PERGAMON

Continental Shelf Research 20 (2000) 1257–1273

CONTINENTAL SHELF RESEARCH

Microbial biofilms in intertidal systems: an overview

Alan W. Decho*

Department of Environmental Health Sciences, School of Public Health, University of South Carolina, Columbia, SC 29208, USA

Received 11 May 1999; received in revised form 11 January 2000; accepted 20 January 2000

Abstract

Intertidal marine systems are highly dynamic systems which are characterized by periodic fluctuations in environmental parameters. Microbial processes play critical roles in the remineralization of nutrients and primary production in intertidal systems. Many of the geochemical and biological processes which are mediated by microorganisms occur within microenvironments which can be measured over micrometer spatial scales. These processes are localized by cells within a matrix of extracellular polymeric secretions (EPS), collectively called a "microbial biofilm". Recent examinations of intertidal systems by a range of investigators using new approaches show an abundance of biofilm communities. The purpose of this overview is to examine recent information concerning the roles of microbial biofilms in intertidal systems. The microbial biofilm is a common adaptation of natural bacteria and other microorganisms. In the fluctuating environments of intertidal systems, biofilms form protective microenvironments and may structure a range of microbial processes. The EPS matrix of biofilm forms sticky coatings on individual sediment particles and detrital surfaces, which act as a stabilizing anchor to buffer cells and their extracellular processes during the frequent physical stresses (e.g., changes in salinity and temperature, UV irradiation, dessication). EPS is an operational definition designed to encompass a range of large microbially-secreted molecules having widely varying physical and chemical properties, and a range of biological roles. Examinations of EPS using Raman and Fourier-transform infared spectroscopy, and atomic-force microscopy suggest that some EPS gels possess physical and chemical properties which may hasten the development of sharp geochemical gradients, and contribute a protective effect to cells. Biofilm polymers act as a sorptive sponge which binds and concentrates organic molecules and ions close to cells. Concurrently, the EPS appear to localize extracellular enzyme activities of bacteria, and hence contribute to the efficient biomineralization of organics. At larger spatial scales, the copious secretion of specific types of EPS by diatoms on the surfaces of

* Corresponding author. Tel.: + 1-803-777-6584; fax: + 1-803-777-3391.
E-mail address: adecho@sph.sc.edu (A.W. Decho).

0278-4343/00/$ - see front matter
PII: S0278-4343(00)00022-4

intertidal mudflats may stabilize sediments against resuspension. Biofilms exert important roles in environmental- and public health processes occurring within intertidal systems. The sorptive properties of EPS effectively chelate toxic metals and other contaminants, which then act as an efficient trophic-transfer vehicle for the entry of contaminants into food webs. In the water column, biofilm microenvironments in suspended flocs may form a stabilizing refugia that enhances the survival and propagation of pathogenic (i.e., disease-causing) bacteria entering coastal waters from terrestrial and freshwater sources. The EPS matrix affords microbial cells a tremendous potential for resiliency during periods of stress, and may enhance the overall physiological activities of bacteria. It is emphasized here that the influences of small-scale microbial biofilms must be addressed in understanding larger-scale processes within intertidal systems.

Keywords: Microbial; Intertidal; Marine; Coastal; Contaminants; Bacteria; Biofilms; EPS; Exopolymers; Bacteria; Pathogens; Metals

1. Introduction

Intertidal systems are a key interface of the ocean, atmosphere, and terrestrial environments, and as such, are characterized by frequent fluctuations in temperature, ion concentration, dessication, UV-irradiation, and wave action. The relative frequency of these fluctuations poses both physical and biochemical challenges to microorganisms which inhabit this environment. The characteristics and intensities of such stresses may vary substantially (see, for review Giller et al., 1994).

Microorganisms have important and established roles which may effect large-scale changes within intertidal systems. Heterotrophic bacteria, for example, are crucial to transformations and remineralization of organic carbon, nitrogen and other nutrients throughout oceans (Azam, 1998; Azam et al., 1993). Autotrophic eukaryotes, such as diatoms, may be important primary producers in these systems (Paerl, 1997). Although net effects of microbial processes may be measured over a range of spatial and temporal scales, most microbial processes are generated within microenvironments having spatial scales, measured in micrometers (Krembs et al., 1998).

1.1. The concept of the microbial microenvironment: biofilms by design

When bacteria and microalgae are associated with surfaces (e.g., sediment particles of intertidal sandflats, plant surfaces) they secrete a matrix of mucilaginous extracellular polymers (EPS) to form a "microbial biofilm" (Fig. 1). Biofilms and their associated mucilaginous secretions form a cohesive matrix surrounding the particles of intertidal sediments. The microbial biofilm is now known to represent a common adaptation, perhaps even a life stage, of most bacteria in natural systems (Costerton et al., 1995). Since an early review of the roles of microbial biofilms in marine systems (Decho, 1990) a wide range of studies have been conducted which focused on many specific aspects of biofilms and mucilage production. Examination of natural sediment environments from a range of investigators using recently developed technologies reveals

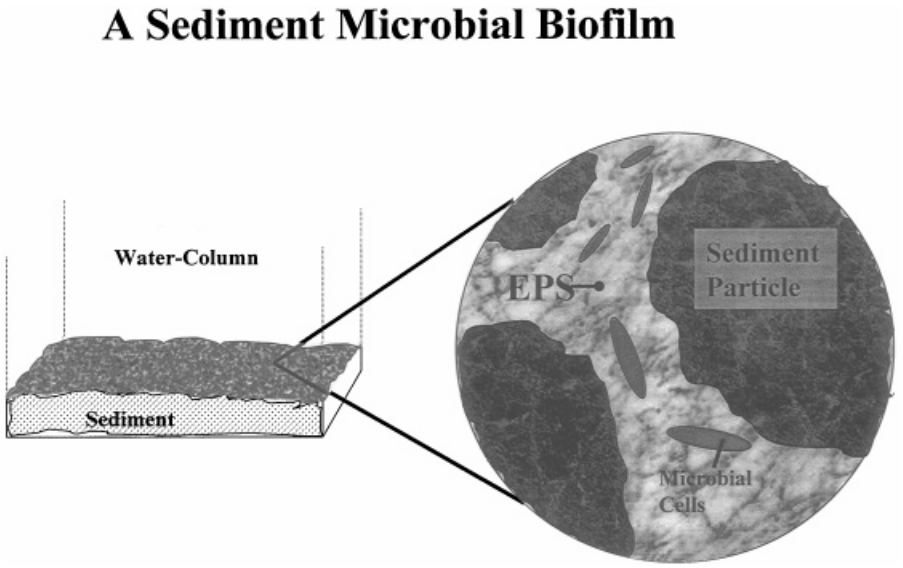

Fig. 1. Conceptual diagram of a microbial biofilm within an intertidal sediment flat. The biofilm consists of microbial cells such as diatoms, cyanobacteria, heterotrophic bacteria embedded within a matrix of extracellular polymeric secretions (EPS) which closely surround sediment particles. A typical mudflat biofilm is most pronounced near the sediment surface, where diatoms and other phototrophs are the major producers of EPS. The EPS contributes to the sediment fabric and may influence the cohesive properties of the sediment. The highly hydrated EPS may also represent a protective adaptation to prevent dessication of cells during prolonged tidal exposure.

the presence and properties of biofilm communities, and exciting revelations concerning biofilms and more generally microbial processes. These studies have greatly increased our understanding of this rather common, but complex microbial assemblage.

EPS are the primary structuring agent for microbial microenvironments and control the physical properties of biofilms. The biofilm is a community of microbial cells whose activities may be structured or enhanced by the EPS matrix surrounding the cells. In general, the matrix forms a stabilizing and protective microenvironment, and may serve a variety of specific functions to cells. The physical state of exopolymers may vary considerably, and range from a tight, cohesive gel to a very loose slime to dissolved or colloidal states (Decho, 1990). EPS capsules, which surround cells and are in direct contact with cell membrane, will exert the most proximate protective effects.

2. New approaches in the analyses of biofilms

A range of relatively new approaches have become available which allow investigators to probe microbial processes and biofilms with unprecedented sensitivity and resolution. Some of these approaches are briefly summarized below. They include:

Confocal scanning laser microscopy, atomic-force microscopy, low-temperature scanning electron microscopy, and Fourier-transform infared spectroscopy.

For the first time intact microbial biofilms within sediments have been examined using confocal scanning laser microscopy (CSLM) (Decho and Kawaguchi, 1999). The application of a hydrated embedding resin (e.g., nanoplast) has allowed the fine structure of EPS to be seen using scanning and transmission electron microscopy (Leppard et al., 1996; Lienemann et al., 1998), and CSLM (Decho and Kawaguchi, 1999). The coupling of specific fluorescent probes with CSLM (Pawley, 1995) permitted biofilm and their interstitial microenvironments to be observed (Caldwell et al., 1992; Lawrence et al., 1991, 1997; Surman et al., 1996). A wide range of fluorescent probes are now available (Haugland, 1996) which will permit specific groups of microorganisms and/or their activities to be quantitatively examined. Such applications will permit, for the first time, quantitation of microbial processes at microspatial (i.e., micrometer) scales in marine sediments. Atomic-force microscopy (AFM) has allowed hydrated polymer gels to be observed and measured with unprecedented clarity. Decho (1999a) used contact-mode AFM to observe single EPS molecules and EPS in a gel state. He found that EPS gels composed of alginate polymers tended to form sturdy gels by formation of dimer bridges. This was concluded by measuring the thickness of polymer molecules in proximity to Ca bridges. Other techniques which have yielded insight have involved the use of Fourier-transform infared spectroscopy (FTIR) in the examination of biofilms communities and carbohydrates (Nichols et al., 1985; Suci et al., 1997, 1998). The application of low-temperature scanning electron microscopy (SEM) has contributed greatly to our understanding of the "sediment matrix fabric" (Stolz, 1994; Paterson, 1995; Défarge, 1997; Défarge et al., 1996; Black et al., 1999). Low-temperature SEM is a technique in which whole or fractured-sediments can be observed while in an intact, hydrated state. Such approaches have provided, for the first time, high-resolution imaging of the natural sediment fabric. Future studies using these techniques will be essential to understanding how the EPS matrix and other important components contribute to sediment stability in intertidal systems.

3. Physical and chemical properties of EPS

The physical states of EPS can be best described as a continuum, ranging from gels to a fully dissolved (i.e., solution) state. In practical operational terms, these polymers exist as capsules, sheaths, looser slimes in biofilms and as DOC in solution. Unfortunately, many descriptive reports often have failed to recognize the relationship between the physical forms of these molecules and the physiological conditions present (Sutherland, 1990).

The chemical properties of EPS are instrumental in effecting the various roles of biofilms in biological and geochemical processes. A detailed literature exists regarding chemical properties of EPS isolated from laboratory cultures of various bacteria (see, for reviews, Decho, 1990; Sutherland, 1990). However, relatively little is known of the chemical properties of natural EPS.

Exciting new work by Vedugo, Orellana and colleagues (Tam and Verdugo, 1981; Verdugo, 1994; Chin et al., 1998) has examined the physical chemistry of polymeric molecule interactions in the formation of gels. Polymer gels are networks of molecules interconnected by chemical or physical crosslinks. These interactions give polymer gels a unique set of physical properties (Verdugo, 1994). Polymer molecules may assemble to form "tangled networks" or "covalently crosslinked networks" stabilized by Ca ion bonds. Electron probe microanalyses reveal that microgels resulting from DOM polymer assembly are generally rich in Ca, and low in Mg and P (Chin et al., 1998). Tanaka's theory of gel-swelling (Tokita and Tanaka, 1991) suggests that the degree of crosslinking changes as a result of Ca sequestration. The polymer gel acts as a Donan trap, similar to an ion-exchange resin, and concentrates cations with various degrees of specificity depending on how the network is assembled (Chin et al., 1998). In laboratory studies it has been shown that slight changes in functional groups may alter the properties of polymers. More heavily crosslinked gels tend to retain water better, and may provide a protective hydration buffer for cells against dessication. In some biofilms, a major function of EPS coatings may be to conserve water during periods of tidal exposure (Potts, 1994).

Recent work by Zhou et al. (1998) showed that the cohesiveness of natural EPS in water-column aggregates was related to an enrichment in specific deoxy sugar monomers (e.g., rhamnose and fucose). This study suggests that the ratios of sugar monomers may be important in the cohesive properties of the EPS, and their ability to form aggregates. EPS polymers often contain hydrophobic and surfactant moieties, which may greatly alter the chemical properties of the polymers (Neu, 1996). These compounds influence the surface characteristics of the polymer and may affect the subsequent colonization of other bacteria.

The polymer network, and hence its ability to concentrate ions, may be altered by ultraviolet (UV) irradiation. Exposure of DOM to UV has been shown to result in cleavage of marine polymers, decreasing their average molecular size (Kieber et al., 1990; Mopper et al., 1991). This can decrease the stability of tangled polymer networks. The EPS matrix is a "pliant" matrix; one which swells and contracts in response to environmental changes, and reorganizes itself constantly. This is predicted by results of polymer chemistry studies.

4. Quorum sensing and biofilm community development

In intertidal systems, microbial biofilms often contain cells densely packed within an EPS matrix. These conditions facilitate or may even represent the result of chemical communication between microbial cells (Decho, 1999b). Biofilms represent groups of partially immobilized cells contained within a gel matrix. The development of biofilms on the surface of a sediment grain, for example, is thought to be mediated in part by density-dependent chemical signal released by bacteria. These signals, called autoinducers, will effect physiological changes in activities of cells, when they reach a threshold concentration. This process is called "quorum sensing" (Fuqua et al., 1996) and allows the potential self-regulation of microbial communities.

Excellent reviews of quorum sensing processes have been provided by Fuqua et al. (1996), Ruby (1996) and Dunlap (1997). It is assumed in quorum sensing that groups of microbial cells act as "coordinated units", rather than as opportunistic individuals. This form of intercellular communication serves to coordinate gene expression, and structures morphological differentiation and development responses of bacterial cells. Much empirical evidence exists to support the process of quorum sensing in a range of microbial systems. Quorum sensing signals influence such important bacterial activities such as pathogenesis, conjugative plasmid transfer, bioluminescence and extracellular enzyme activity. Recently, quorum sensing has been shown to influence the development of biofilm architecture (Davies et al., 1998). Cells which were able to communicate using autoinducer signals, developed biofilms having a very different architecture, than mutant cells which were not able to produce signals. The ability of microbial cells to act as coordinated units is thought to facilitate a more efficient utilization and biotransformations of carbon and other nutrients, a shunting of waste products, and an overall more effective resiliency of microbial communities to potential stressors.

5. Roles in intertidal systems

5.1. Diatom mats and sediment stabilization

The surface sediments of an intertidal mudflat typically experience frequent resuspension by the ebb and flood tides. This frequent physical mixing will prevent large and well-developed layering of microorganisms within these uppermost sediments of the mudflat. This also suggests that biofilms associated with these sediments may be well developed.

In some areas of mudflats, there occurs the development of cohesive diatom mats. These mats act to stabilize the surface sediment layers of the mudflat against resuspension (Paterson, 1995). Diatom mats form on the surfaces of many intertidal mudflats, and often occur with seasonal periodicities (Underwood and Paterson, 1993; Underwood, 1994). The mats consist of dense aggregations of cells and EPS. The EPS component has been considered an important agent in stabilizing the mudflat sediments against resuspension. A recent study by Smith and Underwood (1998) showed that diatom secretion of EPS was closely related to their motility, and occurred predominantly during dark periods. EPS resulting from diatom motility appears to possess very cohesive physical properties, which may contribute to sediment stabilization. Examinations of chlorophyll *a* as an index of diatoms, and concentrations of EPS as measured by the phenol–sulfuric acid technique showed associations of these parameters with sediment stability (Underwood and Smith, 1998). There have been a number of studies which report either positive effects of diatoms mats on sediment stability (Grant et al., 1986; Paterson, 1989, 1995; Underwood and Paterson 1993; Sutherland et al., 1998), or no significant effects. Therefore, the presence of a diatom mat is not always related to increased sediment stability. While the causes of these differences are still uncertain, they may relate, in part, to a range of factors such as

species composition, complexity and physiological states of diatom mats, and the methods used to quantify sediment stabilization.

Diatoms are known to undergo diel vertical migrations, largely in response to light. EPS has been shown to be secreted during motility (Underwood et al., 1995; Smith and Underwood, 1998). It is probable, that diatoms may secrete several types of EPS (Hoagland et al., 1993), some of which are used for motility, and others which are secreted copiously as secondary metabolites or for other functions, as have been found for heterotrophic bacteria (Sutherland, 1995). It is likely that both the composition and cohesive properties of these specific EPS may be quite different. This may, in part, explain the observed differences in sediment stability associated with certain diatom mats. Experimental examinations of the cohesive properties and viscosities of different types of diatom EPS will be important in understanding why some mats may or may not exhibit cohesive stabilizing effects on sediment.

Also, the physiological state of the diatom mat may influence the composition and cohesiveness of diatom EPS. The EPS and overall diatom mat may be more/less cohesive depending on if it is in the early or later stages of mat formation. This requires further investigation. Many diatoms, and other photosynthetic organisms such as cyanobacteria are known to produce different EPS depending on the physiological state of the cells (Hoagland et al., 1993). Since the physical stability of a diatom mat depends on close interactions between cells, EPS and mudflat sediments, no further generalizations could be prudent until further focused studies have been conducted.

Another potential effect resulting from the physically stabilizing conditions of diatom mats is one that should potentiate development of more organized bacterial biofilms and microbial communities. A typical diatom exhibits a relatively high photosynthetic production. This production shows a diel periodicity, with generally higher production during daylight hours (Paerl, 1997). Also resulting from this production is the release of a significant amount of large and small molecular weight (MW) exudates which may potentially fuel an active heterotrophic community. Such a community would include a range of aerobic heterotrophic bacteria and sulfate-reducing bacteria (SRB). Both of these are geared to quickly utilize the photosynthetic exudates of the mat. While SRB are generally associated with anoxic sediments, their activity has now been demonstrated to occur in oxic conditions (Canfield and Des Marais, 1991; Jφrgensen, 1994). The cohesive properties of EPS will also influence the characteristics of sediment which is resuspended from the mudflat. Cohesive properties should result in enhanced floc formation even when sediments are eroded.

5.2. Intertidal stromatolites

Stromatolites are layered sediment macrostructures which are formed through the interactions of biological organisms, such as bacteria, and geochemical processes (Reid et al., 2000). Fossil stromatolites represent the oldest known macroscopic evidence of life on earth and were the dominant life form, as evidenced by the fossil record, for over 2.5 billion years (Awramik, 1992). Today, marine stromatolites are

forming only in a few isolated parts of tropical intertidal (and subtidal) systems in the Bahamas and Western Australia.

Marine stromatolites represent an extreme example of biologically mediated sediment stabilization. The intertidal stromatolites, in the Exuma Cays, Bahamas exist in high-energy environments (Reid and Browne, 1991). The stromatolite community consists of apparently closely interacting but widely diverse groups of prokaryote microorganisms (e.g., cyanobacterial autotrophs, aerobic heterotrophs and sulfate-reducing bacteria) (Stal, 1995). Cyanobacteria biofilms comprise a major structuring component in the formation and growth of Exuma stromatolites (Reid et al., 1995). They are responsible for a majority of organic carbon production and nitrogen fixation (Pinckney et al., 1994), and secrete much of the EPS (Decho and Kawaguchi, 1999) which may initially stabilize cells and sediment against resuspension. The interactions of sulfate-reducing bacteria (SRB) and autotrophic production have been suggested to result in lithified well-sorted sedimentary structures consisting of layers of precipitated $CaCO_3$, and $CaCO_3$ sand grains (Visscher et al., 1998). The binding of Ca^{2+} ions by EPS inhibits the geochemical precipitation of $CaCO_3$ which are near (or above) saturation concentrations. Partial degradation of EPS by SRB may result in precipitated $CaCO_3$ in restricted areas (i.e., laminae) of the biofilm. Stromatolites represent a relatively clear example of how biological, chemical and geological factors may interact to alter (i.e., stabilize) a physical environment in intertidal systems.

5.3. Localization of extracellular enzymes

A potentially important, but surprisingly less-explored aspect of biofilms is their role in the localization of extracellular enzymes in proximity of microbial cells. Extracellular enzymes, defined here as secreted enzymes which are not in direct contact with cell membranes, are secreted by cells to hydrolyze large molecular-weight (MW) organic matter into smaller oligomers and monomeric components which can be directly taken up by cells (Hoppe, 1991). The presence of polysaccharides in the EPS matrix may provide a protective molecular microenvironment for bacterial extracellular enzymes. This process is important in the efficiencies of larger-scale transformations of organic matter by bacteria, and in the conversion of dissolved (DOC) to particulate (POC) forms of organic matter. While this process has been suggested to occur (Decho, 1990), direct evidence supporting this process has been tenuous at best.

A wide range of studies have examined extracellular enzyme activities within marine sediments (King, 1986; Henrichs and Reeburgh, 1987; Mayer, 1989; Meyer-Reil, 1991; Arnosti, 1995, 1996; Belanger et al., 1997). These studies collectively suggest that rates with which organic macromolecules (and monomers) are remineralized vary widely. Arnosti (1996) found rapid degradation of polysaccharides within anoxic marine sediments.

Activities of extracellular enzymes in sediments and water-column environments have been observed by measuring corresponding fluorescence associated with methyl-umbelliferal (MUF) substrates (Smith et al., 1992). These studies have largely addressed aggregates in marine planktonic systems. Another interesting approach

developed by Arnosti (1996) uses specific fluorescently-labeled (FLA-) polysaccharides. The size(s) of the polysaccharides before and after microbial hydrolyses are quantitatively examined, and hydrolysis rates can be measured.

Extracellular enzymes may play important roles in the dispersion or release of microbial cells from an attached state. A wide range of extracellular enzymes are produced by microorganisms having a range of hydrolysis functions and specificities (Sutherland, 1995; Warren, 1996). It has been demonstrated that in the opportunistic pathogen *Pseudomonas aeruginosa*, dispersal of cells from a biofilm is accomplished using specific lytic enzymes which hydrolyzed the EPS surrounding the cells (Boyd and Chakrabarty, 1995). This releases cells from the attached biofilm state.

It is not known how extracellular enzymes may be associated with the EPS matrix. Hoffman and Decho (1999) have postulated that enzymes may be localized within hydrophilic microdomains or regions which occur within a larger EPS matrix. This can be determined using laser confocal microscopy (CLM). Recent evidence suggests that enzymes may be attached to polysaccharides via sulfhydral linkages, since incubation of EPS in mercaptoethanol results in the release of smaller MW bands of proteins using polyacrylamide gel electrophoresis (PAGE) separations (Kawaguchi and Decho, in press). Glycosylation and/or localization within a polysaccharide gel, is known to confer some protective effects to proteins (Ortega et al., 1998). However, it is not known how changes in salinity, temperature and other fluctuations will affect extracellular enzyme activities within intertidal systems. It is possible that specific associations of enzymes with the EPS matrix may prolong their activities and resiliency during environmental fluctuations.

5.4. Protective refugia for pathogens entering marine systems

Pathogenic (i.e., disease-causing) bacteria enter intertidal systems from terrestrial and freshwater sources (Payment et al., 1989). Many of these, upon entering marine waters, are immediately stressed by salinity shock and/or UV irradiation. However, the ability of pathogenic bacteria to survive and propagate after entering marine waters represents a significant public health concern (Stanwell-Smith, 1991). This relates to both the recreational use of marine waters, and to the human consumption of seafood harvested from these waters. The survival of some pathogens upon entering marine waters, may be linked to their association with aggregate flocs. Increasing salinity, going from freshwater to full seawater results in a substantial increase in the cations Na^+, Ca^{2+}, and Mg^{2+}. Upon reaching saline water, enhanced floc formation and aggregation of suspended dissolved organic carbon (DOC), silts and clays is generally observed in most intertidal systems.

When cells are enclosed within EPS, the matrix provides a "protective refugia" which will buffer cells against potential stresses. The idea of the biofilm as a protective refugia is not a new one. Studies of bacteria in other systems (see for review, Costerton et al., 1995) have shown that cells enclosed within a biofilm are generally more resistant to antibiotics, toxic metals, chlorination, and other potential anti-microbial agents. The exact mechanisms which influence the enhanced resiliency of cells are uncertain but have been attributed to such factors as: the direct binding of toxic agents

Table 1
Major binding ligands associated with EPS polymers

Major binding ligand	Monomeric components of EPS molecules	Reference(s)
Hydroxyls	Neutral sugars, etc.	Sutherland (1990)
Carboxyls	Uronic acids	Geesey and Jang (1989)
Phosphates		Lindberg (1990)
Sulfates, sulfonates	Cyanobacteria Polysaccharides	de Philippis and Vincenzini (1998)
Ketal-linked pyruvates	Hexose sugars	Smith et al. (1990)
Acetyl groups	Neutral sugars	Lindberg (1990), and Sutherland (1990)
Amine groups	Amino sugars	Lindberg (1990) and Sutherland (1990)

by EPS; a reduction in physiological rates of biofilm cells; and enhanced horizontal exchange of resistance-containing plasmids. The binding of transition metals to EPS may further condense and stabilize the EPS, and enhance the protective nature of the EPS for cells.

5.5. *Binding and concentration of metal contaminants*

A growing body of literature shows evidence that EPS bind and concentrate a range of metal ions, metalloids and molecules. Many metal ions, such as Cd^{2+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, etc., are efficiently chelated by EPS. This occurs primarily through the formation of unidentate, bidentate and multidentate complexes of cations with anionic groups on EPS molecules (Geesey and Jang, 1989). The strength of the binding affinity in the complex will depend largely on ion size/charge ratios, and a number of other factors, such as EPS composition, physical gel state, pH, and ionic salinity.

The complexation ligands on EPS are critical to their ability to bind and concentrate ions. Microbial exopolysaccharides contain a range of ester-linked groups and pyruvate ketals (Lindberg, 1990; Sutherland, 1990) (Table 1). Ester-linked groups, such as acetate, do not contribute to the overall charge of the macromolecule, while pyruvate ketals, which are normally associated with neutral hexose sugars, add to the overall anionic nature of the EPS. Uronic acids, such as D-guluronate, also contribute to the anionic binding capacity of EPS. Sulfate groups may be present in some prokaryote EPS, such as those isolated from cyanobacteria. Phosphate groups may also contribute to the binding capacity of EPS. Their occurrence in bacterial EPS is relatively common (Sutherland, 1990).

Factors such as pH could be easily modified in seawater environments, especially at microenvironmental spatial scales, and this could strongly influence binding and release of metals from EPS. While the pH of bulk seawater is generally between 8.0 and 8.2, it may vary considerably within the localized microenvironments of a biofilm due to the photosynthetic and respiratory activities of microorganisms. In general, a lower pH (i.e., acidic conditions) will result in a release of ions from a bound state, while higher pHs (i.e., basic conditions) tend to favor their chelation. It follows that

within the surface sediments of an intertidal mudflat, for example, the strength of metal chelation to polymers may vary over a diel cycle in response to net peaks in photosynthesis (during daylight) and respiration (during darkness). Salinity changes also could affect the binding capacities of metals such as Cd. Schlekat et al. (1998) isolated an estuarine bacterium which produced EPS and exhibited a very strong Cd chelation capacity. They found that across a wide range of salinity (e.g., 3–30 ppt) and pH conditions (pH 5.0–9.0), over 90% of Cd (0.038 mg/L) remained bound by EPS. At higher Cd concentrations (50 mg/L), however, a significant salinity effect was observed, with some release of Cd from EPS at higher salinities. They attributed this to the association of Cd with secondary ligands having weaker affinities for the ion. The results of the study suggest that the binding (and release) of certain metal ions from microbial biofilms may occur with regular periodicities, i.e., over tidal or light–dark cycles.

The binding capacity of a given EPS polymer may be further influenced by its physical state (i.e., gel vs slime vs. dissolved state) when it binds the ion(s). For example, EPS polymers, when in a gel state, may exhibit stronger binding affinities for a given cation than similar polymers in a looser slime state. This would relate to the ability of the former to more readily form stronger multidentate complexes with ions (Geesey and Jang, 1989). Caution must be exercised in extrapolating the results of sorption experiments, conducted using polymers isolated from laboratory cultures of bacteria, since EPS polymers may be partially degraded soon after their secretion in natural environments.

Another potential factor which may influence metal chelation is an indirect one, involving the post-secretive alteration of EPS polymers by UV irradiation. Laboratory studies show that there is an enhancement of available carboxyls on EPS and other organic molecules after exposure to UV-irradiation (Kieber et al., 1990). This may occur due to splitting of glucose rings by UV wavelength photons. An increase in carboxyl groups following UV exposure may increase its potential to bind metal ions. This will potentially increase its stabilizing effect on sediment resuspension.

5.6. Trophic transfer of metal contaminants to food webs

In examining the factors affecting the bioavailability of metal ions to consumer animals, organic carbon has represented a confounding factor; one which may enhance or reduce bioavailability (see, for review, Tessier and Campbell, 1987; Tessier and Turner, 1995). This has been largely because the operational term "organic carbon" represents many forms of carbon molecules which have very different chemical and compositional properties, and range in digestibility to consumer animals from refractory to labile.

The trophic implications of biofilms and their EPS are significant to intertidal ecosystems, and potentially complex. It is now known that the association of metal contaminants with EPS may significantly enhance the bioavailability of the metals in marine systems (Schlekat et al., 1998, 1999, 2000; and others). However, EPS occurs in a range of physical states (capsules, gels, loose slime, DOC) and chemical compositions;

all of which may influence its complexation capacities of metals, and digestibilities by consumer animals through feeding processes.

As a form of potentially labile carbon, the EPS of biofilms constitutes an efficient trophic transfer vehicle to food webs (Decho, 1990). Earlier studies found that EPS, specifically its carbon, isolated from laboratory cultures of bacteria and diatoms, had varying degrees of digestibility by consumer animals. Decho and Lopez (1993) found that capsular EPS appeared more refractory to the deposit- and suspension-feeding polychaete *Streblospio benedicti*. They postulated that the higher content of protein in capsular EPS, compared with loose slime EPS, contributed to its denser gel structure, and greater refractivity to digestion by the consumer.

Several patterns are emerging, all of which, however, must be tested more rigorously before firm generalization can be made: (1) Although the toxicity and bioavailability of many metals have been traditionally related to aqueous-phase concentrations, it is now known that particulate forms of metals may represent a significant route of uptake for consumers (sensu Luoma et al., 1992). (2) Bacteria cells enclosed in capsules are generally less digestible than cells having not obvious capsules (Plante and Shriver, 1998). (3) Certain compositional types of EPS appear less digestible than others (Decho and Lopez, 1993). (4) Certain metals, such as Cd, may exhibit fluctuating bioavailabilities, depending on the ambient salinity. (5) Microenvironments within biofilms, may result in significant pH variations, which may in turn affect the binding and release of metals from particles. (6) EPS–metal associations may exhibit "*reciprocal effects*" on the bioavailability and trophic transfer of metals and carbon. That is, EPS which is a labile carbon food source, may enhance the bioavailability of metals. However, high metal concentrations associated with the EPS may decrease the bioavailability of the EPS carbon. The use of radioisotopes has greatly increased the sensitivity of measuring bioavailability and the uptake of metal ions in marine systems.

5.7. Plankton blooms

In water-column environments of estuaries and other intertidal systems, temporary blooms of photosynthetic diatoms, cyanobacteria and flagellates often occur in response to nutrient inputs. The carbon-rich organics (i.e., mainly cells and EPS) resulting from this overproduction may be deposited on intertidal sandflats and mudflats. This may result in quickly developing hypoxia, or anoxia conditions, once heterotrophic degradation of this material commences. When the mucilage is more resistant to degradation, it may result in expansive floating mats which have been periodically observed in the northern Adriatic Sea (Stachowitsch et al., 1990; Muller-Niklas et al., 1994; Herndl, 1988), and seafoam which typically washes ashore on intertidal sand beaches of the North Sea (Lancelot and Mathot, 1987). The adverse effects of blooms may be further complicated when the major bloom microorganism(s) produce and/or release toxins which adversely affect other organisms and/or human. These have been the subject of much study in recent years.

A characteristic of many blooms is the later-stage release of abundant extracellular mucilage, which are typically rich in polysaccharides (Alldredge et al., 1993; Passow

et al., 1994; Lavoie et al., 1995). The overproduction of extracellular polysaccharides has been generally attributed to a secondary metabolite response of cells to the unbalanced nature of the ambient nutrient conditions (Myklestad, 1995). This results from a continued abundance of C (i.e., as carbonate), while concurrent limitations in other nutrients (N, Si or P) develop. Such responses can be easily reproduced in laboratory cultures of microorganisms (Myklestad, 1995). However, some adaptive features of this have been proposed. The aggregation of cells and EPS, often leads to flocculation to the sediment surface (Passow et al., 1994), although prolonged suspension of flocs may also occur (Passow and Alldredge, 1994).

6. Summary

The microbial biofilm matrix effects a number of changes on the physical and biological properties of intertidal sediment environments. These range from the macro-scale stabilization of sediments to the micro-scale stabilization of microbial microenvironments. While the biofilm is a common microbial adaptation in intertidal systems, the complexity of biofilms and their resulting effects on sediment systems are not well understood. New imaging and analytical approaches have provided important insight into their functioning, resiliency and the resulting effects on intertidal processes. The microbial biofilm represents an important parameter for consideration during the experimental investigation, and the interpretation of biological, chemical, and sedimentological data in intertidal systems.

Acknowledgements

The author acknowledges the scientists of the INTRMUD Group for their helpful discussion of this work during a conference on Intertidal Mudflats sponsored with support from the European Union. The helpful comments of Professor K. Dyer (University of Plymouth), Dr. D.M. Paterson (Univ. Aberdeen), and one anonymous reviewer have contributed to the development of this manuscript, and is graciously acknowledged. This work was partially supported by grants from the National Science Foundation (OCE 95-23712), and Office of Naval Research (N0001497-10024 and N0001497-0806). This represents RIBS Contribution No. XXX.

References

Alldredge, A.L., Passow, U., Logan, B.E., 1993. The abundance and significance of a class of large transparent organic particles in oceans. Deep-Sea Research 40, 1131–1140.

Arnosti, C., 1995. Measurement of depth- and site-related differences in polysaccharide hydrolysis rates in marine sediments. Geochimica et Cosmochimica Acta 59, 4247–4257.

Arnosti, C., 1996. A new method for measuring polysaccharide hydrolysis rates in marine environments. Organic Geochemistry 25, 105–115.

Awramik, S.M., 1992. The history and significance of stromatolites. In: Schidlowski, M., et al. (Ed.), Early Organic Evolution: Implications for Mineral and Energy Resources. Springer, Berlin, pp. 435–449.

Azam, F., 1998. Microbial control of oceanic carbon flux: the plot thickens. Science 280, 694–696.

Azam, F., Smith, D.C., Steward, C.F., Hagstrom, A., 1993. Bacteria–organic matter coupling and its significance for ocean carbon cycling. Microbial Ecology 28, 167–179.

Belanger, C., Desrosiers, B., Lee, K., 1997. Microbial extracellular enzyme activity in marine sediments: extreme pH to terminate reaction and sample storage. Aquatic Microbial Ecology 13, 187–196.

Black, D.M., Paterson, D.M., Davidson, I.D., 1999. Sediment micro-fabric of oil rig drill spoil heaps: preliminary observations using low-temperature scanning electron microscopy. Environmental Science and Technology 33, 1983–1990.

Boyd, A., Chakrabarty, A.M., 1995. Pseudomonas aeruginosa biofilms: role of the alginate exopolysaccharide. Journal of Industrial Microbiology 15, 162–168.

Caldwell, D.E., Korber, D.R., Lawrence, J.R., 1992. Confocal laser microscopy and digital image analysis in microbial ecology. Advances in Microbial Ecology 12, 1–67.

Canfield, D.E., Des Marais, D.J., 1991. Aerobic sulfate reduction in microbial mats. Science 251, 1471–1473.

Chin, W.-C., Orellana, M.V., Verdugo, P., 1998. Formation of microgels by spontaneous assemble of dissolved marine polymers. Nature 391, 568–572.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M., 1995. Microbial biofilms. Annual Review of Microbiology 49, 711–745.

Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., Greenberg, E.P., 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280, 295–298.

Decho, A.W., 1990. Microbial exopolymer secretions in ocean environments : their role(s) in food webs and marine processes. Oceanography and Marine Biology: an Annual Review 28, 73–153.

Decho, A.W., 1999a. Imaging of an alginate polymer gel using atomic force microscopy. Carbohydrate Research 315, 330–333.

Decho, A.W., 1999b. Chemical communication within microbial biofilms: chemotaxis and quorum sensing in bacteria cells. In: Wingender, J., Neu, T., Flemming, H.-C. (Eds.), Microbial Extracellular Polymer Substances. Springer-Verlag, Berlin, pp. 155–169.

Decho, A.W., Kawaguchi, T., 1999. Confocal imaging of in situ natural microbial communities and their extracellular polymeric secretions (EPS) using nanoplast resin. Biotechniques 27, in press.

Decho, A.W., Lopez, G.R., 1993. Exopolymer microenvironments of microbial flora: multiple and interactive effects of trophic relationships. Limnology and Oceanography 38, 1633–1645.

Défarge, C., 1997. Cryoscanning electron microscopy and high resolution scanning electron microscopy of organic matter and organomineral associations in modern microbial sediments. Geomaterials Petrology and Sedimentology 324 (2a), 553–561.

Défarge, C., Trichet, J., Jaunet, A., Robert, M., Tribble, J., Sansone, F.J., 1996. Texture of microbial sediments revealed by cryo-scanning electron microscopy. Journal of Sedimentary Research 66, 935–947.

Dunlap, P.V., 1997. N-acyl-L-homoserine lactone autoinducers in bacteria. In: Shapiro, J.A., Dworkin, M. (Eds.), Bacteria as Multicellular Organisms. Oxford University Press, New York, pp. 451.

Fuqua, C., Winans, S.C., Greenberg, E.P., 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum sensing transcriptional regulators. Annual Review of Microbiology 50, 727–751.

Geesey, G.G., Jang, L., 1989. Interactions between metal ions and capsular polymers. In: Beveridge, T.J., Doyle, R.J. (Eds.), Metal Ions and Bacteria. Wiley, New York, pp. 325–357.

Giller, P.S., Hildrew, A.G., Raffaelli, D.G. (Eds). 1994. Aquatic Ecology: Scale, Pattern and Processes. Blackwell Publishers, Oxford, 649 pp.

Grant, J., Bathmann, U.V., Mills, E.L., 1986. The interaction between benthic diatom films and sediment transport. Estuarine, Coastal and Shelf Science 23, 225–238.

Haugland, R.P., 1996. Handbook of fluorescent probes and research chemicals, 6th Edition Molecular Probes, Inc., Eugene, OR.

Henrichs, S.M., Reeburgh, W.S., 1987. Anaerobic mineralization of marine sediment organic matter: rates and the role of anaerobic processes in the oceanic carbon economy. Geomicrobiology Journal 5, 191–237.

Herndl, G.J., 1988. Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. II. Microbial density and activity in marine snow and its implication to overall pelagic processes. Marine Ecology Progress Series 48, 265–275.

Hoagland, K.D., Rosowski, J.R., Gritz, M.R., Roemer, S.C., 1993. Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. Journal of Phycology 29, 537–566.

Hoffman, M., Decho, A.W., 2000. Extracellular enzymes within microbial biofilms and the role of the extracellular polymer matrix. In: Wingender, J., Neu, T., Flemming, H.-C. (Eds.), Microbial Extracellular Polymer Substances. Springer, Berlin (in press).

Hoppe, H.-G., 1991. Microbial extracellular enzyme activity: a new key parameter in aquatic ecology. In: Chrost, R.J. (Ed.), Microbial Enzymes in Aquatic Environments. Springer, Berlin, pp. 60–83.

Jϕrgensen, B.B., 1994. Sulfate reduction and thiosulfate transformations in a cyanobacterial mat during a diel oxygen cycle. FEMS Microbial Ecology 13, 303–312.

Kawaguchi, T., Decho, A.W., 2000. Biochemical characterization of cyanobacterial extracellular polymers (EPS) from modern marine stromatolites. Preparative Biochemistry and Biotechnology (in press).

Kieber, R.J., Zhou, X., Mopper, K., 1990. Formation of carbonyl compounds from UV-induced photodegradation of humic substances in natural waters: fate of riverine carbon in the sea. Limnology and Oceanography 35, 1503–1515.

King, G.M., 1986. Characterization of B-glucosidase activity in intertidal marine sediments. Applied and Environmental Microbiology 51, 373–380.

Krembs, C., Juhl, A.R., Long, R.A., Azam, F., 1998. Nanoscale patchiness of bacteria in lake water studied with the spatial information preservation method. Limnology and Oceanography 43, 307–314.

Lancelot, C., Mathot, S., 1987. Dynamics of a Phaeocystis-dominated spring bloom in Belgian coastal waters. I. Phytoplankton activity and related parameters. Marine Ecology Progress Series 37, 239–248.

Lavoie, D.M., Little, B.J., Ray, R.I., Bennett, R.H., Lambert, M.W., Asper, V., Baerwald, R.J., 1995. Environmental scanning electron microscopy of marine aggregates. Journal of Microscopy 178, 101–106.

Lawrence, J.R., Korber, D.R., Hoyle, B.D., Costerton, J.W., Caldwell, D.E., 1991. Optical sectioning of microbial biofilms. Journal of Bacteriology 173, 6558–6567.

Lawrence, J.R., Korber, D.R., Wolfaardt, G.M., Caldwell, D.E., 1997. Analytical imaging and microscopy techniques. In: Hurst, C.J., et al. (Ed.), Manual of Environmental Microbiology. American Society of Microbiology Press, Washington, DC, pp. 29–51.

Leppard, G.S., Heissenberger, A., Herndl, G.J., 1996. Ultrastructure of marine snow. I. Transmission electron microscopy methodology. Marine Ecology Progress Series 135, 289–298.

Lienemann, C.P., Heissenberger, A., Leppard, G.G., 1998. Optimal preparation of water samples for the examination of colloidal material by transmission electron microscopy. Aquatic Microbial Ecology 14, 205–213.

Lindberg, B., 1990. Components of bacterial polysaccharides. Advances in Carbohydrate Chemistry and Biochemistry 48, 279–318.

Luoma, S.N., Johns, C., Fisher, N.S., Steinberg, N.A., Oremland, R.A., Reinfelder, J.R., 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Environmental Science and Technology 26, 485–491.

Mayer, L.M., 1989. Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. Limnology and Oceanography 34, 973–981.

Meyer-Reil, L.A., 1991. Ecological aspects of enzymatic activity in marine sediments. In: Chrost, R.J. (Ed.), Microbial Enzymes in Aquatic Environments. Springer, Berlin, pp. 84–95.

Mopper, K., Zhou, X., Kieber, R.J., Sikorski, R.J., Jones, R.D., 1991. Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. Nature 353, 60–62.

Muller-Niklas, G., Schuster, S., Laltenbock, E., Herndl, G.J., 1994. Organic content and bacterial metabolism in amorphous aggregations in the northern Adriatic Sea. Limnology and Oceanography 39, 58–68.

Myklestad, S., 1995. Release of extracellular products of phytoplankton with special emphasis on polysaccharides. Science of the Total Environment 165, 155–164.

Neu, T.R., 1996. Significance of bacterial surface-active compounds in interactions of bacteria with interfaces. Microbiology Reviews 60, 151–170.

Nichols, P.D., Henson, J.M., Guckert, J.B., Nivens, D.E., White, D.C., 1985. Fourier transformed-infared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria polymer mixtures and biofilms. Journal of Microbiological Methods 4, 79–94.

Ortega, N., Busto, M.D., Perez-Mateos, M., 1998. Stabilisation of beta-glucosidase entrapped in alginate and polyacrylamide gels towards thermal and proteolytic deactivation. Journal of Chemistry, Technology and Biotechnology 73, 7–12.

Paerl, H.W., 1997. Primary productivity and producers. In: Hurst, C.J., Hudson, G.R., McInerney, M.J., Stetzenbach, L.D., Walter, M.V. (Eds.), Manual of Environmental Microbiology. American Society of Microbiology Press, Washington, pp. 252–262.

Passow, U., Alldredge, A.L., 1994. Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. Marine Ecology Progress Series 113, 185–198.

Passow, U., Alldredge, A.L., Logan, B., 1994. The role of particulate carbohydrate exudates in the flocculation of diatom blooms. Deep-Sea Research 41, 335–357.

Paterson, D.M., 1989. Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behavior of epipelic diatoms. Limnology and Oceanography 34, 223–234.

Paterson, D.M., 1995. Biogenic structure of early sediment fabric visualized by low-temperature scanning electron microscopy. Journal of Geological Society of London 152, 131–140.

Pawley, J., 1995. Handbook of Biological Confocal Microscopy, 2nd Edition, Plenum Press, New York.

Payment, P., Gramade, F., Paquette, G., 1989. Microbiological and virological analysis of water from two water filtration plants and their distribution systems. Canadian Journal of Microbiology 34, 1304–1309.

de Philippis, R., Vincenzini, M., 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiology Reviews 22, 151–175.

Pinckney, J., Paerl, H.W., Reid, R.P., Bebout, B., 1994. Ecophysiology of stromatolitic mats Stocking Island, Exuma Cays, Bahamas. Microbial Ecology 29, 19–37.

Plante, C.J., Shriver, A.G., 1998. Differential lysis of sedimentary bacteria by *Arenicola marina* L.: examination of cell wall structure and exopolymeric capsules as correlates. Journal of Experimental Marine Biology and Ecology 229, 35–52.

Potts, M., 1994. Desiccation tolerance of prokaryotes. Microbiology Reviews 58, 755–805.

Reid, R.P., Browne, K.M., 1991. Intertidal stromatolites in a fringing Holocene reef complex. Bahamas. Geology 19, 15–18.

Reid, R.P., MacIntyre, I.G., Browne, K.M., Steneck, R.S., Miller, T., 1995. Modern marine stromatolites in the Exuma Cays. Bahamas: uncommonly common. Facies 33, 1–18.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J., Bebout, B., Macintyre, I., Dupiaz, C., Pinckney, J., Parel, H., Prnfert-Beburt, L., Steppe, T., Desmarais, D., 2000. Microbial Lithification in modern marine stromatolites. Nature, in press.

Ruby, E.G., 1996. Lessons from a cooperative bacterial-animal association. The *Vibrio fischeri-Euprymna scolopes* light organ symbiosis. Annual Review of Microbiology 50, 591–624.

Schlekat, C.E., Decho, A.W., Chandler, G.T., 1998. Sorption of cadmium to bacterial extracellular polymeric sediment coatings under estuarine conditions. Environmental Toxicology and Chemistry 17, 1867–1874.

Schlekat, C.E., Decho, A.W., Chandler, G.T., 1999. Dietary assimilation of cadmium associated with bacterial exopolymer sediment coatings by the estuarine amphipod, *Leptocheirus plumulosus*: effects of Cd concentration and salinity. Marine Ecology Progress Series 183, 205–216.

Schlekat, C.E., Decho, A.W., Chandler, G.T., 2000. Bioavailability of particle-associated Ag, Cd, and Zn to the estuarine amphipod, Leptocheirus plumulosus, through dietary ingestion. Limnology and Oceanography 45, 11–21.

Smith, D.J., Underwood, G.J.C., 1998. Exopolymer production by intertidal epipelic diatoms. Limnology and Oceanography 43, 1578–1591.

Smith, J.J., Qunitero, E.J., Geesey, G.G., 1990. A sensitive chromatographic method for the detection of pyruval groups in microbial polymers from sediments. Microbial Ecology 19, 137–147.

Smith, D.C., Simon, M., Alldredge, A.L., Azam, F., 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359, 139–142.

Stachowitsch, M., Fanuko, N., Richter, M., 1990. Mucus aggregates in the Adriatic Sea: an overview of stages and occurrences. PSZNI Marine Ecology 11, 327–350.

Stal, L.J., 1995. Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytologist 131, 1–32.

Stanwell-Smith, R., 1991. Recent trends in the epidemiology of waterborne disease. In: Morris, R., Alexander, L.M., Wyn-Jones, P., Sellwood, J. (Eds.), Proceedings of the UK Symposium on Health Related Water Microbes. IAWPRC, Glasgow, pp. 44–52.

Stolz, J.F., 1994. Light and electron microscopy in microbial mat research : an overview. In: Caumette, P., Stal, L.J. (Eds.), Microbial Mats, NATO ASI Series, Vol. 35, pp. 173–182.

Suci, P.A., Siedlecki, K.J., Palmer, R.J., White, D.C., Geesey, G.G., 1997. Combined light microscopy and attenuated total reflection fourier transform infared spectroscopy for integration of biofilm structure, distribution and chemistry at solid–liquid interfaces. Applied and Environmental Microbiology 63, 4600–4603.

Suci, P.A., Vrany, J.D., Mittleman, M.W., 1998. Investigation of interactions between antimicrobial agents and bacterial biofilms using attenuated total reflectance fourier transform infared spectroscopy. Biomaterials 19, 327–339.

Surman, S.B., Walker, J.T., Goddard, D.T., Morton, L.H.G., Keevil, C.W., Weaver, W., Skinner, A., Hanson, K., Caldwell, D., Kurtz, J., 1996. Comparison of microscopic techniques for the examination of biofilms. Journal of Microbiological Methods 25, 57–70.

Sutherland, I.W., 1990. Biotechnology of microbial exopolysaccharides. Cambridge University Press, Cambridge, 163pp.

Sutherland, I.W., 1995. Polysaccharide lyases. FEMS Microbial Reviews 16, 323–347.

Sutherland, T.F., Amos, C.L., Grant, J., 1998. The effect of buoyant biofilms on the erodibility of sublittoral sediments of a temperate microtidal estuary. Limnology and Oceanography 43, 225–235.

Tam, P.Y., Verdugo, P., 1981. Control of mucus hydration as a Donnan equilibrium process. Nature 292, 340–342.

Tessier, A., Campbell, P.G.C., 1987. Partitioning of trace metals in sediments: relationships with bioavailability. Hydrobiologica 149, 43–52.

Tessier, A., Turner, D.R., 1995. Metal Speciation and Bioavailability in Aquatic Systems. Wiley, Ltd., New York

Tokita, M., Tanaka, T., 1991. Friction coefficient of polymer networks of gels. Journal of Chemistry and Physics 95, 4613–4619.

Underwood, G.J.C., 1994. Seasonal and spatial variations in epipelic diatom assemblages in the Severn Estuary. Diatom Research 9, 451–472.

Underwood, G.J.C., Paterson, D.M., 1993. Seasonal changes in diatom biomass, sediment stability and biogenic stabilisation in the Severn estuary. Journal of Marine Biological Association of United Kingdom 73, 871–887.

Underwood, G.J.C., Paterson, D.M., Parkes, R.J., 1995. The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnology and Oceanography 40, 1243–1253.

Underwood, G.J.C., Smith, D.J., 1998. Predicting epipelic diatom exopolymer concentrations in intertidal sediments from sediment Chl-*a*. Microbial Ecology 35, 116–125.

Verdugo, P., 1994. Polymer gel phase transition in condensation–decondensation of secretory products. Advances in Science 110, 145–156.

Visscher, P.T., Reid, R.P., Bebout, B.M., Hoeft, S.E., MacIntyre, I.G., Thompson, J.A., 1998. Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): The role of sulfur cycling. American Mineralogist 83, 1482–1493.

Warren, R.A.J., 1996. Microbial hydrolysis of polysaccharides. Annual Review of Microbiology 50, 183–212.

Zhou, J., Mopper, K., Passow, U., 1998. The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. Limnology and Oceanography 43, 1860–1871.