Chapter 9

# Microbial Effects

*Hans-Curt Flemming*

Chapter 9 deals with a very important and often neglected component involved in pollutant mobility: the microorganisms. They are ubiquitous. They colonize sediment surfaces in contact with water more or less densely and are present in the water phase, mainly as flocs. Of course, they represent a dynamic phase in the system which has to be considered. However, their influence is very difficult to asses as microorganisms do not represent a chemically homogeneous phase but are spatially heterogeneous and vary in time in terms of population composition, density, activity and presence of extracellular polymeric substances (EPS). This results in a very complex network of interactions as microorganisms respond to the conditions of their environment, and, in addition, can possibly transform pollutants – be it by degradation of organic substances or by changing the species of metals, e.g., by alkylation. Little wonder that such aspects have not been exhaustively addressed scientifically and it is a particular feature of this book to specifically deal with the aspects provided by microorganisms.

The first section deals generally with the most frequent microbial phenomenon which is biofilms. It is important to understand the basic laws of biofilm development and dynamics in order to understand their impact on pollutant mobility, including sediment stability. The nature of EPS will influence biofilm sorption properties as well as biofilm cohesion and "gluing" of sediment particles. The interaction with ions is of particular interest here as bridging effects due to ionic interactions with charged groups in EPS play an important role. Aspects of sorption are addressed as well as the various stabilizing and destabilizing effects on sediments. Sorption properties of biofilms are equally complex as there are different sorption sites such as cells, cell walls, and EPS with different sorption capacities.

A very important step for understanding the effect of microorganisms is the comparison of non-sterile to sterile systems. This is addressed in laboratory systems in Sect. 9.2 in detail, using mucoid and non-mucoid strains of *Pseudomonas aeruginosa* in order to obtain quantitative data. Of course, much care has to be applied when extrapolating these results to natural systems, however, surprisingly consistent parallels could be drawn from slow sand filters samples from a drinking water plant. Interestingly, biofilms can contribute to higher erosion rates when growing directly at the sediment-water interface ("fluffy layer"), disrupting sediment particles from the surface and carrying them into the water phase, contributing to sediment-related pollutant mobility while in greater depth, it could be clearly shown that biofilms increase sediment cohesion.

In Sect. 9.3, again sterile vs. non-sterile experiments were carried out, indicating the cohesive effect of biofilms to sediments and increasing the settlement of suspended particles. In these experiments, sterilization was performed by gamma-irradiation of environmental samples, leaving dead biomass in place which, however, cannot further multiply. When particulate metals are transported in the water phase, bacteria obviously contribute to their accelerated sedimentation which has been confirmed in field investigations: Bacterial cell content, suspended matter content and heavy metal concentrations followed similar patterns during settling after re-suspension.

In general, this chapter contributes an interesting aspect which is far from being fully understood and can represent a problem for modeling as the dynamics of microbial influence are so complex. However, "black-box" approaches neglecting biotic influences cannot the solution of the problem as the sometimes insufficient predictive power of modeling demonstrates.

*Hans-Curt Flemming · Martin Strathmann · Carlos Felipe Leon Morales*

## 9.1 Biofilms and Their Role in Sediment Dynamics and Pollutant Mobility

### 9.1.1 Introduction

Natural sediments are not sterile but inhabited by a large range of microorganisms (Riding and Awramik, 2000) and higher forms of life. As a consequence, these organisms participate in many chemical processes in sediments, in the interaction between sediments and the water phase and in sediment dynamics. In fluvial environments, the interface between the major water body and the sediment, is a very active zone both in physicochemical and biological terms. Especially in highly permeable sediments, the dynamic flux of energy, nutrients, metabolites and particles (including microorganisms) is interdependent with local hydrodynamics (Huettel et al. 2003). Due to their slime matrix, active microbial communities at the water-sediment interface, develop into macroscopic scale structures which modify sediment topography and frictional resistance. These surface alterations have repercussions in fluid flow, shear forces and other physical parameters, especially at the benthic boundary layer. Microbial colonization is not limited to the sediment-liquid interface; equally important on their effect on river sediment hydrodynamics, is their ability to develop at significant sediment depths. At this level, permeability and hydraulic conductivity changes caused by microbial colonization, can have a profound effect on sediment cohesion and sorption/desorption processes (Leon-Morales et al. this vol.).

A key feature for understanding the influence of microorganisms on sediments is their common form of life which is biofilms. Microorganisms in nature do not occur as pure cultures and single organisms but in mixed communities, organized in aggregates. Such aggregates are termed somewhat inaccurate as "biofilms" and include films, colonizing solid surfaces in water but also on liquid-liquid and liquid-gaseous interfaces as well (e.g., at the water-air-interface or water-oil-interface). The term also embraces microbial mats, sludges and flocs (which can be considered as floating biofilms). The justification for this is that all these phenomena have one aspect in common which is

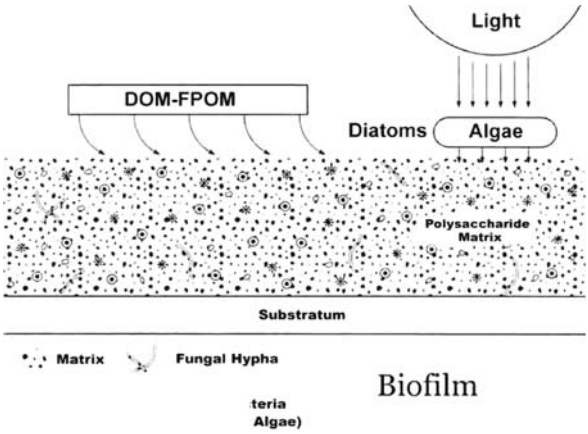


**Fig. 9.1.**
Sediment biofilm (after National Park Service, U.S. Department of the Interior)

that the cells in the aggregates are kept together by a matrix of extracellular polymeric substances (EPS) which provide some basic advantages for the cells, such as a long retention time in a stabile position to each other, allowing for the formation of synergistic microconsortia. Due to their physiological activity, gradients develop in pH-value, redox potential as well as in the concentration of electron acceptors and donators.

Biofilms can form on virtually all surfaces exposed to non-sterile environments, provided enough water and nutrients. They can be found in soil and aquatic environments, on plant surfaces, on tissues of animals as well as in technical systems such as filters, pipes and reactors, and they play a pivotal role in medical context, in particular, in infections (Costerton et al. 1995). Environments in which biofilms have been investigated include not only solid-liquid but also air-liquid (Spiers et al. 2003), liquid-liquid (Macedo et al. 2005) interfaces, among others. Biofilms have to be considered in attempts to understanding transport, immobilization and remobilization processes of sediment particles as well as of pollutants as they form a dynamic interphase between solid surfaces and the water phase. In modeling approaches, this aspect is usually completely neglected as it is difficult to include and to predict, in particular, if the basic rules of biofilm development and properties are not taken into account. In order to account for such influences, experiments on sediment and pollutant mobility, therefore, have to be carried out (see Leon-Morales et al. this volume; Neto et al. this volume). In sediments with light access, diatoms usually contribute the main component of biofilm populations (Stal and de Brouwer, 2003). A typical sediment biofilm is depicted in Fig. 9.1.

### 9.1.2 Extracellular Polymeric Substances (EPS)

One key aspect for understanding the role of biofilms is the "extracellular polymeric substances" (EPS). These are highly hydrated biopolymers of microbial origin, embedding the biofilm organisms; for a detailed overview see Wingender et al. (1999). EPS are defined as "extracellular polymeric substances of biological origin that participate in the formation of microbial aggregates". EPS represent the construction material of biofilms and the immediate environment for biofilm inhabitants. In general, the pro-

portion of EPS in biofilms can vary between 50 and 90% of the total organic matter. Detailed reviews on EPS can be found in (Wingender et al. 1999) and in (Flemming and Wingender 2002). EPS are mainly responsible for the structural and functional integrity of biofilms and are considered as the key components that determine the physico-chemical and biological properties of biofilms (Allison 2003; Flemming and Wingender 2003). EPS create a microenvironment for sessile cells which is conditioned by the nature of the EPS matrix.

Regardless of their origin, EPS are located at or outside the cell surface. This extracellular localization and the composition of EPS may be the result of different processes: active secretion, shedding of cell surface material, cell lysis, and adsorption from the environment. Microbial EPS are biosynthetic polymers (biopolymers) which consist mainly of polysaccharides and proteins, but can also contain substantial amounts of DNA, lipids, glycolipids and humics. Most bacteria are able to produce EPS, whether they grow in suspension or in biofilms. Cell surface polymers and EPS are of major importance for the development and structural integrity of flocs and biofilms. They mediate interactions between the microorganisms and maintain the three-dimensional arrangement.

Much information has been collected about the chemical nature and physico-chemical properties of extracellular polysaccharides, since they are abundant in many bacterial EPS. Specific polysaccharides (e.g., xanthan) are only produced by individual strains, whereas non-specific polysaccharides (e.g., levan, dextran or alginate) are found in a variety of bacterial strains or species. Non-carbohydrate moieties like acetyl, pyruvyl and succinyl substituents can greatly alter the physical properties of extracellular polysaccharides and the way in which the polymers interact with one another, with other polysaccharides or proteins, and with inorganic ions (Sutherland 1994). The network of microbial polysaccharides displays a relatively high water-binding capacity and is mainly responsible for acquisition and retention of water to form a highly hydrated environment within flocs and biofilms (Chamberlain 1997).

Extracellular polysaccharides are believed to have the main structural function within biofilms by forming and stabilizing the biofilm matrix.

It must be pointed out that polysaccharides are not necessarily the main EPS component. However, not much is known about synergistic gelling of polysaccharides, proteins and humic substances. In many cases of environmental biofilm samples, proteins prevail, and humic substances are also integrated in the EPS matrix, being considered by some authors as belonging to the EPS (Wingender et al. 1999). Although mostly a minor component, lipids can make up a significant proportion of the EPS in some cases. This has been shown in the case of strongly acidophilic organisms, colonizing and leaching pyrite (Gehrke et al. 1998). The role of proteins, however, is mostly considered in terms of their enzymatic activity. Only a few authors speculate that extracellular proteins may also have structural functions. For example, the bridging of extracellular polysaccharides by lectin-like proteins is discussed (Dignac et al. 1998). Furthermore, the role of lectins (proteins with specific binding-sites for carbohydrates) in adhesion of bacterial cells and biofilm formation has been investigated (Tielker et al. 2005). A part of the extracellular proteins has been identified as enzymes. An overview about extracellular enzymes can be found in Wingender and Jaeger (2002). Enzyme activities in biofilms include among others aminopeptidases, glycosidases, esterases,

lipases, phosphatases and oxidoreductases (Frølund et al. 1995). Most of these enzymes are an integrated part of the EPS matrix (Frølund et al. 1995). It is believed that their main function is the extracellular degradation of macromolecules into low molecular weight compounds which can then be transported into the cells and are available for microbial metabolism. The degradation and utilization of particulate matter is performed by colonization of the material and the secretion of extracellular enzymes. The EPS matrix prevents the enzymes and the degradation products from loss and keeps them in close proximity to the biofilm cells. Moreover, specific interactions between extracellular enzymes and other EPS components have been observed resulting in the protection and localization of the enzyme (Wingender et al. 1999). It is suggested, that the structure of the EPS matrix might not be purely random but is involved in the regulation and activity of extracellular enzymes. Thus, the cell maintains a certain level of control over enzymes which otherwise are out of reach.

In order to degrade hydrophobic compounds, microorganisms excrete surface-active polymers. An overview of the various types of EPS, their properties and their significance can be found in Neu (1996). The production of those biosurfactants can be induced by hydrophobic carbon sources (Ramsay et al. 1987), indicating the potential of microorganisms to secrete certain EPS when required. It is well known that hydrophobic surfaces can be colonized easily as demonstrated in nature by biofilm formation on leaves during biological degradation.

### *Adhesion and Cohesion*

A major ecological advantage of the biofilm mode of life is that consortia of various organisms can establish and maintain their position over a long period of time, compared to the planktonic form of life. This applies not only to biofilms but also to flocs and allows for the development of synergistic relationships. A classical example is nitrification which takes place in biofilms and allows the spatial closeness of ammonia oxidizers to nitrite oxidizers. The EPS molecules which keep the organisms together and, if they form a biofilm, are responsible for adhesion to a given surface, provide this advantage. There is literally no surface material which cannot be colonized sooner or later, but there are strong differences in the colonization kinetics. In some cases, attachment was found to stimulate the synthesis of EPS (Vandevivere and Kirchman 1993). Both adhesion and cohesion are based on weak physico-chemical interactions and not on covalent bonds.

Three major kinds of forces can be distinguished: electrostatical interactions, hydrogen bonds and London dispersion forces (Mayer et al. 1999; Flemming et al. 2000). This is symbolized in Fig. 9.2 (Flemming et al. 2000).

The individual binding force of any type of these interactions is relatively small compared to a covalent C-C bond. However, the total binding energies of weak interactions between EPS molecules multiply with the large number of binding sites available in the macromolecules and add up to bond values exceeding those of covalent C-C bonds. The matrix network is formed by fluctuating adhesion points and the resulting matrix can behave as a gel as long as a certain shear stress is not exceeded. In this phase, the adhesion points flip back to their original arrangement. Above that point ("yield point"), new adhesion points assemble and the matrix behaves as a highly viscous fluid (Körstgens et al. 2001).

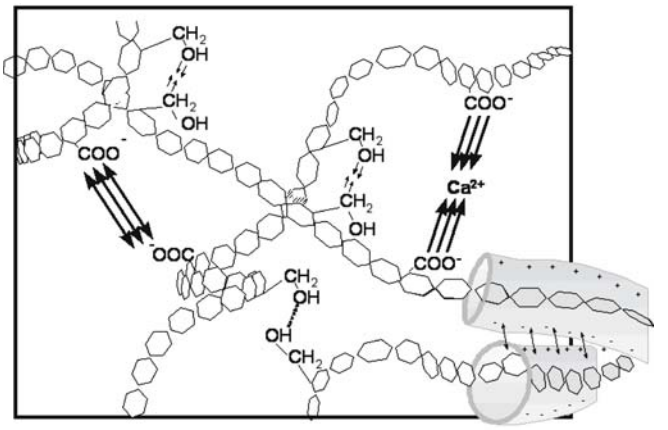


**Fig. 9.2.**
Weak physico-chemical interactions between polysaccharide strains in the EPS matrix (after Flemming et al. 2000)

### *Biofilm Architecture and Mass Transport*

The architecture of the EPS matrix influences the processes within biofilms profoundly. Costerton et al. (1994) have shown that pores and channels occur in which convective transport is possible to a certain extent. Hoffman and Decho (1999) postulated areas of different density of the matrix, which have been observed experimentally. These features result in an extremely heterogeneous structure. This structure is dynamic; Schmitt et al. (1995) demonstrated in a *P. putida* biofilm which was charged with toluene that a rising concentration of toluene caused the formation of more polysaccharide, and furthermore, these compounds contained more carboxyl groups. In sediment biofilms with access of light, algae are major contributors to EPS production (see Fig. 9.1).

An important question is whether the EPS matrix acts as a diffusion barrier. The major component of that matrix is water. It could be shown by NMR measurements that the self-diffusion coefficient of water within the biofilm is practically the same as in free water, and only a very small fraction, less than 0.1%, displays a significantly lower diffusion coefficient (Vogt et al. 2000). There is evidence that non-charged molecules up to a molecular mass of around 10 000 Dalton experience practically no diffusion limitation. However, if they are consumed, as is the case with oxygen, gradients arise because oxygen consumption by aerobic organisms can occur faster than oxygen can follow the diffusion gradient. This is how anaerobic zones in biofilm arise and why anaerobic organisms can find suitable habitats directly below respiring aerobic colonies. Charged molecules can interact with charged groups of the EPS, which may slow down their mobility to a certain extent. This makes perfect sense from an ecological point of view because the mobility of nutrients, exoenzymes and other products is not restricted within the matrix, which is of great importance for cells located in the center of clusters.

Mass transport is influenced not only by the internal architecture of biofilms but also by their interface to the water phase. Some biofilms have a highly filamentous appearance while others are smooth. It is obvious that a large number of filaments will increase the surface at which interactions with components of the water phase are possible.

It could be demonstrated that in this biofilm matrix which is dominated by polysaccharides with carboxyl groups, calcium acts as an important bridging ion which increases the stability of the network significantly. This is also the case for copper and iron but not for magnesium. In such cases, surfactants will not contribute to the dissolution of biofilms. However, if other biopolymers dominate, it is possible that surfactants have a more significant effect. Hydrogen bonds are also part of the overall binding force. They can be influenced by so-called chaotropic agents which have a high affinity for water, thus interfering with the water shell around the biopolymers. In some cases, this type of bond dominates the binding forces. The extent to which each bond contributes to the cumulative binding force depends strongly of the nature of the EPS molecules. As different strains can produce different EPS, the variety is considerable, suggesting that not all biofilms can be dissolved by means of only one cleaning formulation. This coincides well with observations from practice. EPS are not totally insoluble in water. A certain amount of EPS is continuously lost to the water phase. In wastewater, this contributes to important measures of process parameters such as chemical oxygen demand (COD).

### 9.1.3 Biofilm Role on Sediment Stability

In sediments, sand grains are at least partially colonized by biofilms (Decho 1994). However, the extent of colonization can range from minute specks (Fig. 9.3) to complete coverage and clogging of the sediment particles (Fig. 9.4). It is long known that biofilms influence the entrainment of sand (Dade et al. 1990), mainly due to the cohesive forces between biofilm-covered sand grains.

Massive colonization of sediments can result in clogging and has been reported by Battin and Sengschmitt (1999). Here, algae were the main causes for copious EPS production which resulted in clogging. In addition, detrital material can accumulate in sediment biofilms, contributing to further clogging (Neu and Lawrence 1997).

When light has access to sediments, algae develop and contribute to biofilm mass. De Brouwer et al. (2005) have investigated in particular the role of the benthic diatoms *Nitzschia* cf. *brevissima* and *Cylindrotheca closterium*. They determined critical shear stress in presence and absence of these organisms and found a significant correlation of extracellular carbohydrate to critical shear stress for *N.* cf. *brevissima* but not for

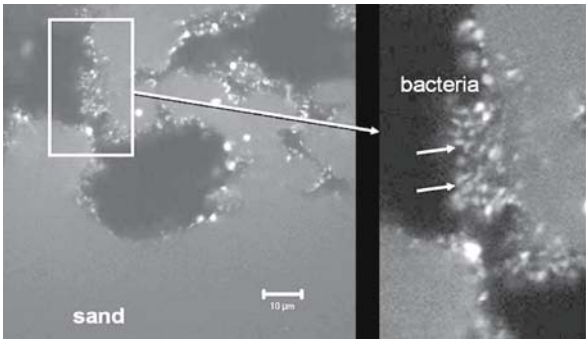


**Fig. 9.3.**
Patchy biofilm on the surface of sand grains. The cells were stained with the DNA specific fluorochrom SYTO 9 (molecular probes). Epifluorescence microscopic image; *bar* = 10 µm (after Strathmann et al. in press)

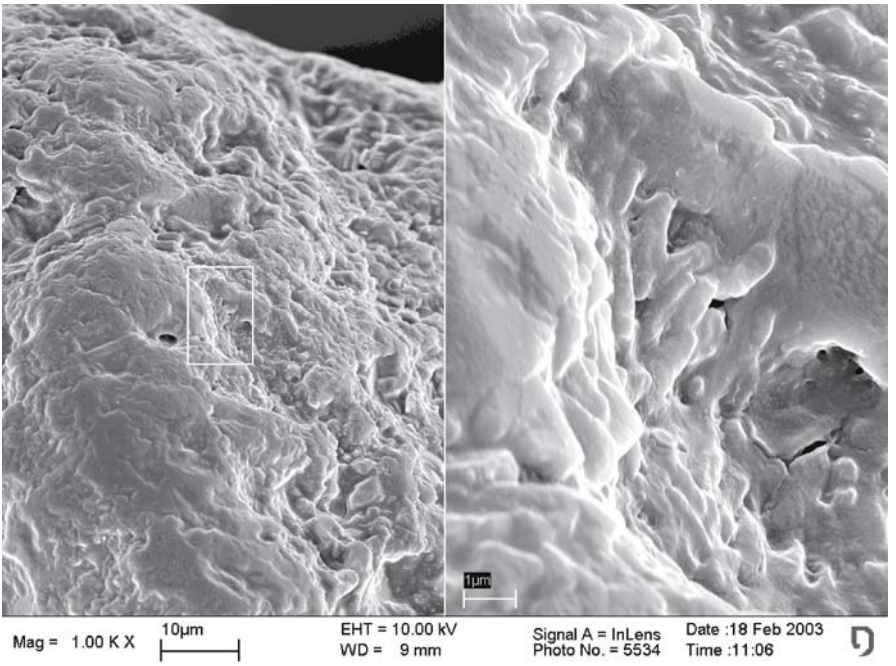


**Fig. 9.4.** SEM of a continuos biofilm on sand grains, formed by the mucoid strain *Pseudomonas aeruginosa* SG 81; the cells can be seen embedded in a thick alginate matrix (Leis unpublished)

*C. closterium*, although they could not give an explanation but only the coinciding observation that both organisms formed biofilms of different morphology as visualized by confocal laser scanning microscopy. However, the finding illustrates the fact that the carbohydrate content alone cannot be taken as an indicator for sediment stability; therefore, diatoms cannot be fully accountable for biostabilization in natural sediments as suggested by some authors (Madsen et al. 1993; Yallop et al. 2000). Neto et al. (this vol.) showed that settling of sand was slower with sterilized sediments, indicating the biotic component in settling. De Brouwer et al. (2005) conclude that "it is clear that stability of the sediments is poorly explained by simple indicators such as chlorophyll a and the extracellular carbohydrates. This again indicates that compositional characteristics of the biofilm matrix may be important to explain the stabilizing effect exerted by diatom biofilms". It has been generally considered that the action of EPS in stabilization of sediments involves chemical interactions between functional groups in the EPS and sediment particles in order to physically bind sediment particles together (Paterson 1997; Yallop et al. 1994). De Brouwer et al. (2005) found clear indications that $Ca^{2+}$ ions favored the adsorption of EPS to sediment particles when compared to $Na^{+}$ ions. This suggests that cation divalent bridging is an important process mediating adsorption of EPS to sediment particles (Decho 1994). In Table 9.1, an overview on stabilizing and destabilizing effects of biological components is given (after Black et al. 2002, with results from Leon Morales, this vol.).

**Table 9.1.** Aspects of stabilizing and destabilizing biological effects on natural cohesive sediment (after Black et al. 2000, with results from Leon-Morales, this vol.)

| Stabilizing | Destabilizing |
|---|---|
| EPS secretion: EPS by bacteria and microphytobenthos enhances cohesion, promotes flocculation and, hence, deposition | Blistering: Trapping of oxygen bubbles in biofilms increases buoyancy of the biofilm to such an extent that it pulls away from sediment |
| Sediment compaction: Burrowing macrofauna increase sediment density and hence stability | Grazing: Organisms feeding on biofilms cause physical disturbance and reduce cohesion of sediment |
| Increased drainage: Burrow and channel formation promotes dewatering and higher density of sediment material | Burrow cleaning: Some benthic fauna clean tubes they inhabit in the sediment, giving rise to a localized benthic flux |
| Flow effects: Plants and animal tubes from dense fields that induce skimming flow in the overlying water, protecting the sediment bed from erosion | Boundary layer effects: Burrows, tubes and tracking of the sediment surface increase bed roughness and hence near bed turbulence so enhancing erosion |
| Sediment compaction: In deeper sediment layers, EPS lead to stronger cohesion | Fluffy layer: Loose parts of biofilms at the sediment surface are easily sheared away, taking bound superficial sediment material with them |

### 9.1.4 Role of Biofilms As Sink and Source of Pollutants

Dissolved and particulate matter in water is continuously interacting at the interfaces with other phases in terms of sorption and desorption. If a biofilm is present, it will participate in such processes either by accumulation or by biochemical transformation of substances – basically, the ability to sequester matter from the water phase is the key mechanism of nutrient acquisition for biofilm organisms. But even if substances trapped in the EPS network do not interact with biofilms at all, they will have to pass through the biofilm when interacting with the underlying surfaces. In the case of large molecules, they can experience significant diffusion resistance. The term "sorption" refers to *adsorption*, *absorption* and *desorption. Adsorption* implies the retention of a solute on the surface of the particles of a material. *Absorption* in contrast involves the retention of a solute within the interstitial molecular pores of such particles (Skoog 1996). Biofilms are involved in all of them. A closer look at the dynamic role of biofilms in terms of sorption sites reveals a complex system (Fig. 9.5).

When a dissolved or particulate substance, transported by the water phase, meets a biofilm, it will not encounter a uniform structure but a highly heterogeneous hydrogel with very different sorption sites. These include:

- *Extracellular polymer substances* (EPS), mainly consisting of polysaccharides and proteins:
  - Charged groups, e.g.,: –COO–, –SH–, $-SO_4^{2-}$, $-H_2PO_4-$, $-NH_4^+$, $-NRH_2^+$
  - Apolar groups, e.g.,: aromatics, aliphatics such as found in proteins; also: hydrophobic regions in polysaccharides

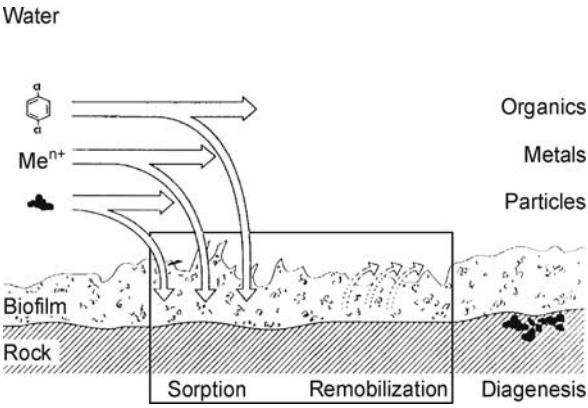


**Fig. 9.5.**
Role of biofilms in sorption and desorption processes (modified after Flemming and Leis 2002)

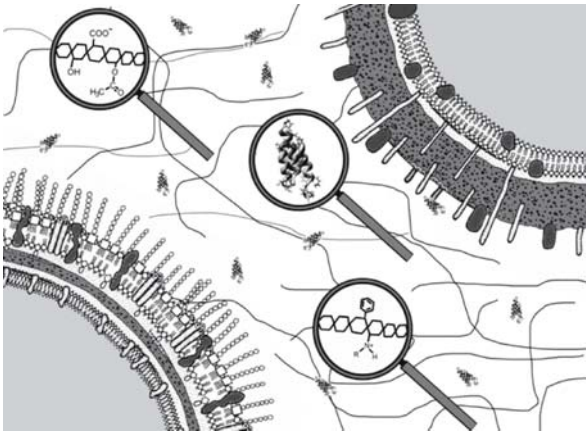

**Fig. 9.6.**
Various sorption sites in the EPS matrix (from top to bottom): *(i)* charged groups of polysaccharides, *(ii)* proteins with polar and apolar sites, *(iii)* apolar and anionic substituents on polysaccharides, additional sorption sites are the cell walls, the membranes and the cytoplasm (from Strathmann et al. in press)

- Cell walls
  - Outer membrane of Gram-negative cells (lipids)
  - Murein or teichoic acid layer of Gram-negative resp. Gram-positive Bacteria
  - Cytoplasmatic membrane (lipids)
- Cytoplasm

The EPS represent the major component of biofilm organic carbon. In Fig. 9.6, the sorption sites within the hydrogel matrix are schematically depicted.

It is obvious that each of these sites has different sorption mechanisms and capacities. Furthermore, the system is dynamic (Sutherland, 2001). Sorption characteristics in bacteria as living organisms can change depending on a great number of factors (Langley and Beveridge 1999). For example, the extent of sorption by some heavy metals will depend on nutritional factors. Nickel uptake in *Pseudomonas aeruginosa* can be increased or lowered depending on the carbon source supplied during growth (Sar 1998). Another example: a biofilm of *Pseudomonas putida* which was exposed to toluene responded in an increase of charged groups in the EPS, providing more ionic binding sites (Schmitt et al. 1995).

Many authors do not differentiate between various sorption sites in biofilms when investigating metal sorption (e.g., Mages et al. this vol.). EPS seem to be a highly plausible binding site from a mechanistic point of view, considering the charged groups of the EPS and their ionic binding capacity. However, in spite of the large body of references confirming this, Späth et al. (1998) separated EPS from cells after charging activated sludge with $Cd^{2+}$. Most of the metal was bound to the cell surfaces and not to the EPS as could have been expected considering the charged nature of many EPS components. The same was true for $Ni^{2+}$ and $Zn^{2+}$. These findings demonstrated that bacterial cell walls can act as templates for metal deposition.

### 9.1.5 Microbial Mineralization and Sediment Formation

Numerous bacteria in aquatic sediments encounter and bind a wide variety of metals in their environment. As the sediments accumulate, they and their bacterial components become subject to geological forces that eventually result in rock formation. During this time, chemical and physical changes occur within the sediments as diagenetic processes. The cell walls of bacteria, present in these sediments, make suitable biological templates for the concentration of metals and the nucleation of crystals and can often greatly influence the initial mineralization process (Krumbein et al. 1994). Carbonate formation in bacteria is another important sorption process mediated by bacterial surfaces. It happens due to the production of alkaline microenvironments near the cell surfaces as a consequence of physiological activities of the cell (for example, bicarbonate use as carbon source, yielding $OH^-$ groups) and also due to the ability of S-layers to bind available $Ca^{2+}$ which together allows gypsum ($CaSO_4 \cdot 2\,H_2O$) and/or calcite ($CaCO_3$) to precipitate. Silicate formation by bacteria has been a very common phenomenon since the beginning of life. It is very likely that the direct participation of bacteria in the formation of these compounds rather than just a silicate after-formation binding with bacterial surfaces. The phenomenon of formation of *microfossils* is thought to be related to silicate interaction with bacteria (Schultze-Lam et al. 1996). Biofilm cells in drinking water pipes seem to be suitable nucleation sites for mineral deposition. Figure 9.7 is a scanning electron micrograph showing microorganisms as templates for the deposition of iron oxides.

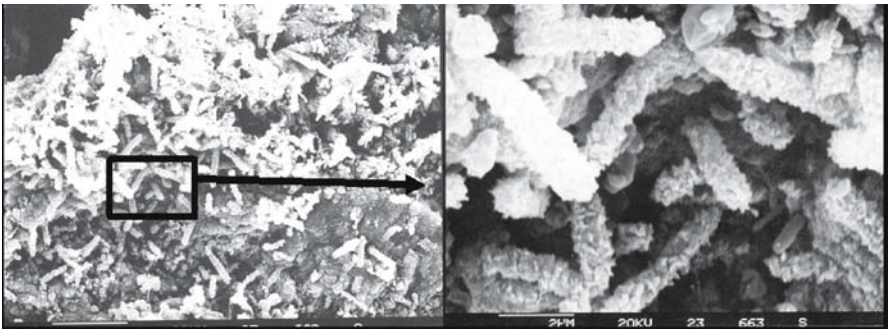


**Fig. 9.7.** Iron oxides precipitated on bacteria in corrosion products of a drinking water pipe (modified from Flemming and Leis 2002)

The external environment and internal metabolism of living bacteria often exert a profound influence on the chemistry of bound metals. These influences include changes in oxidation state, formation of organometallic compounds and formation of precipitates due to detoxification or energy-yielding mechanisms of the bacterial protoplast. Alternatively, the metals may be affected indirectly by the production of metabolic end-products such as $SO_4^{2-}$ and $S^{2-}$ or an alteration in the local pH and/or $E_h$. The results of microbial activity may ultimately lead to metal immobilization, remobilization and/or the formation of metal aggregates.

### 9.1.6 Desorption Processes

Bacterial surfaces and biofilms are not inert chemical structures. They represent a dynamic system in which the various components are synthesized, assembled, modified and finally broken down by autolysins and sloughed off into the environment. Thus, they may contribute to the remobilization of the sorbed substances.

By nature, the immobilization of metal ions in biomass cannot be irreversible. The biological binding sites sooner or later will be degraded. Fate and transport of the metal is directly related to the fate and transport of the bacterial cell. When the cell dies the metal is released. In some instances, this process will lead to mineral formation and is responsible for the deposition of large ores. However, in other cases the sorbed metal ions will return in their more soluble form and be remobilized. Experiments with cell walls of *Bacillus subtilis* and *E. coli* envelopes adsorbed to kaolinite and smectite clays and with the corresponding organic material-clay aggregates showed the complexicity of remobilization processes. Bound to these substances were Ag(I), Cu(II) and Cr(III). The sorbed metals were then leached with $HNO_3$, $Ca(NO_3)_2$, EDTA, fulvic acids and lysozyme at several concentrations. The findings on remobilization of the sorbed metals, in general showed the order $Cr^{2+} < Ag^{+} < Cu^{2+}$. In the wall, clay and composite systems, $Cr^{3+}$ was very stable; at pH 3, 500 micromolar EDTA, 120 ppm fulvic acid and 160 ppm $Ca^{2+}$ released less than 32% (wet weight) of the sorbed chromium. Ag (45–87%) and Cu (up to 100%) were readily removed by these agents. The organic chelators were in general less effective at mobilizing certain metals than elevated $Ca^{2+}$ or low (acidic) pH values. Lysozyme digestion of *Bacillus* walls remobilized $Cu^{2+}$ from walls and Cu-wall-kaolinite composites. $Ag^{+}$ and $Cr^{3+}$ smectite inhibited enzyme activity to some extent, and the metals remained insoluble.

Interesting is the comparison of the stability of metal complexes of low molecular weight complexing agents with the stability of metal-EPS-complexes. The EDTA complexes have stability constants of up to $10^{18}$, the complex of $Cu^{2+}$ with 1-hydroxyethane-1, 1-diphosphonic acid (HEDP) is about $10^{19}$. If these complexing agents occur in the water phase, it should be expected that eventually sorbed metal ions are rapidly complexed. However, the remobilization in this case is unexpectedly low.

As desorption does usually not occur very fast, biofilms have a "memory effect" for pollutants with which they were in contact. This fact has been utilized for localizing industrial and municipal discharges in sewers, using sorption data from sewer biofilms. Using systematic upflow analysis it is possible to reveal the points at which pollutants

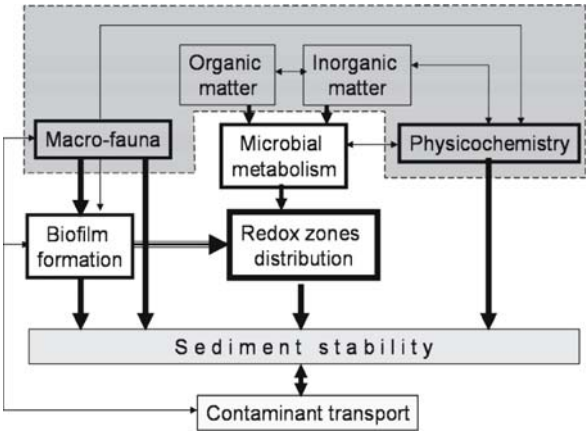


**Fig. 9.8.**
Complete overview of biogeochemical factors to be taken into account when making predictions on sediment transport in natural aquatic environments. Shaded area: covered by conventional studies

were discarded into the sewer system. This method is applied in order to identify point sources for waste water pollution.

### 9.1.7 Conclusions

From the information presented above, the complex influence of biofilms on sediment stability and pollutant mobility is obvious (Fig. 9.8). The problem for all attempts to model biofilm influence arises from the vast spatial and temporal heterogeneity of biofilms. In all non-sterile systems, biofilms are present. However, their influence on sediment stability and sorption/desorption of pollutants depends upon many factors such as:

- Nutrient availability (for heterotrophic organisms: biodegradable substances, for phototrophic organisms: access of light and limiting factors such as phosphate), and, as a consequence, biomass concentration
- Composition of the population (predators can change composition dramatically)
- Amount and nature of EPS
- Hydrodynamic conditions (higher shear stress will select for more cohesive EPS and specialized organisms)
- Sediment compartment considered (hydrodynamic influence can change with sediment depth)

It is clear that this is not an exhaustive list of biological factors involved but it is also clear that they cannot be neglected. Unless they are understood and acknowledged for, predictive modeling will be always crude and not really effective. In this respect, Sects. 9.2 and 9.3, explore both the influences of a biogenic component on the stability and settling characteristics of sediments as well as in terms of retention-remobilization processes. Section 9.4, in contrast, deals with a specific case of biofilm influence on the distribution of bound and dissolved metal species in environments directly linked with fluvial systems.

## References


Allison D (2003) The biofilm matrix. Biofouling 19(2):139–150

Battin TJ, Sengschmitt D (1999) Linking sediment biofilms, hydrodynamics, and river bed clogging: evidence from a large river. Microb Ecol 37:185–196

Black KS, Tolhurst DJ, Paterson DM, Hagerthey SE (2002) Working with natural cohesive sediments. J Hydraul Eng, pp 2–8

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial Biofilms. Annual Review of Microbiology 49:711–745

Costerton JW, Lewandowski Z, Debeer D, Caldwell D, Korber D, James G (1994) Biofilms, the Customized Microniche. Journal of Bacteriology 176(8):2137–2142

Chamberlain AHL (1997) Matrix polymers: the key to biofilm processes. In: Wimpenny J, Handley PS, Gilbert P, Lappin-Scott H, Jones M (eds) Biofilms: Community Interactions and Control., UK, BioLine, pp 41–46

de Brouwer JFC, Wolfstein K, Ruddy GK, Jones TER, Stal LJ (2005) Biogenic Stabilization of Intertidal Sediments: The Importance of Extracellular Polymeric Substances Produced by Benthic Diatoms. DOI 49:501–521

Dade WB, Davis JD, Nichols PD, Nowell ARM, Thistle D, Trexler MB, White DC (1990) Effects of bacterial exopolymer adhesion on the entrainment of sand. Geomicrobiol 8:1–16

Decho AW (1994) Molecular-scale events influencing the macroscale cohesiveness of exopolymers. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments, BIS-Verlag, Oldenburg, pp 135–148

Dignac MFU, Rybacki D, Bruchet A, Snidaro D, Scribe P (1998) Chemical description of extracellular polymers: implication on activated sludge floc structure. Wat Sci Tech 38:45–53

Flemming HC, Leis A (2002) Sorption properties of biofilms. In: Bitton (ed) Encyclopedia of environmental microbiology, Vol. 5. John Wiley & Sons, Inc., New York, pp 2958–2967

Flemming HC, Wingender J (2002) Extracellular polymeric substances (EPS): Structural, ecological and technical aspects. In: Bitton G (ed) Encyclopedia of Environmental Microbiology, vol. 4. John Wiley & Sons, Inc, New York, pp 1223–1231

Flemming HC, Wingender J (2003) The crucial role of extracellullar polymeric substances in biofilms. In: Wuertz S, Bishop P, Wilderer P (eds) Biofilms in wastewater treatment. An interdisciplinary approach., IWA Publishing, London, pp 401

Flemming H-C, Wingender J, Mayer C, Körstgens V, Borchard W (2000a) Cohesiveness in biofilm matrix polymers. In: Lappin-Scott H, Gilbert P, Wilson M, Allison D (eds) Community structure and co-operation in biofilms, SGM symposium 59, Cambridge University Press, pp 87–105

Flemming H-C, Wingender J, Griebe T, Mayer C (2000b) Physico-chemical properties of biofilms. In: Evans LV (ed) Biofilms: recent advances in their study and control, Harwood academic publishers

Frølund BG, Nielsen PH (1995) Enzymatic activity in the activated-sludge floc matrix. Appl Microbiol Biotechnol 43:755–761

Gehrke T, Telegdi J, Thierry D, Sand W (1998) Importance of extracellular polymeric substances from Thiobacillus ferrooxidans for bioleaching. Appl Environ Microbiol 64(7):2743–2747

Hoffman M, Decho AW (1999) Extracellular enzymes within microbial biofilms and the role of the extracellular polymeric matrix. In: Wingender J, Neu TR, Flemming HC (eds) Microbial extracellular polymeric substances. Springer-Verlag, pp 217–230

Huettel M, Røy H, Precht E, Ehrenhauss S (2003) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia 494:231–236

Körstgens V, Flemming H-C, Wingender J, Borchard W (2001) Uniaxial compression measurement device for the investigation of the mechanical stability of biofilms. J Microbiol Meth 46:9–16

Krumbein WE, Paterson DM, Stal LJ (1994) General discussion. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. BIS Oldenburg, pp 433–435

Langley S, Beveridge TJ (1999) Metal binding by Pseudomonas aeruginosa PAO1 is influenced by growth of the cells as a biofilm. Canadian Journal of Microbiology 45(7):616–622

Leon Morales CF, Leis AP, Strathmann M, Flemming HC (2004) Interactions between laponite and microbial biofilms in porous media: implications for colloid transport and biofilm stability. Water Research 38(16):3614–3626

Macedo AJ, Kuhlicke U, Neu T, Timmis KN, Abraham W-R (2005) Three stages of a biofilm community developing at the liquid-liquid interface between polychlorinated biphenyls and water. Applied and Environmental Microbiology 71(11):7301–7309

Madsen KN, Nilsson P, Sundbäck K (1993) The influence of benthic microalgae on the stability of a subtidal sediment. J Exp Mar Biol Ecol 170:159–177
Mayer C, Moritz R, Kirschner C, Borchard W, Maibaum R, Wingender J, Flemming HC (1999) The role of intermolecular interactions: studies on model systems for bacterial biofilms. Int J Biol Macromol 26(1):3–16
Neu T (1996) Significance of bacterial surface active compounds in interaction of bacteria with surfaces. Microbiological Reviews 60:151–166
Neu TR, Lawrence JD (1997) Development and structure of microbial biofilms in river water studied by confocal laser scanning microscopy. FEMS Microbiol Ecol 24:11–25
Paterson DM (1997) Biological mediation of sediment erodibility. In: Burt N, Pareker R, Watts J (eds) Cohesive sediments. Wiley, New York, pp 215–229
Ramsay BR, de Tremblay M, Chavarie C (1988) A method for the quantification of bacterial protein in the presence of Jarosite. Geomicrobiol J 3:171–177
Riding R, Amrawik SM (2000) Microbial sediments. Springer-Verlag, New York, Heidelberg, 331 pp
Sar P, Kazy SK, Asthana RK, Singh SP (1998) Nickel uptake by Pseudomonas aeruginosa: role of modifying factors. Current Microbiology 37:306–311
Schmitt J, Nivens D, White DC, Flemming H-C (1995) Changes of biofilm properties in response to sorbed substances – an FTIR-ATR study. Water Science and Technology 32(8):149–155
Schultze-Lam S, Fortin D, Davis BS, Beveridge TJ (1996) Mineralization of bacterial surfaces. Chem Geol 132:171–181
Skoog DA, West DM, Holler FJ (1996) Fundamentals of analytical chemistry, Saunders college publishing
Späth R, Flemming HC, Wuertz S (1998) Sorption properties of biofilms. Water Science and Technology 37(4–5):207–210
Spiers AJ, Bohannon J, Gehrig SM, Rainey PB (2003) Biofilm formation at the air-liquid interface by the Pseudomonas fluorescens SBW25 wrinkly spreader requires an acetylated form of cellulose. Mol Microbiol 50(1):15–27
Stal LJ, de Brouwer JFC (2003) Biofilm formation by benthic diatoms and their influence on the stabilization of interdital mudflats. Ber Forsch.-Zentr., Terramare 12:109–111
Strathmann M, Leon Morales CF, Flemming H-C (2006) Influence of biofilms on colloid mobility in the subsurface. In: Frimmel HF., Flemming H-C, Förstner U (eds) Colloid mobility, Springer-Verlag, Heidelberg, in press
Sutherland IW (1994) Structure-function relationships in microbial exopolysaccharides. Biotech Adv 12:393–448
Sutherland IW (2001) The biofilm matrix – an immobilized but dynamic microbial environment. Trends in Microbiology 9:222–227
Tielker D, Hacker S, Loris R, Strathmann M, Wingender J, Wilhelm S, Rosenau F, Jaeger K-E (2005) *Pseudomonas aeruginosa* lectin LecB is located in the outer membrane and is involved in biofilm formation. Microbiology 151:1313–1323
Vandevivere P, Kirchman DL (1993) Attachment stimulates exopolysaccharide synthesis by a bacterium. Appl Environ Microbiol 59(10):3280–3286
Vogt M, Flemming HC, Veeman WS (2000) Diffusion in *Pseudomonas aeruginosa* biofilms: a pulsed field gradient NMR study. J Biotechnol 77(1):137–146
Wingender J, Jaeger K-E (2002) Extracellular enzymes in biofilms. In: Bitton G (ed) Encyclopedia of Environmental Microbiology, vol. 3. John Wiley & Sons, Inc, New York, pp 1207–1223
Wingender J, Jäger K-E, Flemming H-C (1999) Interactions between extracellular enzymes and polysaccharides. In: Wingender J, Neu T, Flemming H-C (eds) Microbial extracellular polymer substances, Springer-Verlag, Heidelberg, Berlin, pp 231–251
Wingender J, Neu TR, Flemming H-C (1999) What are bacterial extracellular polymeric substances? In: Wingender J, Neu TR, Flemming H-C (eds) Microbial Extracellular Polymeric Substances. Springer-Verlag, Berlin, pp 1–19
Yallop ML, de Winder B, Paterson DM, Stal LJ (1994) Comparative structure, primary production and biogenic stabilization of cohesive and non-cohesive marine sediments inhabited by microphyto-benthos. Estuar Coast Shelf Sci 39:565–582
Yallop ML, Paterson DM, Wellsbury P (2000) Interrelationships between rates of microbial production, exopolymer production, microbial biomass, and sediment stability of intertidal sediments. Microb Ecol 39(2):116–127

*Carlos Felipe Leon Morales · Martin Strathmann · Hans-Curt Flemming*

## 9.2 Role of Biofilms on Sediment Transport – Investigations with Artificial Sediment Columns

### 9.2.1 Introduction

Sediments are a sink for contaminants which can be accumulated for long periods of time. The so-called sediment transport cycle in rivers starts with erosion caused by the action of the flowing water body. Normally, this is a constant but slow process, drastic events however, can result in the sudden remobilization and distribution of accumulated contaminants. Subsequent deposition of aggregates in the river bed closes the cycle. The subject can be approached from a wide variety of disciplines (Förstner 2004) and, traditionally physical and engineering sciences have helped understanding many key physical parameters on the study of sediment transport such as settling and erosion rates, boundary layer shear stresses, etc. In natural sediments, biological influence must be considered too, which applies for almost every aspect of the sediment transport cycle. Sediment ecology is complex with many trophic levels involved. Biofilms are a common way of microbial aggregated life in sediments. Biofilm microorganisms are embedded in a matrix of extracellular polymeric substances (EPS) (Flemming and Wingender 2002). Hydrodynamics determine greatly mass transfer processes in river sediment biofilms as well as their structure. Biofilms are not confined to the sediment-liquid interface but are also distributed along considerable sediment depths (Battin and Sengschmitt 1999). Benthic biofilms, composed mainly of microalgae (but also bacteria) have been recognized as important factors on sediment stability (Sutherland et al. 1998). In terms of retention-remobilization processes, biofilms have considerable and complex sorption properties and capacities. Biofilms contribute to the net capacity of the sediment to retain, transform, and liberate migrating substances. Anionic groups in EPS such as carboxyl, phosphoryl, and sulfate groups can represent important cationic exchange sites. It is not entirelly clear however, how changes on fluid dominant ionic species which commonly occur in subsurface and other environments, contribute to the influence exerted by biofilms. This is especially true in highly permeable sediments were advection and dispersion can represent an important mixing and transport mechanism. The aim of this work is to investigate the influence of biofilms on sediment stability and retention-remobilization processes in model sediment columns, at two commonly found fluid ionic conditions. Biofilm influence is expressed in terms of the relative contribution of two major EPS components: extracellular carbohydrates and extracellular proteins. The collected data is expected to contribute to the understanding of complex interaction processes which determine biofilm influence in sediment transport.

### 9.2.2 Materials and Methods

#### *Organisms and Influent Solutions*

*Pseudomonas aeruginosa* SG81 and its alginate deficient mutant, *P. aeruginosa* SG81R1 were used as model biofilm forming microorganisms. *P. aeruginosa* SG81 is a mucoid,

highly EPS producing bacterium (Grobe et al. 1995). The organism was kept on Pseudomonas Isolation Agar, PIA (Difco$^{TM}$). Liquid cultures were maintained in Triptic Soy Broth, TSB (Merck). For biofilm growth inside sediment columns (1, 2 or 3 weeks), a defined salts medium, APM50 was used. This medium consisted of 50 mM sodium gluconate, 1 mM $KNO_3$, 0.1 mM $MgSO_4 \cdot 7\,H_2O$, 0.05% yeast extract and 0.2 M NaCl.

Rhodamine 6G was used as a model organic pollutant. It is used in a wide variety of applications going from ground water tracer experiments to fingerprint detection technology in forensics. The compound was detected by fluorescence ($\lambda_{ex}$ 480 nm $\lambda_{em}$ 541 nm) (SFM 25, Kontron Instruments fluorometer). $CaCl_2$ or NaCl based solutions were used at 70 mM concentration as influent solutions either when APM50 media or the model contaminant were not the influents. For bacterial transport experiments various NaCl concentrations were used, ranging from 0.6 mM up to 1 M. Desionized water was the lowest ionic strength influent solution used.

### *Sediment Columns and Main Experimental Setting*

To determine biofilm influence on sediment transport processes, experiments were performed in columns representing both the sediment-liquid interface ("flume columns") and the sediment porous matrix ("PM columns") (Fig. 9.9), in absence (sterile) and presence of biofilms (non-sterile). Organic-free (550 °C, 2 h) sand $F_{36}$ with an average diameter of 0.1 mm was used as model sediment. Flume columns were horizontally positioned, glass columns, half-filled with the model sediment. PM columns in contrast, were completely filled and consisted on vertically positioned glass columns as well as microscopy flow cells which were used for direct visualization and quantification of transport processes inside the sediment porous matrix. The columns were con-

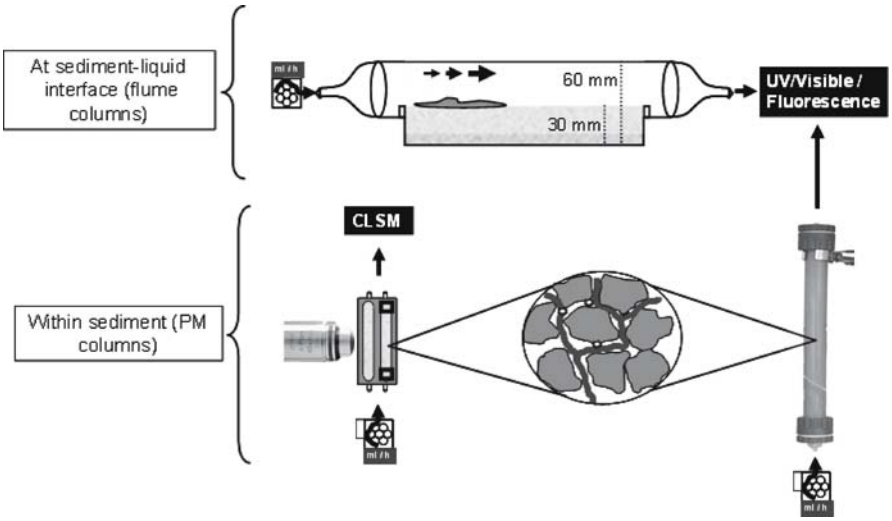


**Fig 9.9.** Main experimental setting. *Upper section:* "Flume columns" representing the sediment-liquid interface. *Lower section:* "PM columns" both sediment-filled microscopy flow cells (*left*) and completely filled sediment columns (*right*) representing the sediment porous matrix

nected using PVC or tygon tubing (Novodirect, Kehl, Germany) to peristaltic pumps (Ismatec SA, Switzerland). Columns and accompanying tubing were sterilized by autoclaving (121 °C, 20 min) or by dry heat (250 °C, 4 h). Columns for biofilm experiments were inoculated with *P. aeruginosa* suspensions (approx. $10^9$ cells $ml^{-1}$) and then fed with APM50 as specified before. After the completion of the experiments (except for the microscopy flow cells), the remaining sand was removed from the column for further analysis. All described experiments were done at least in triplicate and mean values are presented. Error bars represent the standard deviation around these mean values. Pearson correlation coefficients, *r*, were calculated with the open source spreadsheet Gnumeric and considered only significant for $p < 0.05$ (*t*-test distributions).

### *Stability and Retention-Remobilization Experiments*

Stability experiments were done in "flume columns" only. Shear stress was applied to the sediment bed by water flow (flume-like stress) (704S, Watson Marlow peristaltic pump). The starting point of sediment particle movement was assessed at increasing flow velocities using a video camera. The velocities ranged from 1 cm $s^{-1}$ up to 21 cm $s^{-1}$ set at equivalent intervals. *P. aeruginosa* SG81 was used for these experiments. APM50 as influent medium was replaced the day before by either $CaCl_2$ or NaCl solutions as background solutions. The ionic strength of these solutions was kept constant all the time.

For retention-remobilization experiments the flow rate was maintained constant and mobility was stimulated by decreasing the ionic strength of the background solutions. Flume columns were exposed to the same amount of contaminant influent at a concentration of $6 \times 10^{-6}$ M. This was followed by pollutant-free influent until effluent pollutant concentration was stable and low. *P. aeruginosa* SG81 as well as its mutant were used in these experiments. For experiments with "PM columns", colloid-associated pollutant and bacterial mobility through the sediment were assessed as collision efficiencies. Collision efficiency, $\alpha$, is defined as the probability of a migrating particle to attach, upon collision with sediment particles. It was obtained as the ratio between deposition rate constants at increasing salt concentrations (up to 70 mM NaCl for colloid-associated pollutant and up to 1 M NaCl for bacteria) and those at high salt concentrations (= 1M NaCl) where deposition is independent of the salt concentration. Due to the reason that deposition is always higher at high salt concentrations, $\alpha$ values closer to 1 indicate high deposition while those closer to 0 indicate low deposition, for further details see (Leon Morales et al. 2004).

### *Analyses*

Both the liquid phase and the sediment matrix were used to obtain information about the model contaminant and bacterial concentrations. Cell enumeration was done by total cell counts (TCC) using thoma cell chambers. EPS material was quantified as total extracellular carbohydrates and total extracellular proteins as described in (Wingender et al. 2001). Biofilms were separated from the sand matrices by mechanical shear stress (stomacher® 400 circulator) in the presence of a cation exchange resin, CER (DOWEX $50 \times 8$, Fluka) in a modified version of the procedure described by (Frølund et al. 1996).

A UV-VIS spectrophotometer (Cary 50, Varian Inc) was used to monitor bacterial and contaminant concentrations in columns effluents. Confocal laser scanning microscopy, CLSM (LSM510, Zeiss) was used on the microscopy flow cells to observe at the pore scale, the fluorescence of labeled bacteria and colloidal tracers as described in (Leon Morales et al. 2004).

### 9.2.3 Results

#### *Sediment-Liquid Interface – "Flume Columns"*

**Sediment stability.** The influence of biofilm growth and the ionic nature of the background solution on sediment stability were investigated. Higher shear stress (created by step-wise increasing influent flow rates) was necessary for the initiation of sediment particle movement in the presence of 5 day-old biofilms as compared with sterile, organic-free columns (Fig. 9.10). After 15 days of biofilm development, a fluffy layer developed on top of the sediment which was sheared off at low flow rates (1–5 cm $s^{-1}$). The underlying sediment, however, displayed an elevated stability. This stabilizing effect could be correlated with increasing protein and carbohydrate concentrations.

An increase on the critical shear stress was observed when the background solution was $CaCl_2 + NaHCO_3$ instead of $NaCl + NaHCO_3$ even though all other parameters including time of biofilm growth were kept constant. There was a higher correlation of carbohydrates to the critical shear stress ($r = 0.89$) as compared with protein ($r = 0.60$). The correlation of carbohydrates to the critical shear stress is further increased and found to be significant ($p = 0.02$), when the background solution was $CaCl_2 + NaHCO_3$.

The ratio carbohydrate/protein, found in the extruded sediment, increased with time of biofilm growth: from 1.58 after 5 days of growth to 2.63 after 15 days of growth and after $Na^+$ based background solutions and to 2.27 for $Ca^{2+}$ based background solutions.

**Retention-remobilization.** Model pollutant retention-remobilization was investigated in the presence of $Ca^{2+}$ based background solutions, for columns inoculated with *P. aeruginosa* SG81 and its alginate deficient mutant. After model contaminant column saturation (Fig. 9.11) and the subsequent stimulated remobilization, fractions were

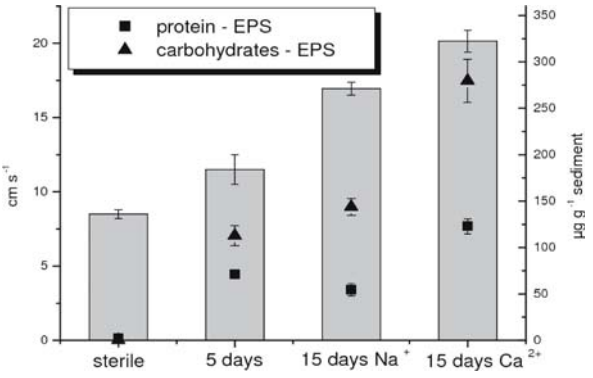


**Fig 9.10.** Biofilms induced sediment stability. The *bars* indicate the linear velocity (*left y-axis*) at which sediment particle movement started. *Squares* and *triangles* represent micrograms of extracellular proteins and extracellular carbohydrates respectively per gram of extruded wet sediment

collected from sediment columns effluents at fixed time intervals and flow rates. Relative fluorescence of each fraction was plotted against time of elution. Integration of these breakthrough curves was used to quantify the amount of remobilized pollutant.

Sterile columns resulted in the higher remobilization rates in triplicate columns as compared to the columns inoculated with the mutant and the alginate producing bacterium (Fig. 9.12).

Figure 9.12 shows that pollutant remobilization in the presence of both *P. aeruginosa* SG81 and SG81R1 biofilms is significantly decreased. Taking the integrated remobilization of the sterile columns as a starting point, model pollutant remobilization from the alginate-deficient mutant inoculated columns was 52% while it was 23% in presence of a biofilm with the alginate-producing wild strain. The correlation of EPS to pollutant retention is high for both proteins and carbohydrates ($r = 0.90$ and $r = 0.97$ respectively), however, the ratio carbohydrate/protein is higher (2.83) in the mucoid columns as compared to the non-mucoid ones (0.70).

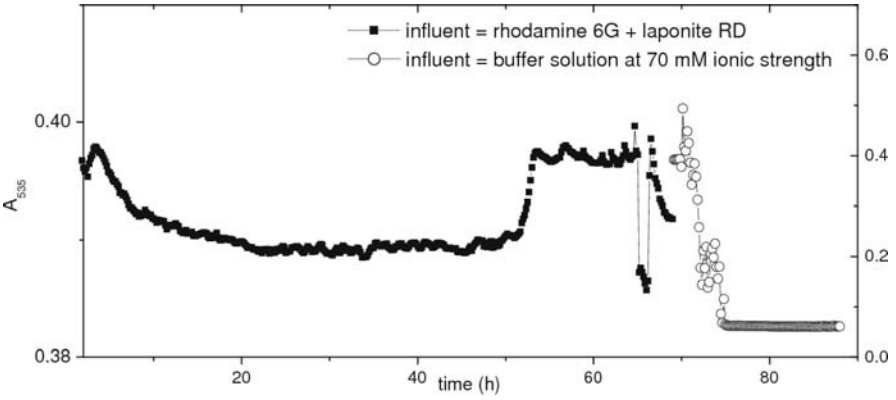


**Fig 9.11.** Process of column exposure to the model contaminant used. This is the concentration of contaminant as detected in the column effluent. The concentration in the influent was constant until around 70 hours time after which it was changed by pollutant-free solution at high ionic strength

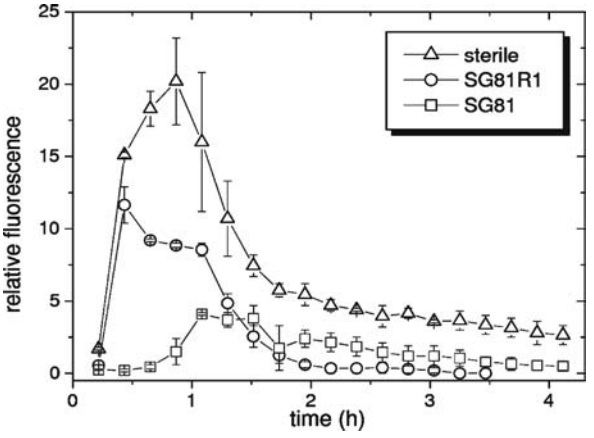


**Fig 9.12.**
Remobilization patterns of rhodamine 6G from sterile sediment columns and columns grown with *P. aeruginosa* SG81 and *P. aeruginosa* SG81R1 biofilms

### *Processes within the Sediment – "PM Columns"*

**Bacterial transport and biofilm formation in porous media.** Biofilms developed also between sediment particles even at considerable sediment depths. These experiments therefore investigate the role of biofilms in pollutant transport and hydrodynamics at this level. For these experiments, only *P. aeruginosa* SG81 was used. Data from the completely saturated columns and from the sand-packed microscopy flowcells showed that the saturated hydraulic conductivity decreased over time when columns were inoculated and fed with nutrients. After an instability period on the measured hydraulic conductivity, it decreased steadily until a more or less constant lower plateau was reached for the rest of the observation period. This plateau was observed after approximately 7 days of constant nutrient influent. In non-inoculated columns a reduction in saturated hydraulic conductivity was observed after the first hours of column packing. The measured hydraulic conductivity remained more or less stable at a higher value than obtained with the biofilm growing columns (Fig. 9.13).

The attachment or collision efficiencies of biofilm bacteria give information on the first steps of biofilm formation in a porous medium. Relatively high collision efficiencies were found for the model microorganism at a wide range of salt concentrations as compared with clay colloids (Fig. 9.14).

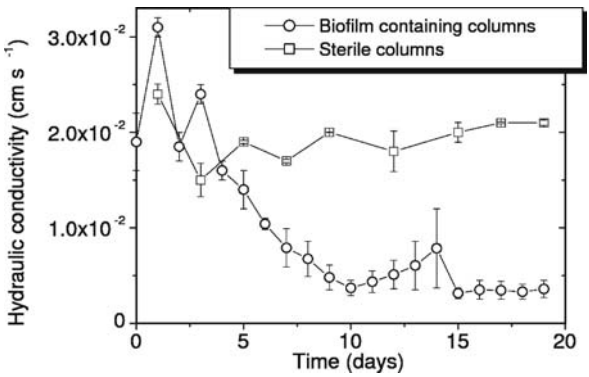


**Fig 9.13.**
Changes in porous matrix hydraulic conductivity induced by biofilm growth. Hydraulic conductivity was measured using a constant head permeameter

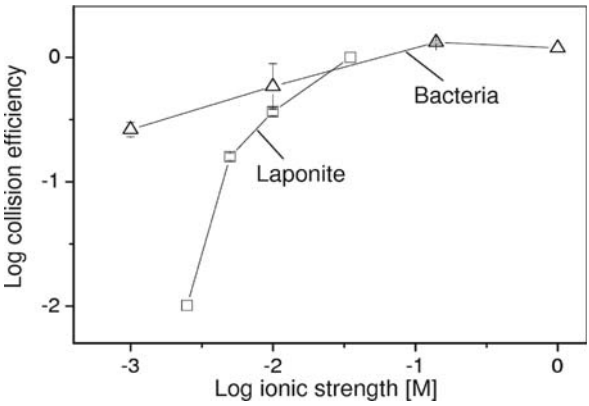


**Fig 9.14.**
Collision efficiencies obtained from image analysis of bacterial and colloid transport experiments done with sediment packed microscopy flowcells and CLSM

Bacterial attachment occurred even at low ionic strengths (1 mM in Fig. 9.14 and 9.15), however, drastically decreasing ionic strength in the presence of $Na^+$ ions, results in remobilization of retained microorganisms (Fig. 9.16b). In the presence of clay-like colloids, in contrast, sudden increments on ionic strength resulted in clogging of the porous matrix and remobilization of attached bacteria (Fig. 9.16c). Additionally, biofilm growth was confirmed in the surface of sand grains by CLSM (Fig. 9.16a).

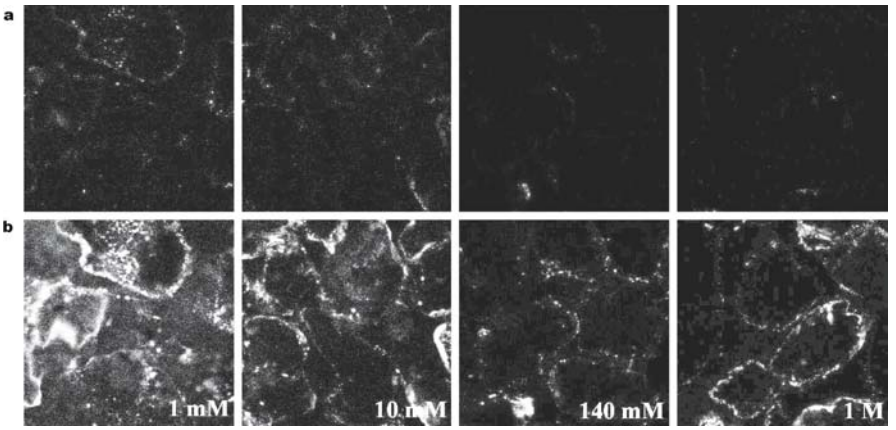


**Fig 9.15.** Pore scale visualization of bacterial attachment to sediment grains. Bacteria were labeled with SYTO 9 and injected as a pulse. The pictures represent before (**a**) and after (**b**) bacterial elution at the same distance from the flow-cell inlet and at the same range of ionic strengths as depicted in Fig. 9.14

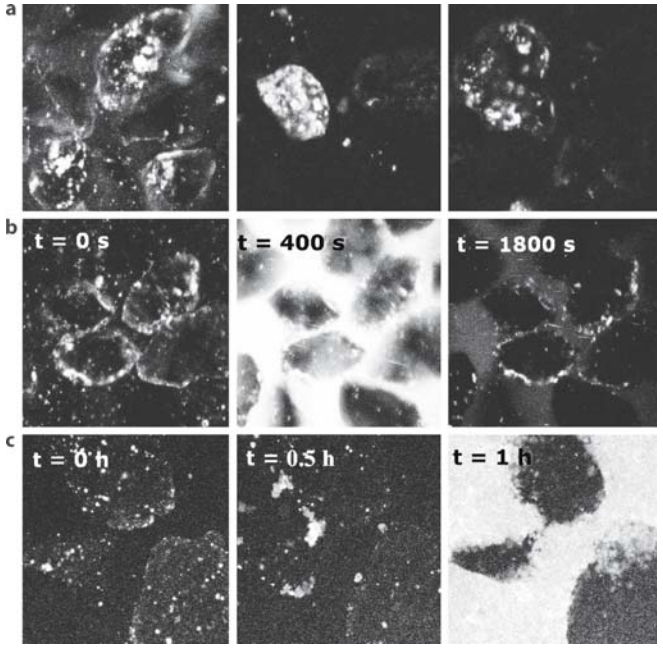


**Fig 9.16.**
Visualization of porous medium hydraulic conditions. **a** Biofilm formation on top of sand grains evidenced by detection of fluorescently labeled (SYTO 9) bacterial cells using CLSM. Uncovered sand grains remain dark. **b** Remobilization of bacterial cells (lighter zones) after drastically decreasing ionic strength in the influent solution. **c** Clogging of the porous matrix with colloidal aggregates at high ionic strength conditions. The aggregates are stained with rhodamine 6G which is also detected by fluorescence (lighter zones)

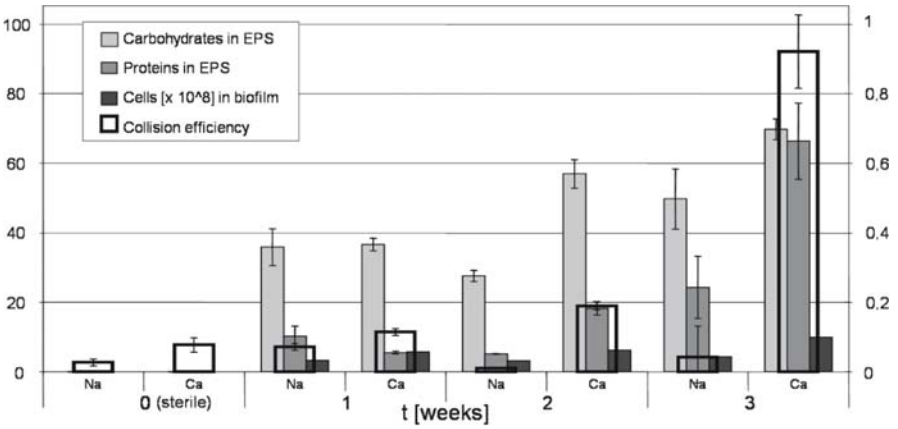


**Fig 9.17.** Collision efficiencies (*right y-axis*) of colloid-associated rhodamine 6G and corresponding EPS and cell counts (*left y-axis*) remaining in saturated sediment columns (porous matrix) after colloid mobility experiments. *x-axis* represents weeks of biofilm growth

### *EPS, Electrochemical Conditions and Pollutant Retention*

After characterization of biofilm formation and bacterial attachment and transport through the target sediment, biofilm influence on the mobility of the model pollutant was investigated using background solutions dominated by two different ionic species. The amount of EPS especially proteins and cell numbers increased with time in inoculated columns (Fig. 9.17). The impact of biofilm growth on colloid and colloid-bound model pollutant transport depended, however, greatly on the type of cation dominating the background solution previous to pollutant injection. This is especially evident after 3 weeks of biofilm growth (Fig. 9.17).

Pollutant retention increased with time and EPS content in columns with $Ca^{2+}$ dominated background solutions. There was some correlation of carbohydrates to pollutant retention ($r = 0.47$) in these systems, however, the correlation of proteins to retention was significantly higher ($p = 0.02$). In the case of columns with $Na^{+}$ dominated background solutions, pollutant retention did not increase with time. The combined EPS production in the porous matrix environment was lower (as much as 1 order of magnitude) as compared to the liquid sediment interface. In contrast to what happened at the sediment-liquid interface, inside the porous medium, carbohydrate/protein ratio decreased with time of biofilm growth (from around 6 in the first week to around 1 in the third week) when $Ca^{2+}$ dominated the background solution. It remained constant, however, when $Na^{+}$ was the dominant ion in the background solution.

## 9.2.4 Discussion

### *Sediment Stability*

At the sediment water interface, biofilm growth had a clear influence on sediment stability, i.e. on the stress necessary to start sediment particle movement. Plateau phase

biofilm growth at this interface (15 day biofilms) had a stabilizing effect only after a layer of low density EPS was eliminated at low flow rates. Visual observations showed that this "fluffy" layer carried with it associated sediment material but this was not quantified. It was found that sediment stability is positively correlated with an increased production of EPS. Especially in the case of $Ca^{2+}$ dominated background solutions, extracellular carbohydrates were highly correlated to critical shear stress (Fig. 9.10). The carbohydrate/protein ratio increased with time at the sediment-water interface. This suggests a calcium stabilizing and cementing effect, within carbohydrates and between carbohydrates and sediment particles. The influence of calcium ions in the viscoelastic properties of *P. aeruginosa* SG81 biofilms has been demonstrated already in other studies using rheological methods (Körstgens et al. 2001). In the mentioned studies, calcium was available in the growth medium during all the time of biofilm development. In the present study, in contrast, calcium was available only after biofilm growth. This shows that calcium can be incorporated and can exert an influence in relatively short periods of time. In natural sediments, de Brouwer et al. (2002) found a strong interaction between extractable carbohydrate and sediment particles. Part of this EPS was irreversibly bound to sediments (not re-extracted by 0.1M EDTA). Furthermore, the amount of irreversibly bound EPS increased 50% in the presence of $Ca^{2+}$ ions.

EPS production in PM columns was generally lower than in flume sediment columns suggesting isolated and patchy biofilms and demonstrating the importance of the habitat on biofilm development. In these systems, carbohydrate/protein ratio decreased with time and at constant ionic strength conditions, biofilms affected the hydraulic conductivity of the porous medium. The effect on sediment cohesiveness was not assessed but the retention capacity particularly of clay-like minerals suggests a positive effect on sediment stability. EPS production within the sediment and mobile EPS fractions permeating sediment voids can increase the cohesiveness of sediment grains which can be bonded in bigger aggregates. Under these conditions, rates of sediment mobilization will depend not only on the size of the sediment grains but also on EPS content. At changing electrochemical conditions (e.g., decreasing ionic strength), especially in the presence of monovalent cations, biofilm stability as well as rates of microbial attachment can be drastically affected. This will have obvious consequences on sediment stability. Furthermore, in natural environments, biofilm distribution in the porous medium is not homogeneous and can change depending on depth, ecology and on the biogeochemistry of the site (Yallop et al. 2000). The type of biofilm and the relatively fast changes that can occur in biofilm stability and EPS production rates at changing electrochemical conditions, are determinant for sediment transport in natural environments. This dynamic behavior and the resulting distributions in erosion rates cannot be easily included in sediment transport prediction models.

### *Retention Remobilization Processes*

At the sediment-liquid interface, the absence of alginate (*P. aeruginosa* SG81 mutant) resulted in higher pollutant remobilization as compared with the alginate producing wild strain. In both cases, however, there was less remobilization than in the sterile columns. Although, it is very likely that the excess of carbohydrate was responsible for lower remobilization rates in the wild type inoculated columns, it is not possible to rule

out the role of proteins in retention. This is evidenced in the fact that pollutant retention was also highly correlated with protein content. Previous studies (data not shown) have demonstrated that after predominance of $Ca^{2+}$ ions, remobilization of retained particles and biofilm components occurs very slowly. Only after several pore volumes of deionized water, remobilization events start to take place. It is plausible then to assume, that after prolonged periods of a very low ionic strength influent, accumulated pollutant, not remobilized in the time frame of our experiments (Fig. 9.12), could start to be remobilized. This can also be truth in the case of monovalent ion exchange which is realistic in nature e.g., sea water infiltration near coasts.

In PM columns, the correlation of proteins to retention was significantly higher than carbohydrates and $Ca^{2+}$ played an important role both in biofilm stability and pollutant retention. Furthermore, the ratio carbohydrate/protein decreased with time which suggests an important role of proteins in pollutant retention within the sediment matrix. In the case of $Na^{+}$ background solutions, the lack of retention could be attributed to biofilm instability, detachment and co-elution effects.

In summary, carbohydrates are probably more important than proteins in sediment stability at the sediment-liquid interface. In terms of retention-remobilization processes at this level there was no evidence of a predominant carbohydrate role. Within the sediment, in contrast, proteins played a predominant role when conditions were appropriate for pollutant retention (i.e. dominance of $Ca^{2+}$ in the background solutions).

### 9.2.5 Conclusions

The experimental data from sterile/non-sterile sediment systems show that:

- *P. aeruginosa* biofilms increase the stability of model sediments. The extent of this effect increases with time and with the presence of calcium ions.
- The remobilization of model contaminants is inhibited in the presence of biofilms. This inhibition is higher in the alginate producing mucoid *P. aeruginosa* SG81 strain as compared to its alginate-deficient mutant.

In the porous matrix, transport can be highly influenced by biofilm formation and fluid ionic composition, both in terms of changes in hydraulic conditions (permeability, hydraulic conductivity, dispersivity, etc.) and retention-remobilization of migrating pollutants.

Biofilm influence on sediment transport processes is not limited to the major sediment-water phase interface. Processes occurring in the depth of the sediment must also be taken into account.

### Acknowledgments

This work is part of the cooperative research project SEDYMO – "Sediment Dynamics and pollutant Mobility in river basins" financed by the German Federal Ministry of Education and Research (BMBF). We thank all our project partners especially Professors U. Förstner and B. Westrich and their groups, for constructive input.

## References

Battin TJ, Sengschmitt D (1999) Linking sediment biofilms, hydrodynamics, and river bed clogging: evidence from a large river. Microb Ecol 37:185–196

de Brouwer JFC, Ruddy GK, Jones TER, Stal LJ (2002) Sorption of EPS to sediment particles and the effect on the rheology of sediment slurries. Biogeochemistry 61(1):57

Flemming H-C, Wingender J (2002) Extracellular polymeric substances (EPS): Structural, ecological and technical aspects. In: Bitton G (ed) Encyclopedia of Environmental Microbiology, vol. 4. John Wiley & Sons, Inc., New York, pp 1223–1231

Förstner U (2004) Sediment dynamics and pollutant mobility in rivers: an interdisciplinary approach. Lakes and Reservoirs: Research and Management 9:25–40

Frølund B, Palmgren R, Keiding K, Nielsen PH (1996) Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res 30(8):1749–1758

Grobe S, Wingender J, Trüper HG (1995) Characterization of mucoid *Pseudomonas aeruginosa* strains isolated from technical water systems. J Appl Bacteriol 79:94–102

Körstgens V, Flemming H-C, Wingender J, Borchard W (2001) Influence of calcium ions on the mechanical properties of a model biofilm of mucoid *Pseudomonas aeruginosa*. Wat Sci Tech 43(6):49–57

Leon Morales CF, Leis AP, Strathmann M, Flemming H-C (2004) Interactions between laponite and microbial biofilms in porous media: implications for colloid transport and biofilm stability. Water Res 38(16):3614–26

Sutherland T, Grant J, Amos C (1998) The effect of carbohydrate production by the diatom *Nitzschia curvilineata* on the erodibility of sediment. Limnology Oceanography 43:65–72

Wingender J, Strathmann M, Rode A, Leis A, Flemming H-C (2001) Isolation and Biochemical Characterization of Extracellular Polymeric Substances from *Pseudomonas aeruginosa*. Methods Enzymol 336(25):302–314

Yallop ML, Paterson DM, Wellsbury P (2000) Interrelationships between rates of microbial production, exopolymer production, microbial biomass, and sediment stability in biofilms of intertidal sediments. Microb Ecol 39(2):116–127

*Manuelle Neto · Jean-Philippe Bedell · Rémy Gourdon · Guy Collilieux Antonio Bispo*

## 9.3 Role of Bacteria in Heavy Metal Transport during the Dredging in the Rhône River

### 9.3.1 Introduction

Dredging activities have a wide range of applications, permitting to: limit flooding, restore water flow, restore navigation and improve the quality of water when contaminated sediments are extracted. The number of dredging is increasing due to human activities. Dredging activities have potential economic and environmental impacts, such as ecological or health risk since the pollution detected in these sediments comes from various origins.

Between 1990 and 2000, the quantity of sediments dredged in France amounted to 2.8 million $m^3\,yr^{-1}$ (Hardy 2002). Dredging activities on the Rhône River represent 46% of the total volume of sediments dredged in France.

Over the 1997–2002 period, the average volume of sediments dredged from the Rhône River was around 885 000 $m^3\,yr^{-1}$. The volume of silt dredged in that period was estimated to 3.9 million $m^3$, i.e., an average of 665 000 $m^3\,yr^{-1}$ (ca. 70 to 75% of the total volume dredged annually in the Rhône River). Nevertheless, this volume represents

only about 6% of the total suspended matter transported by the Rhône during a year between Génissiat (department 01, France) and Vallabrègues (department 30, France), which are, respectively, the upstream and downstream reference points.

Dredging on the Rhône River is usually done with a suction dredge followed by a discharge into the water flow. A mixture of water (70 to 90%) and sediment (10 to 30%) is pumped from the sediment and re-suspended in the water flow. The concentration of suspended matter downstream a suction dredge typically ranges between 100 and 300 $g\,l^{-1}$ depending on the material dredged and the type of machine used.

In situ measurements were realized during a re-suspension dredging operation (on the Vaugris site, Isère, 38, France). It was observed that: *(i)* the plume generated was detectable over 1 000 m downstream, and *(ii)* a high bacterial population was present over 500 m downstream.

Bacteria, bio-colloids, are important heavy metal carriers (see the review of Sen and Khilar 2006) whose study appears important in works related to heavy metals transport.

The questions tackled in this document are:

- Does the re-suspended matter transport pollutants or are pollutants rather dissolved?
- What is the role of bacteria in the transport of polluants?

The present study focuses on the processes of re-suspension and settling of dredged sediments. Laboratory experiments were conducted with the sediment dredged in the St. Vallier site. This sediment was firstly suspended in a known volume of water, and then allowed to settle. The concentration of suspended particles in the water column was time-monitored during the settling process. Samples were extracted at different times and to separated in distinct grain size compartments, in order to measure bacterial cell density and heavy metals concentration in each of them. Results obtained with sterilized and non-sterilized sediments were compared to assess the role of bacterial populations.

## 9.3.2 Materials and Methods

### *Protocol Development and Experimental Protocol*

The experimental approach followed the static protocol described below.

The sediment/water ratio used (in %) is 30/70. This ratio is the one to be used in situ, as indicated by the National Rhône Company. A dry mass of 240 g of sediment was mixed with distilled water (filtered at 0.22 µm on nitrate and acetate filters) in a final volume of 1 l using a rotary shaker, during 24 h. Preliminary experiments were done in triplicates to evaluate the reproducibility of settling and the method of sampling. Sampling from water column during settling was done every 2 min in these experiments and suspended matter concentrations analyzed. These results showed that the suspended matter concentration was the same throughout the whole water column (excepted the water surface). We decided to sample at half the height of the water column.

On the basis of these preliminary experiments, the following sampling times were considered for the different settling phases in the subsequent experiments:

- Initial mix ($t = 0$).
- Rapid settling ($t = 4$ min).
- Progressive settling monitored at times 10, 15 and 30 minutes.
- Approach of stationary phase at times 60 and 120 minutes.

The suspensions were analyzed for suspended matter, bacterial concentration, and heavy metals concentrations.

The mass of sediment and volume of water used in the experiments were defined so that the suspended matter concentration at $t = 0$ was similar to that obtained at the re-suspension point in the in situ experiment (ca. 74 300 mg $l^{-1}$). The rapid settling phase (obtained at $t = 4$ min) was considered to reproduce in situ suspended matter concentrations obtained 3 meters downstream from the re-suspension point (ca. 2 640 mg $l^{-1}$).

### *Characteristics of Selected Sediment*

The sediment used was dredged from the St. Vallier station (department 26, France) on the Rhône River.

Some experiments were run with sterilized sediment to assess the role of microbial biomass.

Two different types of sterilizations were tested: one physical sterilization (by gamma radiation) and one chemical sterilization (by formaldehyde). These two sterilizations are commonly used on soil and sediment studies (McNamara et al. 2003). The sterilization by gamma rays is usually considered as the least destructive mode of sterilization (McNamara et al. 2003; Trevors 1996). The sterilization by formaldehyde is used to confirm or infirm some of the results obtained with gamma rays sterilization.

These different sterilization stages were carried out as follows:

- *Gamma rays:* gamma rays sterilization was done by the "Ionisos" company (Dagneux, department 01, France) at 40 KGy, followed by a 3-week rest at 4 °C to destroy residual enzymatic activities.
- *Formaldehyde:* the sterilization by formaldehyde was realized by mixing (with several vigorous shakings) 0.5 ml of formaldehyde solution at 30% per dry g of sediment, followed by a 3-week rest at 4 °C.

### *Analytical Methods*

All experiments were triplicated (unless otherwise stated). The glassware used was washed with $HNO_3$ at 5% overnight, and rinsed several times with distilled water.

*Suspended matter concentrations* were measured according to the French standard NF EN 872, by filtration at 1.2 µm on microfiber filter (Whatman).

*Filtrations* of aqueous suspensions were done with nitrate and acetate filters of 8 µm pore-size as a pre-filtration step to avoid clogging in subsequent filtrations, followed by 1.2 and 0.45 µm pore-size filtrations for particle size fractionation (Millipore filters SCWP, RAWP and HAWP, respectively). Before filtration, an aliquot of suspension was sampled and kept for analysis.

*Heavy metal concentrations*

- In the suspensions, concentrations were determined by mineralizing 3 to 20 ml of suspension (depending on suspended matter concentration) with 2 ml of $HNO_3$ at 65% (Merck Suprapur) and 6 ml of HCl at 30% (Merck ultrapur) following the French standard NF EN ISO 15587-1. The suspensions were filtered before being analyzed by atomic absorption (Hitachi 28200) (detection limit for Cd: 0.1 µg $l^{-1}$ and for Zn: 0.1 mg $l^{-1}$).
- In the filtered solutions, Zn concentrations were measured by ICP/AES (Perking Elmer) following the French standard NF EN ISO 11885 (detection limit: 0.05 mg $l^{-1}$ for Zn).

*Bacterial cell density* was measured using *LIVE/DEAD®* Bac*Light™* protocol: a sample of 100 to 150 µl was incubated with 150 µl of propidium iodide and 200 µl of SYTO 9 during 15 minutes in a total volume of 5 ml completed with water filtered at 0.22 µm. After incubation, the solution was filtered on Millipore isopore filter GTBP (0.22 µm). The filter was placed on a slide between two drops of low fluorescence immersion oil (Zeiss Immersol 518N) and covered with a clear glass cover slip. The observation was then realized on a Zeiss microscope with filter set 09 (Zeiss no.: 488009-0000). Twenty microscopic fields were numbered on each filter. Volumes filtered were performed to 10 at 100 cells per fields. The counting operation was realized on 3 different filters without distinction between viable and not viable cells.

### 9.3.3 Results

#### *Characteristics of Selected Sediment*

The physico-chemical characteristics of the sediment are considered as being representative of the Rhône River's sediments (Table 9.2).

Relatively high concentrations of Zn, Cd, Cu and Fe were observed as shown in Table 9.2.

The bacterial concentration was also found to be relatively high (Table 9.2). Such population exhibited dehydrogenase and denitrification activities. The first one indicates a good respiratory potential and a possible role in the carbon cycle (Engelen et al. 1998). The second one plays a part in the carbon and nitrogen cycles (van Rijn et al. 2006). These results suggest that the pollutants present in the sediment do not significantly inhibit bacterial population, dehydrogenase and denitrifying activities.

**Table 9.2.** Physico-chemical and microbiological characteristics of selected sediment

| Parameters | Protocol used | Values | | | |
|---|---|---|---|---|---|
| pH (water) | NF X31-103 | 7.28 ±0.16 | | | |
| pH (KCl) | NF X 31-104 | 7.16 ±0.11 | | | |
| Water content (%) | Afnor X31-102 | 77.46 ±0.01 | | | |
| TOC (%) | NF ISO 10694 | 1.52 ±0.1 | | | |
| Total hydrocarbons (mg/kg DW)[a] | | 71 | | | |
| Heavy metal concentrations (mg $kg^{-1}$)[a] | ICP/AES after mineralization by micro waves NF EN ISO 11885 | Zn 154 | Cd 1 | Fe 28 382 | Cu 46 |
| Bacterial enumeration (cells/g DW) | Bac light (Soil extraction according to Ranjard et al. 1997) | $7.43 \times 10^9$ | | | |
| Dehydrogenase activity (µg of formed formazan/24 h/g DW) | Brohon et al. 1999 | 7 550 ±245 | | | |
| Denitrifying activity (ppm of $N_2O$ formed/h/g DW) | Poly 2000 | $6.85 \times 10^2$ | | | |

[a] Measures realized by a laboratory certified COFRAC (Comité Français d'Accréditation).

### *Time Evolution of Suspended Matter Concentration*

The evolution of suspended matter concentration in the water column (at half-height of water column) during settling is shown in Fig. 9.18 for the different experiments carried out with non-sterilized (namely "biotic") sediment and with sediments sterilized chemically (using formaldehyde) or by gamma irradiation. In all experiments, initial concentrations of suspended matter were roughly the same (ca. $1.6 \times 10^5$ mg $l^{-1}$).

pH values in the water column for biotic and gamma rays experiments were not differentiated (around 6.9 ±0.02) and relatively steady during all the settling.

#### Settling of Biotic (Untreated) Sediment

Figure 9.18 shows that the settling of biotic sediment is faster than that of sterilized sediments. More than 98.8% of suspended matter was found to settle over the 4 first minutes (suspended matter concentrations were $1.59 \times 10^5$ mg $l^{-1}$ and $1.91 \times 10^3$ mg $l^{-1}$ at times 0 and 4 min, respectively).

#### Settling of Sterilized Sediments

Two types of sterilization were tested, using gamma rays or formaldehyde (physical and chemical treatment). The forms of the settling curves obtained with the 2 sterilized sediments are similar to that of the "biotic" sediment, but the speed of settling over the first 30 minutes was found to be significantly different (Fig. 9.18). Sediment sterilized by gamma rays exhibited the slowest settling, and sediment sterilized with formal-

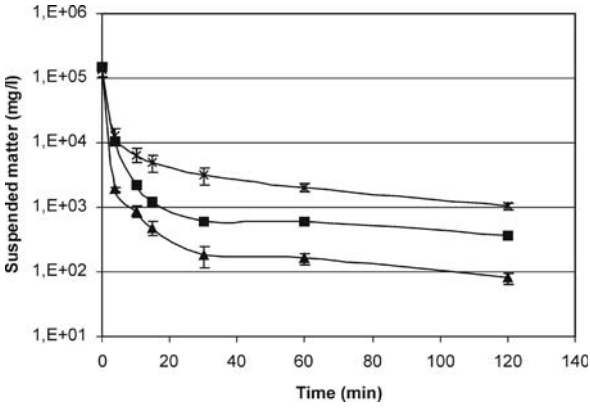


**Fig. 9.18.**
Suspended matter concentration in water column with different treatments. The biotic experiment is represented by a *triangle*, the formaldehyde experiment by a *square*, and the gamma rays experiment by *star*

dehyde showed an intermediate behavior between the "biotic" and the "gamma rays" sediments (Fig. 9.18).

Although the differences observed between the "biotic" and "gamma rays" or "formaldehyde" experiments cannot be exclusively attributed to the presence of microorganisms, results suggest that microorganisms play a role in settling and its velocity. The grain size particles and stability aggregates of biotic sediment and sediment sterilized by gamma radiation showed few differences as underlined before in the review of McNamara (2003) (data not shown). The gamma rays treatment doesn't break aggregates of the sediment. The microbial population via through microbial flocs is known to be involved in the formation of particle aggregates in sediments (Stemmer et al. 1998; van Elsas et al. 1997). The microbial exopolysaccharides bind clay mineral and humic components into microaggreagates (van Elsas et al. 1997). The first hypothesis is that cells death could weaken aggregates. Thus these fragile aggregates could be broken after a long and vigorous shaking as in our experimental protocol. It is assumed that the partial destruction of aggregates results in the formation of smaller particles, which settle at reduced velocity.

Gamma radiation creates free hydrogen and hydroxyl radicals that react as reducing and oxidizing agents and cleave C–C bonds (Trevors 1996). The second hypothesis is that the organic matter of the sediment is transformed, inducing a modification in the settling.

The difference between "biotic" and "gamma rays" sediment may be attributed to these two distinct effects: aggregation and organic matter. This hypothesis is confirmed by the results obtained with the formaldehyde experiment.

### *Bacteria Distribution*

Bacterial counts were done both in the water column sampled at different settling times, and in the different particle size fractions obtained by successive filtrations of the water column at 1.2 μm and 0.45 μm).

Cell counts done in the experiments with sterilized sediments showed that no viable microbial cells were found (data not shown), thereby confirming the efficiency of sterilization treatments.

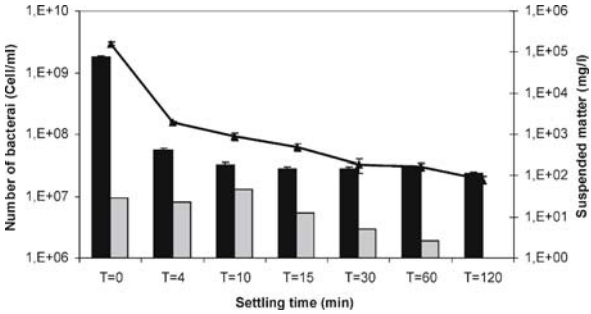


**Fig. 9.19.**
Bacterial population in the water columns (*black bars*) and in the particle size fraction $F < 1.2$ µm (*gray bars*), and the suspended matter concentration in water column (*line*) during settling

Results obtained with "biotic" sediment are shown in Fig. 9.19 where the histograms and the line represent the bacterial cells concentrations and the suspended matter concentration, respectively.

Bacterial concentration was very high at $t = 0$ (initial mix), then decreased rapidly during settling ($1.8 \times 10^9$ at $t = 0$ to $2.34 \times 10^7$ cells ml$^{-1}$ at $t = 120$, see Fig. 9.19), following the same pattern as suspended matter. Heavy metals concentrations also decreased following a similar pattern (see below and Fig. 9.20).

The number of bacteria in the solutions filtered at 1.2 µm varied only slightly during the settling process (from $1.87 \times 10^6$ to $1.30 \times 10^7$ cells ml$^{-1}$). This population (in $F < 1.2$ µm) was found to represent only 0.5% and 6% of the total population at $t = 0$ and at $t = 60$ min, respectively (Fig. 9.19). The cells counted in this fraction correspond to free cells, or cells associated to microparticles or colloïds (particle size less than 1.2 µm).

Cell counts carried out in the solutions filtered at 0.45 µm showed the presence of bacteria at a concentration ranging between $1 \times 10^5$ to $1 \times 10^6$ cells ml$^{-1}$ (data not shown).

### *Heavy Metals Distribution*

Zn, Fe, Cd and Cu concentrations were monitored in the water column and in the solutions filtered at 1.2 µm and 0.45 µm (*i.e.* "Total fraction", "$F < 1.2$ µm" and "$F < 0.45$ µm"). Table 9.3 shows the results obtained in "biotic" and "gamma rays" experiments. Figure 9.20 illustrates the typical data obtained for Zn, which exhibits a behavior representative of that of the other monitored metallic elements. Measurements, of heavy metal distribution, for "formaldehyde experiment" are underway. These complementary results will permit us to confirm or infirm the tendency obtained with the biotic and gamma rays experiments.

Table 9.3 and Fig. 9.20 show a fast decrease in heavy metals concentration both with biotic and sterilized sediments, with a time course very similar to the drop in suspended matter concentration (Fig. 9.18) and bacterial population (Fig. 9.19). Zinc concentration in the water column was divided by 75 during the first 4 minutes in the biotic experiment, and by 12 in the same time for the abiotic experiment (Table 9.3).

Figure 9.20 shows that although zinc concentration was initially slightly higher in the biotic experiments, the concentrations dropped drastically over the 4 first minutes to become 4 times smaller than in the "abiotic" experiments. After the 4 first

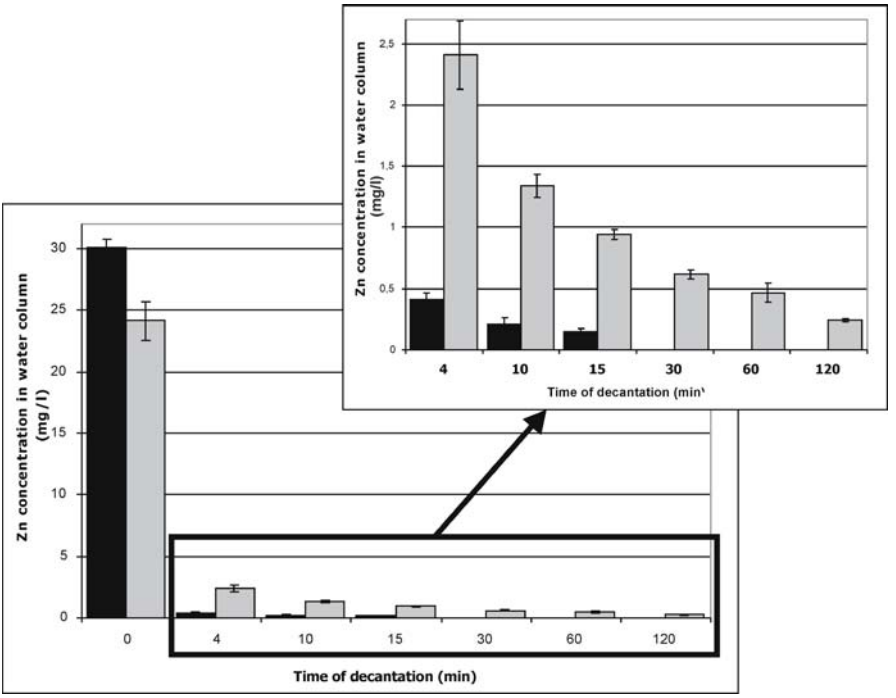


**Fig. 9.20.** Zinc concentration in the water column during settling in "biotic" (*black bars*) and "abiotic" (*gray bars*) experiments

minutes, the focus in Fig. 9.20 shows that the concentration still continued to decrease in both experiments (below detection limit in biotic experiments), but at a much smaller rate.

The same observations can be done for Cd, Cu and Fe. Consequently, Table 9.3 shows that during the first 4 minutes of the settling process, Cd concentration was divided by 50 and 11 in the biotic and abiotic experiments, respectively. For Cu (respectively Fe), the reduction factors of the concentration in the first 4 minutes are 74 (respectively 62) in the biotic experiments, versus 10 (respectively 7) in abiotic experiments.

Heavy metal concentration in the water column during settling after re-suspension of biotic sediment were therefore much smaller than with sterilized sediment.

Correlation analyses (linear correlation) showed that Zn, Cu, Cd and Fe concentrations were significantly correlated with suspended matter concentration in water column ($0.96 < r < 1$ and $p < 0.01$). This result is consistent with those of Carpentier et al. (2002) and Pettine et al. (1994).

Indeed, experiments done with biotic sediment showed that both suspended matter concentration and heavy metals concentrations were significantly smaller than with sterilized sediments.

Oxidation of anoxic sediment (influx of dissolved oxygen) results in positive change in the redox potential and in a decrease in sediment pH (mainly due to the oxidation of sulfide). This decrease in pH doesn't happen in buffered sediment. In our experiment,

**Table 9.3.** Zn, Fe, Cu, Cd concentrations in water column ($mg\,l^{-1}$) during settling (time is given in minutes). Details on Zn concentration for $F < 1.2$ µm and $F < 0.45$ µm (other metals show similar trends). *ND:* Not detected

| | | | $T = 0$ | $T = 4$ | $T = 10$ | $T = 15$ | $T = 30$ | $T = 60$ | $T = 120$ |
|---|---|---|---|---|---|---|---|---|---|
| Zn | Biotic | *F* total | 30.056 $\pm 7 \times 10^{-1}$ | 0.408 $\pm 5.77 \times 10^{-2}$ | 0.208 $\pm 5.2 \times 10^{-2}$ | 0.150 $\pm 2.50 \times 10^{-2}$ | ND | ND | ND |
| | | $F < 1.2$ | 0.11 | ND | ND | ND | ND | ND | |
| | | $F < 0.45$ | 0.12 | ND | ND | ND | ND | ND | |
| | Abiotic | *F* total | 24.139 $\pm 1.56 \times 10^{0}$ | 2.408 $\pm 2.81 \times 10^{-1}$ | 1.342 $\pm 9.46 \times 10^{-2}$ | 0.942 $\pm 3.82 \times 10^{-2}$ | 0.617 $\pm 3.82 \times 10^{-2}$ | 0.467 $\pm 7.64 \times 10^{-2}$ | 0.242 $\pm 1.44 \times 10^{-2}$ |
| | | $F < 1.2$ | 0.15 | 0.11 | ND | ND | ND | ND | ND |
| | | $F < 0.45$ | 0.16 | 0.13 | ND | ND | ND | ND | ND |
| Fe | Biotic | | 4013.9 $\pm 8.86 \times 10^{1}$ | 63.83 $\pm 1.08 \times 10^{1}$ | 38.79 $\pm 1.43 \times 10^{1}$ | 20.25 $\pm 5.41 \times 10^{0}$ | 11.83 $\pm 2.67 \times 10^{0}$ | 7.17 $\pm 7.64 \times 10^{-1}$ | 4.17 $\pm 7.64 \times 10^{-1}$ |
| | Abiotic | | 3100 $\pm 2.14 \times 10^{2}$ | 456.46 $\pm 5.21 \times 10^{1}$ | 267.33 $\pm 2.70 \times 10^{1}$ | 190.42 $\pm 1.23 \times 10^{1}$ | 128.42 $\pm 1.22 \times 10^{1}$ | 85.33 $\pm 1.57 \times 10^{1}$ | 36.33 $\pm 5.55 \times 10^{0}$ |
| Cd | Biotic | | 0.100 $\pm 7.74 \times 10^{-3}$ | 0.002 $\pm 2.78 \times 10^{-4}$ | 0.001 $\pm 1.50 \times 10^{-4}$ | 0.001 $\pm 1.61 \times 10^{-4}$ | ND | ND | ND |
| | Abiotic | | 0.091 $\pm 2.20 \times 10^{-3}$ | 0.008 $\pm 7.1 \times 10^{-4}$ | 0.006 $\pm 3.54 \times 10^{-3}$ | 0.003 $\pm 1.3 \times 10^{-4}$ | 0.002 $\pm 1.28 \times 10^{-4}$ | 0.002 $\pm 3.62 \times 10^{-4}$ | 0.001 $\pm 1.32 \times 10^{-4}$ |
| Cu | Biotic | | 7.443 $\pm 3.35 \times 10^{-1}$ | 0.101 $\pm 1.91 \times 10^{-2}$ | 0.043 $\pm 1.18 \times 10^{-2}$ | 0.031 $\pm 5.80 \times 10^{-3}$ | 0.026 $\pm 5.31 \times 10^{-3}$ | 0.018 $\pm 3.28 \times 10^{-3}$ | 0.013 $\pm 2.44 \times 10^{-3}$ |
| | Abiotic | | 6.534 $\pm 3.19 \times 10^{-1}$ | 0.658 $\pm 5.50 \times 10^{-2}$ | 0.433 $\pm 8.80 \times 10^{-2}$ | 0.266 $\pm 3.81 \times 10^{-2}$ | 0.163 $\pm 2.28 \times 10^{-2}$ | 0.113 $\pm 3.05 \times 10^{-2}$ | 0.056 $\pm 6 \times 10^{-3}$ |

the pH of sediment was 7.2 vs. 6.9 in the water column. We can consider that the low pH variation during the oxidation phase was probably due to the dissolution of mineral carbonate (166 mg $kg^{-1}$ of calcareous in sediment) as described by Caille et al. (2003). Metals co-precipitated with or adsorbed to FeS and MnS are rapidly oxidized. The released Fe and Mn are rapidly re-precipitated and deposited as insoluble oxides/hydroxides to which newly released metals can become adsorbed at varying rates and extents (Calmano et al. 1993; Stephens et al. 2001; Caetano et al. 2003). Caille et al. (2003) showed that during an extended aeration and after a rapid release of metals (Zn,Cu), a decrease in the metal solubility is observed probably due to their co-precipitation with carbonates or oxides. The low concentration in Zn, Cu and Cd in $F < 1.2$ µm and $F < 0.45$ µm may be attributed to the same phenomena as described above.

### 9.3.4 Conclusions

A laboratory protocol was developed to simulate dredging by re-suspension and investigate the role of microbial cells in settling and heavy metals distribution. Bacterial cell concentration, suspended matter concentration, and heavy metals concentrations followed similar patterns during settling after re-suspension. A very fast settling phase was observed over the first 4 minutes after re-suspension, followed by a slower phase, approaching stability within ca. 30 minutes. Moreover, we show a significant bacterial population in the studied grain size compartments ($F < 1.2$ µm and $F < 0.45$ µm), and a low Zn concentration.

Settling was found to occur at a smaller rate with sterilized sediments. Zn, Cu, Cd and Fe concentrations in the water column showed a faster decrease during the settling of biotic sediment as compared to the sediment sterilized by gamma rays.

The concentrations of Zn, Cu, Cd and Fe were correlated to the suspended matter concentration in water column in both treatments (biotic and sterilization by gamma rays).

These first results describe the influence of bacteria on settling of suspended matter. The texture and structure of sediment was not modified by the gamma rays treatment (data not shown). However, from the difference in settling we can conclude that the absence of bacteria, which keep the soil structure "cement", induced a fragility of the aggregates after a long and vigorous agitation (the fragility was not observed with a stability test which is sweeter than our 24 h of vigorous agitation). The absence of bacteria does not permit a constant concentration in exopolysaccharides and thus a keep in stability. Therefore the role of bacteria is to maintain aggregation by the production of polysaccharides. This aggregation induces a rapid settling of particles with heavy metals attached.

In order to better elucidate the mechanisms by which microbial cells influence settling of suspended matter and heavy metals distribution, further studies are underway to investigate particle size distribution of "biotic" vs. "abiotic" experiment and evaluate bacterial diversity by DGGE or SSCP in order to characterize the evolution of the bacterial population during settling. These experiments will permit us to better know the microorganisms part in the particles settling by physico-chemical or biological processes, and so the metals settling during the sediment dredging in the Rhône River.

## References

Bedell JP, Neto M, Pressiat F (2005) Opérations de dragage, pollution potentielle et enjeux environnementaux. Les sédiments du Rhône Grands enjeux, premières réponses. Journée ZABR, Valence, France. 10 juin 2005. pp113–121

Brohon B, Delolme C, Gourdon R (1999) Qualification of soils through microbial activities measurements: influence of the storage period on INT-reductase, phosphatase and respiration. Chemosphere 38:1973–1984

Caetano M, Madureira MJ, Vale C (2003) Metal remobilisation during resuspension of anoxic contaminated sediment: short-term laboratory study. Water Air Soil Poll 143:23–40

Caille N, Tiffreau C, Leyval C, Morel JL (2003) Solubility of metals in an anoxic sediment during prolonged aeration. Sci Total Environ 301:239–250

Calmano W, Hong J, Forstner U (1993) Binding and mobilization of heavy metals in contaminated sediments affected by pH and redox potential. Water Sci Technol 28:223–235

Carpentier S, Moilleron R, Beltran C, Herve D, Thevenot D (2002) Quality of dredged material in the river Seine basin (France). II. Micropollutants. Sci Total Environ 299:57–72

Engelen B, Meinken K, von Wintzingerode F, Heuer H, Malkomes H-P, Backhaus H (1998) Monitoring impact of a pesticide treatment on bacterial soil communities by metabolic and genetic fingerprinting in addition to conventional testing procedures. Appl Environ Microbiol 64:2814–2821

Hardy D (2002) Historique National des opérations de curage et perspectives, Ministère de l'écologie et du développement durable, pp17

McNamara NP, Black HIJ, Beresford NA, Parekh NR (2003) Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Appl Soil Ecol 24:117–132

Pettine M, Camusso M, Martinotti W, Marchetti R, Passino R, Queirazza G (1994) Soluble and particulate metals in the Po River: Factors affecting concentrations and partitioning. Sci Total Environ 145:243–265

Poly F (2000) Réponses des communautés bactériennes telluriques à des perturbations chimiques complexes: Activités potentielles et empreintes génétiques, Université Claude Bernard Lyon 1, Lyon, pp159

Ranjard L, Richaume A, Jocteur-Monrozier L, Nazaret S (1997) Response of soil bacteria to Hg(II) in relation to soil characteristics and cell location. FEMS Microbiol Ecol 24(4):321–331

Sen TK, Khilar KC (2006) Review on subsurface colloids and colloid-associated contaminant transport in saturated porous media. Advances in Colloid and Interface Science 119:71–96

Stemmer M, Gerzabek MH, Kandeler E (1998) Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. Soil Biol Biochem 30:9–17

Stephens SR, Alloway BJ, Parker A, Carter JE and Hodson ME (2001) Changes in the leachability of metals from dredged canal sediments during drying and oxidation. Environ Pollut 114:407–413

Trevors JT (1996) Sterilization and inhibition of microbial activity in soil. J Microbiol Methods 26:53–59

van Elsas JD, Trevors JT, Wellington EMH (1997) Modern soil microbiology. Marcel Dekker, INC, pp 683

van Rijn J, Tal Y, Schreier HJ (2006) Denitrification in recirculating systems: Theory and applications. Aquacult Eng 34:364–376