SEDIMENT ENTRAINMENT AND FLOCCULATION MEDIATED BY MICROBIAL PRODUCED EXTRACELLULAR POLYMERIC SUBSTANCES (EPS)

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Abstract

The stabilizing effects of natural biofilm on erosional processes have been increasingly recognized in the last decades. In riverine systems these effects not only influence sediment dynamics, but also the remobilization of pollutants. Due to the complex interaction between the biofilm, sediment, and hydraulics, an interdisciplinary approach is of great importance.

To investigate the erosional processes of fine sediments due to the impacts of naturally grown biofilm, novel flumes are developed and described. The experimental setup consists of a straight glass flume, with adjustable bottom shear stresses. The biofilm is grown on glass beads (diameter: 40 – 70 µm) in the test section of the flume using suspended cells as an inoculum from natural stream water and an artificial nutrient supply.

To simulate different growth conditions the setup additionally contains adjustable illumination and a water temperature control system.

Biofilm consists of microbes and their metabolic products, the "extracellular polymeric substances (EPS)". The EPS is often referred to as the "glue" which sticks the cells and the sediment together, thus providing stability. In the growth phase, the adhesive forces are measured using a magnetic particle induction device (MagPI) which will be validated against critical shear stresses. The critical shear stress of the sediment stabilized by fully grown biofilm is determined by applying increasing bottom shear stresses with the SETEG flume.

Hydraulic and biologic models still lack a suitable implementation of parameters describing the interrelationship between these two fields. Moreover, the remobilization of pollutants, biologically bound in biofilm, is a focus of future research. This amongst others, emphasizes the importance of an interdisciplinary research approach.

Introduction

The term biofilm (see Figure 1) describes a heterogeneous biological structure consisting of microorganisms and their secreted extracellular polymeric substances (EPS). A biofilm forms on boundary surfaces (sediment-water, water-air, water-oil) (Kraus, 2002) and is often related to the term "slime" or "microbial mat" (Flemming & Wingender, 2001).

Figure 1: Biofilm (greenish-brown) found on cobbles of the streambed in High Ore Creek (USGS)

A well-known example of biofilm is activated sludge used in sewage plants.

Depending on biotic and abiotic conditions (intensity and wavelength of light, temperature, availability of nutrients, oxygen, pH, etc.) suspended heterotrophic bacteria, cyanobacteria and eukaryotic algae attach to a surface and become sessile. In the growth phase they secrete a "glue-like" viscoelastic substance (EPS) (Lewandowski et al., 1993) which is primarily composed of polysaccharides, proteins, water, lipids and nuleic acids (Stoodley et al., 2002). This viscoelastic substance enhances the binding

forces between sediment grains and thus impacts sediment stability ((Gerbersdorf, 2010), see Figure 2).

Detachment mechanisms are erosional processes or active dispersal from the biofilm (called sloughing), that for example, is caused by nutrient limitation. In both cases the bottom shear stress plays an important role.

The biofilmstructure is often referred to as "microarchitecture", or even as a "city of microbes" (Watnick & Kolter, 2000) because of its complex three dimensional structure, including infrastructural elements (transportation channels, nutrient storage, pores, diffusional limitations), mechanical stability (adaption to applied forces, spatially separated habitats for different microorganisms) and communication mechanisms (Flemming & Wingender, 2001).

Several authors ((Liu & Tay, 2002), (Pereira et al., 2002), (Wäsche et al., 2002) (Lewandowski et al., 1993)) conclude that this structure is not only dependent on the availability and metabolic build-up of the construction materials (nutrients, water, sediment grains, etc.) but also on the hydrodynamic boundary conditions.

It is difficult to differentiate between the influence of biotic and abiotic factors. For example, high turbulence (i.e. high flow velocity) facilitates diffusion of nutrients into the biofilm (Liu & Tay, 2002) and increases biofilm growth. In contrast, biofilm growth may lead to a denser structure which, decreases the diffusion of nutrients and thus limits biofilm growth. In this way shear stresses do not only form the biofilm by erosional processes but also strengthen the construction material while limiting growth.

This example emphasizes the urgent need for an interdisciplinary approach between biologists and hydraulic engineers to investigate the subject. In the 3-year project "Ecosystem engineering: Sediment entrainment and flocculation mediated by microbial produced extracellular polymeric substances (EPS)", supported by the Deutsche Forschungsgemeinschaft (DFG), biologists and hydraulic engineers formed the project research team at the Institute for Modeling Hydraulic and Environmental Systems, at the University of Stuttgart.

Motivation

Erosional processes of fine sediments as a part of morphology is subject to ongoing research (Partheniades, 2009). This is, because morphological problems are of great importance for the water frame directive and the deposition and dredging of sediments in inland waterways and harbors causes enormous logistic and socio-economic problems (Westrich & Foerstner, 2004). Moreover, fine sediments are heavily polluted in some streams and erosional processes can lead to a re-mobilization of toxic agents and pollution of riverine habitats and floodplains (Wölz et al., 2009).

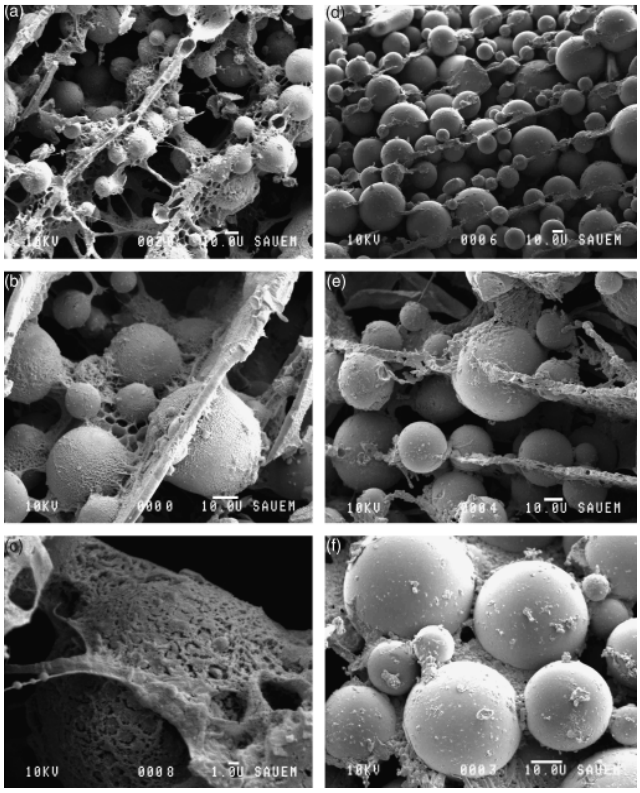

Figure 2: Low temperature scanning electron microscope images of glass beads covered with EPS (Gerbersdorf et al., 2008)

While cohesive sediments provide an excellent substratum for biofilm growth (large surface to volume ratio, rich in nutrients, porous structure) the modeling of erosional processes still lacks a generally accepted implementation of biogenic influences, although a significant impact is reported from several authors ((Prochnow et al., 2001), (Lundkvist et al., 2007), (Lundkvist et al., 2007)). Modeling approaches can be found, for example in (Le Hir et al., 2007) and (Wang & Zhang, 2010).

The aim of the project is to gain fundamental knowledge about the impacts of naturally grown biofilm for freshwater conditions on fine sediment stability.

Experimental Proceedings

Two parameters affecting biostability, the hydrodynamic (scenario 1) and the lightregime (scenario 2) are varied successively in the growth phase, to assign their impacts to erosional processes, the degradation of nutrients in the water column and to the biological composition.

For this reason geometric identical testing flumes are constructed in which the biofilm is grown on fine glass beads under defined conditions.

At present, a prototype flume is finalized and results from erosional studies verify the applicability of the flume. The insights gained from the prototype are used to construct the testing flumes.

Objectives

Three parameters (see Table 1) are identified to have a major impact on biofilm induced sediment stability during biofilm growth:

Table 1: Adjusted boundary conditions in the growth phase

Scenario	Parameter	Low	Medium	High
1	Bottom shear stress τ [N/m²]	0.02	0.15	0.29
2	Intensity of Light I [µE/m²s]	10	20	100
1 + 2	Nutrients	Degradation measurements		

1. The bottom shear stress regulates nutrient support during the growth phase and mediates erosional processes. The adjusted flow conditions are turbulent (Re > 2320) for all applied shear stresses.
2. Light intensity and wavelength is responsible for favoring autotrophic algae/bacteria to grow and produce EPS.
3. Nutrient availability is important for the assembling of organic matter (cells, EPS) and influences biofilm growth.

Two different scenarios to investigate the influence of the three above mentioned parameters are set-up.
In the first scenario the impact of the bottom shear stress to sediment stability is investigated. In one testing flume, a low bottom shear stress (τ = 0.02 N/m²) is applied via regulation of the pumped volume. Parallel to that a medium (τ = 0.15 N/m²) and a high (τ = 0.29 N/m²) shear stress is applied, each to an individual flume. The light intensity is permanently set to a medium level (I = 20 µE/m²s) in the first scenario.
In scenario two, the light intensity is varied in three flumes (low: I = 10µE/m²s; medium: I = 20µE/m²s; high: I = 100µE/m²s) while the shear stress is held constant (τ = 0.15 N/m²).
In both scenarios, the third parameter, the degradation of nutrients in the water column is monitored. In this way the direct impact of hydrodynamics and light regime on the nutrient exchange between water column and biofilm is investigated. The freshwater used in the experiments is natural stream water and thus contains a natural composition of nutrients and organisms/spores which build up the biofilm.
Consumed nutrients are automatically substituted to simulate natural conditions.
Other impact factors (temperature, oxygen, pH, waterdepth), that are held constant in the project, are monitored and adjusted in a natural range (see Table 2).

Table 2: Constant boundary conditions in the growth phase

Parameter	Value/Range
Temperature T [°C]	16 – 18
Oxygen C_{O2} [mg/l]	6
pH	6.5 – 8.5
Waterdepth [m]	0.1

The biofilm is grown on fine inert glass beads with a size range (diameter = 40 - 70 µm) that is comparable to the size range of silt. To ensure reproducibility each experiment is run several times.

Materials and Methods

Construction of the flumes

In order to reproduce testing conditions, all flumes are constructed identically (see Figure 3) and the natural stream water is pumped in a closed circuit to avoid undesired contamination.

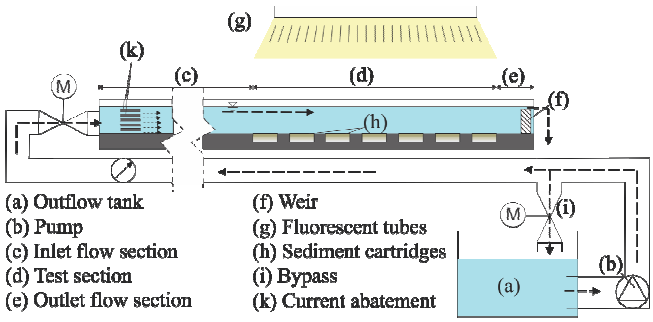

Figure 3: Schematic view of a testing flume

The outflow tank (a) provides the pumping system (b) with 200 l of circulating water flow.
The flumes (L x W x H = 3.00 x 0.15 x 0.15 m) are made of float glass, suitable for Laser Doppler Anemometry (LDA) measurements of the bottom shear stress and monitoring of the biofilm growth.
The test section (d) has a length of 0.90 m and holds the cartridges (h) for biofilm growth. The length of the inlet flow section (L = 1.90 m) (c) is dimensioned to avoid undesired turbulence in the test section and to accomplish a regular distribution of bottom shear stresses. The outlet flow section (e), which ends in a weir (f), has a length of 0.20 m. The light regime is applied with fluorescent tubes (Osram Biolux) (g), which produce a constant intensity,

with wavelengths in the range of 480 nm to 665 nm, important for biofilm growth. The intensity is regulated by adjusting the height of the tubes.

Flume Bottom

The overall duration of the project is 3 years in which several experiments will be conducted. It is especially important to avoid leakage during this time and to have a flexible setup for future research. To achieve this, the bottom of the flume is completely removable. The cartridges, which carry the sediment are removable for external investigations (biological/chemical analysis and stability measurements). They are fitted in the bottom and the sediment surface is on one level with the bottom surface. Thereby the flow lines are assumed to be parallel to the bottom throughout the entire flume and the adjusted bottom shear stress is uniformly applied on the sediment surfaces.

Pumping circuit

The pumping system is composed of a pump (BADU Eco Touch), a pipe leading to the inflow section of the flumes and a regulating valve at the inflow and bypass (i) to regulate the pumped volume.
The chosen pump is suitable for low pumping heads (h > 1.0 m) and a wide range of discharges needed to apply the bottom shear stresses presented in Table 1. The revolution speed of the pump is adjustable in three factory-provided steps. By adjusting the revolution speed to an adequate level the hydraulic losses in the pump can be reduced and the heat evolution can be maintained at an acceptable level. Additionally the water is cooled by a cooling-circuit.

Construction and materials

The flume is sealed with inert aquarium-silicone (OTTO SEAL S28). The inflow adapter, the additional current abatements (k), and the outflow weir (to ensure a defined water depth) are all constructed from stainless steel.
Surfaces that are exposed to low shear stresses and light favor biofilm growth and are not acceptable outside of the test section. To reduce these surfaces, adjustments are constructed for the current abatements and the outflow weir.

Sediment

Because of the complex heterogeneous composition (organic content, size and form of single grains) of natural cohesive sediment, artificial sediment (glass beads) is chosen to account for the geometrical characteristics.
To avoid the destruction of the sediment layer during the insertion in the flume, the glass beads are wettened until saturation and put into cartridges. Geotechnical measurements of the volume, water content, and size range are taken prior to the experiments.

Measurement equipment

Even though each setup is identically constructed, geometrical discrepancies and differences in the pumped volume may lead to undesired deviations in bottom shear stresses for different flumes. Therefore the distribution of the shear stresses and the velocity profiles are measured and determined for each flume using a LDA system.
Once these values are known, they are correlated to the adjusted discharges, which are measured by a mini flow meter (BÜRKERT 8030).
In this way the hydrodynamics of different flumes is compared to each other and the hydraulic regime can be adjusted so reproducibility is achievable. In running investigations, the discharges are monitored individually for each flume. By this means deviations in the shear stress are detected and the results can be evaluated.
Temperature, nutrients, oxygen concentration, and pH are also monitored and adjusted to the values of Table 2.

External stability measurements

The biostability of the sediment can be measured in two ways. During the growth phase, when the stabilization process is just about to begin, a sensitive nondestructive method is chosen. The Magnetic Particle Induction (MagPI) correlates a magnetic force used to attract ferro-magnetic particles with the vertical adhesion strength of the biofilm (see Figure 4).

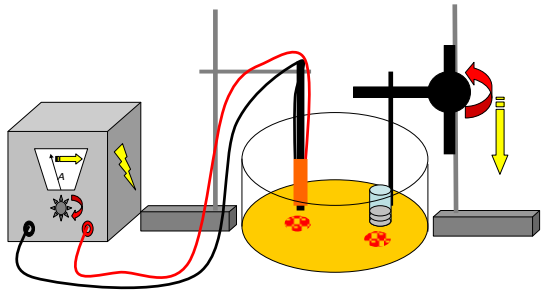

Figure 4: Magnetic particle induction (MagPI) (Larson et al., 2009)

The MagPI consists of an electromagnet which is positioned by a micromanipulator and a power supply unit to set the magnetic force.
Fluorescent ferro-magnetic particles are dropped on the biofilm surface and the MagPI is positioned at a defined vertical distance from the particles. The electromagnetic force is then increased until the particles move. Measurements are repeated on different locations and the results are statistically evaluated (Larson et al., 2009). A correlation between vertical adhesion and critical shear stress will be investigated.

In defined intervals the sediment stability is measured using the SETEG flume (see Figure 5).

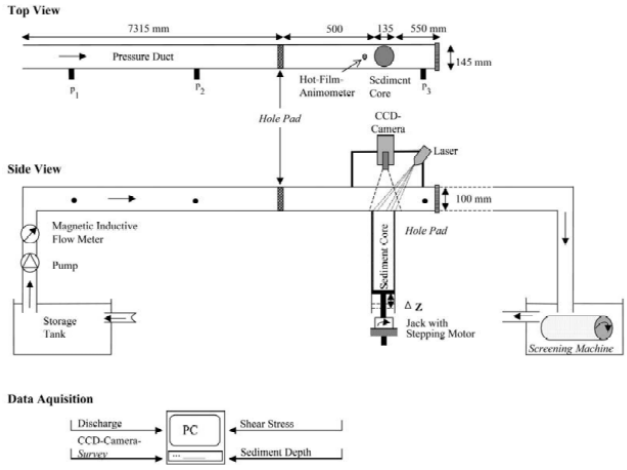

Figure 5: Schematic setup of the SETEG system (Witt & Westrich, 2003)

The SETEG flume is a device to measure critical shear stress and its eroded volume for specific hydraulic conditions of sediment probes. It consists of a straight flume with a total length of 8.5 m and a high resolution camera system with image data processing (Witt & Westrich, 2003).

To evaluate the critical shear stress of the biostabilized sediment, the undisturbed sediment cartridges are fixed at the bottom of the flume. By adjusting the discharge a defined bottom shear stress affects the surface of the sediment until erosion ($\tau_{crit,bio}$). The stability of control samples (without biologic influence) is also determined and the results are compared to evaluate the biological impact on critical shear stresses.

Results and discussion

To verify the functionality and practicability of the prototype a first test is carried out. For this experiment freshwater, with a low content of nutrients, from the source of the River Echaz near Tübingen (Germany) is chosen.

The fluid is then inserted into the prototype system and the sediment is prepared. After carefully flooding the flume, a velocity is adjusted that results in a shear stress (τ = 0.29 N/m²) just below the critical shear stress of the glass beads (τ_{crit} = 0.3 N/m²). No external light is used. The boundary conditions are therefore chosen to be suboptimal growth conditions for the biofilm. After one week of running the experiment, a light brown biofilm is visible. Cartridges for the biological analysis are removed and exchanged with flat plates. After another week of growth the biofilm is clearly visible and the critical shear stress is evaluated using the SETEG system. In comparison to the critical shear stress of the biologic not influenced sediment (τ_{crit} = 0.30 N/m²) the critical shear stress of the biostabilized sediment increased by 170% to $\tau_{crit,bio}$ = 0.82 N/m².

Conclusion & outlook

Biofilm growth is initiated and influenced by multiple boundary conditions. In the DFG project "Ecosystem engineering: Sediment entrainment and flocculation mediated by microbial produced extracellular polymeric substances (EPS)" two major boundary conditions (hydrodynamic and light regime) are varied and their impact on biostabilisation is investigated. The rate of nutrient degradation caused by the above mentioned parameters is monitored and also evaluated. A natural biofilm is grown in specially designed flumes on exactly defined sediment (glass beads) for this reason.

The project aims to gather a fundamental understanding of the biological and hydrodynamic processes involved in biostabilisation, to develop a basis for a numerical model.

Due to complex interactions between biology and hydrodynamics an interdisciplinary approach is chosen and the experimental setup is constructed considering both disciplines equally. The knowledge gained from the construction of a prototype is used for the construction of the testing flumes.

An erosional experiment for verification of the prototype showed an increase of the critical bottom shear stress by 170% for biostabilized sediment in contrast to pure sediment and emphasizes the importance of biostabilisation for morphological processes.

In order to focus on the major influences on biostabilisation, simplifications (stationary flow, artificial sediment, constant temperature etc.) have to be made.

In natural streams high fluctuations in discharges can occur, especially during flood events. Erosional processes are initiated due to higher bottom shear stresses.

The tolerance of the biostabilized sediment to higher shear stresses might be dependent on the specific growth conditions and is supposed to be lower for biofilm grown under lower shear stresses. Investigations where the applied shear stress is varied after the growth phase and evaluation of the $\tau_{crit,bio}$ are planned.

Erosion of fine sediments is closely linked to remobilization of pollutants, so that toxicological research is a future topic.

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