significance. Ecologically, sedimentation of phytoplankton aggregates is considered essential for the survival of phytoplankton cells in marine environments. For example, sedimentation can help phytoplankton escape predation; nutrient depleted conditions and get transported to favourable growth conditions[77]. Moreover, spores of diatoms also aggregate and sink into deeper waters, serving as seed for future blooms.

Since sedimentation serves as a pump for transferring the atmospheric carbon into the deep ocean, the efficiency of carbon pumping plays an important role in the biogeochemical cycling of organic carbon. In the open ocean, only 10% of the photosynthetic carbon may escape the euphotic layer as sinking particles, of which only 1% reaches the deep-sea sediments[78]. One reason for such low organic carbon flux may be the tightly coupled response of grazers and bacterial community to EPS produced by phytoplankton. For example, natural bacterioplankton population can easily hydrolyse dissolved EPS released during photosynthesis, thereby reducing the production of larger aggregates[63]. Similarly, particle associated bacteria might utilize EPS coatings and macroaggregates using an array of extracellular enzymes and release them into dissolved form[79]. On the other hand, the aggregates coated with EPS serve as carbon source for grazers like zooplankton in the water column[80]. The active grazing and repackaging of aggregates as faecal pellets also reduces the amount of organic carbon sedimenting into the pelagic waters. Such a response would lead to disintegration of the sinking macroaggregates releasing nutrients and most of the organic matter might get remineralized in the euphotic zone. Moreover, the presence of TEP-coated diatoms has been found to be more buoyant than non-TEP coated diatoms, thereby reducing the overall sedimentation rates[81]. Such phenomena increase the residential time of the POM, allowing efficient grazing and mineralization within the euphotic zone.

On the contrary, formation of EPS, coated macroaggregates and their sedimentation indicate an uncoupling between the grazers and phytoplankton population. Various factors, including EPS chemistry, resistance to bacterial

breakdown of EPS and low grazing of aggregates are attributed to the formation and sedimentation of macroaggregates in marine waters. For example, EPS produced by *Phaeocystis* might resist bacterial mineralization and lead to the formation of large aggregates[82]. Moreover, depletion of essential nutrients like nitrogen and phosphorus and adsorption of colloids can also limit the bacterial solubilization of phytoplankton EPS. Similarly, the presence of EPS may not always support the grazing activity of higher organisms. For example, feeding experiments showed that particles coated with TEP and free TEP may not be always preferred by zooplankton. TEP produced during blooms of *Thallasiosira* caused aggregation, as grazers like copepods did not prefer to consume free TEP[83]. TEP are generally considered to be highly sticky in nature and this stickiness might be one reason for the avoidance of grazing of free TEP. Due to the accumulation of free TEP, the probability of diatom cells aggregating also increase. On the other hand, diatoms coated with TEP were actively grazed upon by copepods. Such response by grazers in the presence of EPS indicates that the quality of EPS and their fate is directly linked to the quality of particles reaching the sediments, thereby influencing the benthic community structure and biomass.

EPS in microbial loop

Since a major fraction of the photosynthetic carbon (>90%) might be released as soluble EPS[78], much of the photosynthetic carbon should remain unavailable to higher organisms and escape the classical food-chain. However, this does not happen as the soluble form of EPS released by the phytoplankton serves as an organic carbon source and is converted to particulate form by marine bacteria[79]. Although the amount may vary considerably, an average of about 50% of the photosynthetic carbon is channelized through bacteria within the microbial loop[84]. Bacterial conversion of dissolved EPS to POM is critical in meeting the carbon demand of the grazing community. The POM so formed becomes a source of carbon for grazers like ciliates, flagellates, etc. thus channelizing the organic carbon into the classical food-chain[80]. Moreover, bacterial decomposition and dissolution of aggregates with the release of DOM, feeding/grazing of bacterial microaggregates by micro-zooplankton and subsequent feeding by macrozooplankton are the other organic carbon transformations occurring within the microbial loop.

The significance of the microbial loop is not restricted to the marine food-web, but might also help in controlling the atmospheric carbon dioxide levels. Recent evaluation on the production of TEP in relation to CO_2 concentrations indicates that changes in dissolved CO_2 levels positively influenced TEP production[85]. Therefore, the increase in atmospheric carbon levels would be made available to the microbial heterotrophs through the release of EPS, thereby influencing the amount of organic carbon channelized through the microbial loop. Other conservative global esti-

mates indicate that the amount of carbon dioxide assimilated into the microbial loop after photosynthesis is equivalent to five times the anthropogenic carbon dioxide emission[86]. Various physico-chemical and biological factors, including the quality and the quantity of EPS, their residence times, microbial community and the secondary consumers play an important role in regulating the efficiency of the marine microbial loop, which in turn may have significant climatological implications.

Removal of metal ions and its cycling

Microbial EPS are also known for their ability to bind a wide range of metals, including heavy metals like Pb, Co, Sr, Cr, Th and Cd[87,88]. Microbial EPS isolated from biofilms have been often used to study the binding of metals[89]. Binding of metal ions by microbial EPS may have several implications. For example, binding of dissolved ions by EPS is considered essential in the vertical transport of these ions. EPS in aggregates have been reported to contain metal precipitates that accumulate with depth[3]. Once the metal ions reach the sediments, they undergo microbial transformation and may leach back into the overlaying waters. Particulate EPS like TEP can bind more than 90% of the dissolved thorium ions and thereby alter the rate of removal of thorium from pelagic waters[90]. Since thorium is used as a proxy to estimate the flux of organic carbon in the open ocean, such binding of thorium to TEP can lead to discrepancy in the estimates of carbon flux.

Negative impact of EPS

Production of large aggregates in coastal waters can also be a nuisance factor, since it may lead to organic loading in these waters. Mucilaginous polysaccharides of certain phytoplanktons form large aggregates that sink to the bottom sediments[91]. Such accumulation of aggregates increases the oxygen demand, causing anoxic conditions in the bottom waters. These anoxic conditions cause large-scale mortality of benthic fauna in these areas[92]. Similarly, near-bottom anoxic waters would lead to anaerobic degradation of organic matter, leading to increased production of greenhouse gases[93] like H_2S and N_2O. Microbial EPS also play an important role in the fouling and corrosion of economically important submarine structures, thereby causing damage to property and economic losses[94]. The biofilms of surface colonizing bacteria and diatoms are rich in carboxylated sugar residues and sulphate radicals that alter the overall characteristics of the EPS. The acidic polysaccharides in the biofilms tend to ionize metals, thereby causing thinning of metal structures. On the other hand, biofilms of sulphate reducers provide an anaerobic environment wherein aggressive corrosion enhancing processes might take place[8,95]. EPS produced by surface-colonizing microorganisms are generally rich in monosaccharides like glucose and mannose. These sugar components may serve as cues for the settlement and colonization of surfaces by macrofoulers[66].

Since marine biofilms and microbial EPS are good sorbents of metals, they tend to accumulate toxic metals and other pollutants. Similarly, macroaggregates that are rich in EPS also scavenge toxic metals and pollutants from the surface waters before sinking to bottom sediments[68]. These macro-aggregates and biofilms also serve as a good organic carbon source, are actively fed upon by grazers, deposit feeders and filter feeders, thereby gaining entry into the marine food-web[3]. Such ingestion of metal-laden biofilms and aggregates leads to increased accumulation of these compounds in the tissues of the feeders, which can affect the fish catchment due to increased mortality and the health of the people consuming these marine organisms contaminated with toxic chemicals[3].

Future studies

The inter-conversion of DOM–POM is one of the major processes that regulate the fluxes of organic carbon and other essential elements in the marine environment. Intensive research in the last decade suggests that both biotic and abiotic processes contribute to the production of particles in the aquatic environments[11,95,96]. In marine environments, EPS are always associated with other organic matter making it difficult to identify the sources of EPS. Moreover, few studies using sophisticated instrumentation[42], antibody techniques and organic biomarkers[97] have not been able to provide sufficient information on the origin/source of EPS in natural waters. Therefore, concerted efforts are required to study the chemistry of microbial EPS and DOM and evaluate the contribution of EPS to DOM using better methodologies.

It is well-known that microbial EPS (both dissolved and particulate) are degraded by bacterial heterotrophs and consumed by other feeders in the marine food-web. During the last decade, there has been considerable shift in our understanding of the marine food-web and the importance of the microbial loop. Since the bacterial degradation of EPS is regulated by substrate concentration and composition, studies on the chemistry and turnover rates of EPS in natural waters shall help assess the factors that regulate the accumulation and residence time of dissolved EPS in marine waters, their transformation from dissolved to particulate, and sedimentation of adsorbed EPS to the benthos. Another area that has not been explored much is the change in bacterial diversity during degradation of dissolved EPS. Intensive studies using molecular techniques like DGGE or T-RFLP need to be carried out to assess the microbial diversity and reveal the changes in microbial community during the degradation of EPS.

Many organisms are known to feed on microbial EPS and utilize them as an alternate carbon source[3]. Studies carried out till date suggest that microbial EPS are not uniformly labile and their uptake and incorporation by other organisms varies considerably. However, the available information is inadequate to explain the variability in the uptake and incorporation of EPS in both laboratory and field studies. Since marine microbes (bacteria, phytoplankton, fungi, etc.) are constantly interacting with EPS, these interactive sites act as hotspots for the transformation of organic matter, modification of micro-environment and community structure. However, laboratory studies of these micro-environments are few[97] and lack of appropriate tools restrict in situ studies of various processes occurring in these micro-niches. Therefore, newer techniques need to be developed to study the microbial processes that have a significant impact on the ocean–atmospheric exchanges on a global scale.

The recent discovery of EPS binding with ^{234}Th and enhancing the sedimentation hints at the possible discrepancies that might arise while using this isotope as a proxy for the measurement of sedimentation rates in oceanic environments. Similarly, such binding might also occur with metal proxies for production and influence their sedimentation. On the other hand, the bioavailability of essential dissolved metals like Fe that regulate primary production might be influenced by the presence of EPS. The role of EPS in recycling such trace elements and the impact of EPS on palaeoecological studies are other exciting areas of future research. The multiple roles of EPS (dissolved, colloids and particulate) make them an indispensable channel for carbon fluxes and influence the global biogeochemical processes. Hence, the influence of EPS needs to be considered while estimating global carbon budgets. It is also important to include the impact of EPS while developing models on the formation of particles, microbial–particle interactions, marine food-web and biological transformation processes in the marine environment.

1. Hoagland, K. D., Rosowski, J. R., Gretz, M. R. and Roemer, S. C., Diatom extracellular polymeric substances: Function, fine structure, chemistry, and physiology. *J. Phycol.*, 1993, **29**, 537–566.
2. Wingender, J., Neu, T. R. and Flemming, H. C., In *Microbial Extracellular Polymeric Substances – Characterization, Structure and Function*, Springer Publishers, 1999.
3. Decho, A. W., Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Annu. Rev.*, 1990, **28**, 73–153.
4. Decho, A. W., Microbial biofilms in intertidal systems: an overview. *Cont. Shelf Res.*, 2000, **20**, 1257–1273.
5. Prakash, B., Veeregowda, B. M. and Krishnappa, G., Biofilms: A survival strategy of bacteria. *Curr. Sci.*, 2003, **85**, 1299–1307.
6. Sutherland, J. W., Microbial exopolysaccharides from Gram negative bacteria, *Int. Dairy J.*, 2001, **11**, 663–674.
7. Characklis, W. G. and Cooksey, K. E., Biofilms and microbial bio-fouling. *Adv. Appl. Microbiol.*, 1983, **29**, 93–138.
8. Sutherland, I. W., *Microbial Extracellular Polymeric Substances* (eds Wingender, J., Neu, T. R. and Flemming, H. C.), Springer Publications, Berlin, 1999.
9. Weiner, R. M., Biopolymers from marine prokaryotes. *Trends Biotech.*, 1997, **15**, 390–394.
10. De Phillipis, R. and Vincenzinni, M., Exocellular polysaccharides from cyanobacterial and their possible applications. *FEMS Microbiol. Rev.*, 1998, **22**, 151–175.
11. Passow, U., Transparent exopolymer particles (TEP) in aquatic environments. *Prog. Oceanogr.*, 2002, **55**, 287–333.
12. Heissenberger, A., Leppard, G. G. and Herndl, G. J., Ultrastructure of marine snow. 2. Microbiological considerations. *Mar. Ecol. Prog. Ser.*, 1996, **135**, 299–308.

13. Myklestad, S. M., Production of carbohydrates by marine planktonic diatoms. II. Influence of the N/P ratio in the growth medium on the assimilation ratio, growth rate, and production of cellular and extracellular carbohydrates by *Chaetoceros affinis* var. *willei* (Gran) Hustedt and *Skeletonema costatum* (Grev.) Cleve. *J. Exp. Mar. Biol. Ecol.*, 1977, **29**, 161–179.
14. Singh, N., Asthana, R. K., Kayastha, A. M., Pamndey, S., Chaudhary, A. K. and Singh, S. P., Thiol and exopolysaccharide production in a cyanobacterium under heavy metal stress. *Process Biochem.*, 1999, **35**, 63–68.
15. Brachvogel, T., Schweitzer, B. and Simon, M., Dynamics and bacterial colonization of microaggregates in a large mesotrophic lake. *Aquat. Microb. Ecol.*, 2001, **26**, 23–35.
16. Jahn, A. and Nielsen, P. H., Cell biomass and exopolymer composition in sewer biofilms. *Wat. Sci. Tech.*, 1998, **37**, 17–24.
17. Nelson, Y. M., Lion, L. W., Ghiorse, W. C. and Shuler, M. L., Lead binding to metal oxides and organic phases of natural aquatic biofilms. *Limnol. Oceanogr.*, 1999, **44**, 1715–1729.
18. Vandevivere, P. and Kirchman, D. L., Attachment stimulates exopolysaccharide synthesis by a bacterium. *Appl. Environ. Microbiol.*, 1993, **59**, 3280–3286.
19. Williams, A. G. and Wimpenny, J. W. T., Exopolysaccharide production by *Pseudomonas* NCIB 11264 grown in batch culture. *J. Gen. Microbiol.*, 1977, **102**, 13–21.
20. Williams, A. G. and Wimpenny, J. W. T., Exopolysaccharide production by *Pseudomonas* NCIB 11264 grown in continuous culture. *J. Gen. Microbiol.*, 1978, **104**, 47–57.
21. Myklestad, S. M. and Haug, A., Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *willei* (Gran) Hustedt. II. Preliminary investigation of the extracellular polysaccharide. *J. Exp. Mar. Biol. Ecol.*, 1972, **9**, 137–144.
22. Ittekkot, V., Brockmann, U., Michaelis, W. and Degen, E. T., Dissolved free and combined carbohydrates during a phytoplankton bloom in the northern North Sea. *Mar. Ecol. Prog. Ser.*, 1981, **4**, 299–305.
23. Mopper, K., Zhou, J., Sri Ramana, K., Passow, U., Dam, H. G. and Drapeau, D. T., The role of surface active carbohydrates in the flocculation of a diatom bloom in a mesocosm. *Deep-Sea Res.-I*, 1995, **42**, 47–74.
24. Bhosle, N. B., Sawant, S. S., Garg, A. and Wagh, A. B., Isolation and partial chemical analysis of exopolysaccharides from the marine fouling diatom *Navicula subinflata*. *Bot. Mar.*, 1995, **38**, 103–110.
25. Bhosle, N. B., Sawant, S. S., Garg, A. and Wagh, A. B., Chemical characterization of exopolysaccharides from the marine fouling diatom *Amphora coffeaeformis*. *Biofouling*, 1996, **10**, 301–307.
26. Sutherland, I. W., Microbial exopolysaccharide synthesis. In *Extracellular Microbial Polysaccharides* (eds Sanford, P. A. and Laskin, A.), Am. Chem. Soc., Washington, 1977, pp. 40–57.
27. Roberts, I. S., The biochemistry and genetics of capsular polysaccharide production in bacteria. *Ann. Rev. Microbiol.*, 1996, **50**, 285–315.
28. Chester, I. R. and Murray, R. G. E., Protein–lipid–lipopolysaccharide association in the superficial layer of *Spirillum serpens* cell walls. *J. Bacteriol.*, 1978, **133**, 932–941.
29. Efrat, B. S., Susana, C. S. and Yaacov, O., Arabinose content of extracellular polysaccharide plays a role in cell aggregation of *Azospirillum brasilense FEMS Microbiol. Lett.*, 2004 (in press).
30. Zhou, J., Mopper, K. and Passow, U., The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnol. Oceanogr.*, 1998, **43**, 1860–1871.
31. Majumdar, I., D'Souza, F. and Bhosle, N. B., Microbial exopolysaccharides: effect on corrosion and partial chemical characterization. *J. Indian Inst. Sci.*, 1999, **79**, 539–550.
32. Kennedy, A. F. D. and Sutherland, I. W., Analysis of bacterial exopolysaccharides. *Biotechnol. Appl. Biochem.*, 1987, **9**, 12–19.
33. Sutherland, I. W., Microbial exopolysaccharides: control of synthesis and acylation. In *Microbial Polysaccharides and Polysaccharases* (eds Berkeley, R. C. W. *et al.*), Ellis Horwood Limited, Chichester, 1979, pp. 329–338.
34. Marshall, K. C., Stout, R. and Mitchell, R., Mechanism of initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.*, 1971, **68**, 337–348.
35. Cooksey, K. E., Requirement for calcium in adhesion of a fouling diatom to glass. *Appl. Environ. Microbiol.*, 1981, **41**, 1378–1382.
36. Smidsrod, O., Molecular basis of some physical properties of alginate in the gel state. *Faraday Discuss. Chem. Soc.*, 1974, **37**, 263–274.
37. Lalli, C. M. and Parsons, T. R. (eds), *Biological Oceanography: An Introduction*, The Open University Set Book, Butterworth-Heinemann Publications, Oxford, 1997.
38. Sell, A. F. and Overbeck, J., Exudates: phytoplankton–bacterioplankton interactions in Plussee. *J. Plankton Res.*, 1992, **14**, 1199–1215.
39. Raymont, J. E. G. and Sloan, P. R., *J. Fish Res. Biodivers. Can.*, 1965, **22**, 1083–1097.
40. Kirchman, D. L., Meon, B., Ducklow, H. W., Carlson, C. A., Hansell, D. A. and Steward, G. F., Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. *Deep Sea Res.-II*, **48**, 4179–4197.
41. Santschi, P. H., Balonis, E., Wilkinson, K. J., Zhang, J. W. and Buffle, J., Fibrillar polysaccharides in marine macromolecular organic matter as imaged by atomic force microscopy and transmission electron microscopy. *Limnol. Oceanogr.*, 1998, **43**, 896–908.
42. Aluwihare, L. I. and Repeta, D. J., A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae. *Mar. Ecol. Prog. Ser.*, 1999, **186**, 105–117.
43. Ramaiah, N., Raghukumar, C., Sheelu, G. and Chandramohan, D., Bacterial abundance, communities and heterotrophic activities in the coastal waters off Tamil Nadu. *Indian J. Mar. Sci.*, 1996, **25**, 234–239.
44. Bode, A., Barquero, S., Varela, M., Braun, J. G. and De-Armas, D., Pelagic bacteria and phytoplankton in oceanic waters near the Canary Islands in summer. *Mar. Ecol. Prog. Ser.*, 2001, **209**, 1–17.
45. Stoderregger, K. and Herndl, G. J., Production of exopolymer particles by marine bacterioplankton under contrasting turbulence conditions. *Mar. Ecol. Prog. Ser.*, 1998, **189**, 9–16.
46. Cowen, J. P., Massoth, G. and Winn, C. D., Bacterial scavenging of Mn and Fe in mid- to far-field plumes of hydrothermal vent origin. 7th International Symposium Environmental Biogeochemistry, 1985, p. 30.
47. Deming, J. W., Bacterial growth in deep-sea sediment trap and box core samples. *Mar. Ecol. Prog. Ser.*, 1985, **25**, 305–312.
48. Hamamoto, T., Kaneda, M., Kudo, T. and Horikoshi, K., Characterization of a protease from psychrophilic *Vibrio* sp. strain 5709. *J. Mar. Biotechnol.*, 1995, **2**, 219–222.
49. Herndl, G. J., Microbial biomass dynamics along a trophic gradient at the Atlantic barrier reef off Belize (Central America). *P.S.Z.N.-I Mar. Ecol.*, 1991, **12**, 41–51.
50. Heissenberger, A. and Herndl, G. J., Formation of high molecular weight material by free-living marine bacteria. *Mar. Ecol. Prog. Ser.*, 1994, **111**, 129–135.
51. Brophy, J. E. and Carlson, D. J., Production of biologically refractory dissolved organic carbon by natural seawater microbial populations. *Deep-Sea Res.-I*, 1989, **36**, 497–507.
52. Tranvik, L. J., Microbial transformation of labile dissolved organic matter into humic-like matter in seawater. *FEMS Microbiol. Ecol.*, 1993, **12**, 177–183.
53. Benner, R., Pakulski, J. D., McCarthy, M., Hedges, J. I. and Hatcher, P. G., Bulk chemical characterization of dissolved organic matter in the ocean. *Science*, 1992, **255**, 1561–1564.
54. Decho, A. W. and Herndl, G. J., Microbial activities and the transformations of organic matter within mucilaginous material. *Sci. Total Environ.*, 1995, **165**, 33–42.
55. Passow, U., Alldredge, A. L. and Logan, B. E., The role of particulate carbohydrate exudates in the flocculation of diatom blooms. *Deep-Sea Res.-I*, 1994, **41**, 335–357.
56. Alldredge, A. L., Passow, U. and Logan, B. E., The abundance and significance of a class of transparent organic particles in the ocean. *Deep-Sea Res.-I*, 1993, **40**, 1131–1140.
57. Kumar, M. D., Sarma, V. V. S., Ramaiah, N., Gauns, M. and De Sousa, S. N., Biogeochemical significance of transparent exopolymer particles (TEP) in the Indian Ocean. *Geophys. Res. Lett.*, 1998, **25**, 81–84.
58. Krembs, C. and Engel, A., Abundance and variability of microorganisms and transparent exopolymer particles across the ice–water

interface of melting first-year sea ice in the Laptev Sea (Arctic). *Mar. Biol.*, 2001, **138**, 173–185.

59. Garcia, C. M., Prieto, L., Vargas, M., Echevarria, F., Garcia-Lafunte, J., Ruiz, J. and Rubin, J. P., Hydrodynamics and the spatial distribution of plankton and TEP in the Gulf of Cadiz (SW Iberian Peninsula). *J. Plankton Res.*, 2002, **24**, 817–833.

60. Passow, U., Formation of transparent exopolymer particles, TEP, from dissolved precursor material. *Mar. Ecol. Prog. Ser.*, 2000, **192**, 1–11.

61. McKee, M. P., Ward, J. E., Milke, L. M. and MacDonald, B. A., Release of mucopolysaccharides by bivalved molluscs and their contribution to the production of transparent exopolymer particles (TEP) in near shore waters. *J. Shellfish Res.*, 2000, **19**, 664.

62. Ramaiah, N, Yoshikawa, T. and Furuya, K., Temporal variations in transparent exopolymer particles (TEP) associated with a diatom spring bloom in a subarctic ria in Japan. *Mar. Ecol. Prog. Ser.*, 2001, **212**, 79–88.

63. Smith, D. C., Steward, G. F., Long, R. A. and Azam, F., Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep-Sea Res.-II*, 1995, **42**, 75–97.

64. Costerton, J. W., Irvin, R. T. and Cheng, K. J., The bacterial glycocalyx in nature and disease. *Annu. Rev. Microbiol.*, 1981, **35**, 299–324.

65. Swezyk, U., Holmstroem, C., Wrangstadh, M., Samuelsson, M. O., Maki, J. S. and Kjelleberg, S., Relevance of the exopolysaccharide of marine *Pseudomonas* sp. strain S9 for the attachment of *Ciona intestinalis* larvae. *Mar. Ecol. Prog. Ser.*, 1991, **75**, 259–265.

66. Khandeparkar, L., Anil, A. C. and Raghukumar, S., Factors regulating the production of different inducers in *Pseudomonas aeruginosa* with reference to larval metamorphosis in *Balanus amphitrite*. *Aquat. Microb. Ecol.*, 2002, **28**, 37–54.

67. Ford, T. and Mitchell, R., The ecology of microbial corrosion. *Adv. Microb. Ecol.*, 1990, **11**, 231–261.

68. Herndl, G. J., Marine snow in the Northern Adriatic Sea: Possible causes and consequences for a shallow ecosystem. *Mar. Microb. Food-Webs*, 1992, **6**, 149–172.

69. Plante, C. J., Role of bacterial exopolymeric capsules in protection from deposit-feeder digestion. *Aquat. Microb. Ecol.*, 2000, **21**, 211–219.

70. Crocker, K. M. and Passow, U., Differential aggregation of diatoms. *Mar. Ecol. Prog. Ser.*, 1995, **117**, 249–257.

71. Passow, U., Shipe, R. F., Murray, A., Pak, D. K., Brzezinski, M. A. and Alldredge, A. L., The origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. *Cont. Shelf Res.*, 2001, **21**, 327–346.

72. Biddanda, B., Microbial synthesis of macroparticulate matter. *Mar. Ecol. Prog. Ser.*, 1985, **20**, 241–251.

73. Passow, U. and Alldredge, A. L., Aggregation of a diatom bloom in a mesocosm: The role of transparent exopolymer particles (TEP). *Deep Sea Res.-II*, 1995, **42**, 99–109.

74. Grossart, H. P. and Simon, M., Bacterial colonization, microbial decomposition of limnetic organic aggregates (lake snow). *Aquat. Microb. Ecol.*, 1998, **15**, 115–125.

75. Azam, F. and Smith, D. C., Bacterial influence on the variability in the ocean's biochemical state: a mechanistic view, *Particle Analysis in Oceanography* (ed. Demers, S.), Springer-Verlag, Berlin, 1991, pp. 213–233.

76. Honjo, S., Seasonality and interaction of biogenic and lithogenic particulate flux at the Panama basin. *Science*, 1982, **218**, 883–884.

77. Smetacek, V., Role of sinking in diatom life-history cycles: Ecological, evolutionary and geological significance. *Mar. Biol.*, 1984, **84**, 239–251.

78. Wefer, G., *Productivity of the Oceans: Present and Past* (eds Berger, W. H., Smetacek, V. and Wefer, G.), John Wiley, 1989, pp. 139–154.

79. Azam, F., Microbial controls of oceanic carbon flux: The plot thickens. *Science*, 1998, **280**, 694–696.

80. Lampitt, R. S., Hillier, W. R. and Challenor, P. G., Seasonal and diel variation in the open ocean concentration of marine snow aggregates. *Nature*, 1993, **362**, 737–739.

81. Engel, A. and Schartau, M., Influence of transparent exopolymer particles (TEP) on sinking velocity of *Nitzschia closterium* aggregates. *Mar. Ecol. Prog. Ser.*, 1999, **182**, 69–76.

82. Billen, G. and Fontigny, A., Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters. 2. Bacterioplankton dynamics. *Mar. Ecol. Prog. Ser.*, 1987, **37**, 249–257.

83. Prieto, L., Sommer, F., Stibor, H. and Koeve, W., Effects of planktonic copepods on transparent exopolymeric particles (TEP) abundance and size spectra. *J. Plankton Res.*, 2001, **23**, 515–525.

84. Fuhrman, J., *Primary Productivity and Biogeochemical Cycles in the Sea* (eds Falkowski, P. G. and Woodhead, A. D.), Plenum Press, 1992, pp. 361–383.

85. Engel, A., Direct relationship between CO_2 uptake and transparent exopolymer particles production in natural phytoplankton. *J. Plankton Res.*, 2002, **24**, 49–64.

86. Williamson, P. and Holligan, P. M., *Ocean Prod. Climate Change, Tree*, 1990, **5**, 299–503.

87. Roane, T. M. and Pepper, I. L., *Environmental Microbiology* (eds Maier, R. M., Pepper, I. L. and Gerba, C. P.), Academic Press, 2000.

88. Ford, T. E., Maki, J. S. and Mitchell, R., Corrosion of metals by thermophillic microorganisms. Corrosion/87, NACE Publications, California, 1987, 360.

89. Salehizadeh, H. and Shojaosadati, S. A., Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. *Water Res.*, 2003, **37**, 4231–4235.

90. Quigley, M. S., Santschi, P. H., Hung, C. C., Guo, L. and Honeyman, B. D., Importance of acid polysaccharides for ^{234}Th complexation to marine organic matter. *Mar. Chem.*, 2002, **76**, 27–45.

91. Herndl, G. J. and Peduzzi, P., The ecology of amorphous aggregations (marine snow) in the northern Adriatic Sea. 1. General considerations. *P.S.Z.N.-I Mar. Ecol.*, 1990, **11**, 327–360.

92. Rogers, S. I. and Lockwood, S. J., Observations on coastal fish fauna during a spring bloom of *Phaeocystis pouchetii* in the eastern Irish Sea. *J. Mar. Biol. Assoc. UK*, 1990, **70**, 249–253.

93. Naqvi, S. W. A. *et al.*, Increased marine production of N$_2$O due to intensifying anoxia on the Indian continental shelf. *Nature*, 2000, **408**, 346–349.

94. Maki, J. S., The influence of marine microbes on biofouling. In *Recent Advances in Marine Biotechnology* (eds Fingerman, M., Nagabhushanam, R. and Thompson, M.-F.), 1999, vol. 3, p. 147.

95. SIGMA experiment. *Deep-Sea Res.-II*, 1995, **42**, 1–8.

96. Simon, M., Grossart, H. P., Schweitzer, B. and Ploug, H., Microbial ecology of organic macroaggregates in aquatic systems. *Aquat. Microb. Ecol.*, 2002, **28**, 175–211.

97. Janse, I., Production and breakdown of *Phaeocystis* polysaccharides, Ph D thesis, Rijke University, Groningen, The Netherlands, 2000.

98. Bhaskar, P. V., Studies on some aspects of marine microbial exopolysaccharides, 2003, Ph D thesis, Goa University, Goa, India.

99. Bejar, *et al.*, Characterization of exopolymers produced by halophilic strains of *Halomonas eurihalina*. *J. Biotechnol.*, 1998, **61**, 135–141.

100. Guezennec, J., Pignet, P., Lijour, Y., Gentric, E., Ratiskol, J. and Colliec-Jouault, S., Sulfation and depolymerization of a bacterial exopolysaccharides of hydrothermal origin. *Carbohydr. Polym.*, 1998, **37**, 19–24.

101. Rougeaux, H., Pichon, R., Kervarec, N., Raguenes, G., H., C. and Guezennec, J. G., Novel bacterial exopolysaccharides from deep-sea hydrothermal vents, *Carbohydr. Polym.*, 1996, **31**, 237–242.

102. Khandeparkar, R. D. S. and Bhosle, N. B., Extracellular polymeric substances of the marine fouling diatom *Amphora rostrata* Wm. Sm. *Biofouling*, 2001, **17**, 117–127.

103. Pavlova, K. and Grigorova, D., Production and properties of exopolysaccharide by *Rhodotorula acheniorum* MC. *Food. Res. Intl.*, 1999, **32**, 473–477.

104. Staats, N., de Winder, B., Stal, L. J. and Mur, L. R., Isolation and characterization of extracellular polysaccharides from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. *Eur. J. Phycol.*, 1999, **34**, 161–169.