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Investigation on the iron-uptake by natural biofilms

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ABSTRACT

Biofilms are natural communities of microorganisms living in aquatic ecosystems which play an important role in the biogeochemistry of many inorganic elements, including iron. The present work aimed to study the uptake of iron by natural river biofilms (produced in the laboratory) and to examine the relationships between biofilms and iron in water. For that, biofilms were formed from natural water samples collected at different times of the year. Total content and global localization of iron were determined by a combination of chemical analyses and microscopy, which indicated that iron was systematically distributed throughout the biofilm matrix. Depending on the level of iron uptake, iron was diffuse or present as hot spots, was primarily localized to the fraction ascribed to OM compounds (45–60%) or the residual fraction (~14–40%). Additional experiments were conducted using iron–organic complexes with different affinities (log K) to study iron uptake according to the speciation. These experiments suggested the association between iron and organic ligands (i.e. depending on the affinity constant) influenced the uptake of iron, but did not control the biofilm affinity for iron, which appeared to be controlled by chemical-kinetic laws.

1. Introduction

In aquatic ecosystems, the majority of microorganisms live attached to the surface of rocks or plants (Costerton, 1999) rather than as planktonic cells, due to significant benefits (limitation of biocides and heavy metals action, exchange of genes and protection from predators) (Molin and Tolker-Nielsen, 2003). These microbial communities are usually known as biofilms (Watnick and Kolter, 2000) and function as consortia in a relatively complex and coordinated manner (Davey and O'Toole, 2000).

Biofilms are composed primarily of microbial cells (primarily bacteria, but also archaea and eukaryotic microbes), extra-polymeric substances (EPS) and inorganic materials. EPS are a polymeric conglomeration generally composed of water, extracellular DNA, Humic substances, proteins and polysaccharides in which microbial cells and minerals are embedded. EPS may account for 50–90% of the total organic carbon present in biofilms (Flemming et al., 2000) and can be considered the primary matrix material of the biofilm (Flemming et al., 2007). The composition of EPS determines the local biofilm conditions for cells living in the microenvironment by affecting porosity, density, water content, charge, sorption properties, hydrophobicity and mechanical stability (Characklis et al., 1990). This matrix material is a dynamic environment in which the microbial cells appear to reach

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homeostasis and are optimally organized to make use of all available nutrients (Sutherland, 2001).

Biofilms play an important role in the biogeochemistry of both major and trace elements, since microorganisms grow by using mineral and organic elements from water or surfaces (rocks or plants). Some metals, like iron, are essential to almost all organisms (bacteria, algae) living in biofilms. Iron can serve both as an electron donor for lithotrophic growth and an electron acceptor for anaerobic respiration (e.g. Lovely, 1991; Emerson and Moyer, 1997), a major component of certain non-haem iron-containing proteins, as well as a cofactor for certain enzymatic reactions. It is now well accepted that there is a significant interdependence between microbes and iron speciation (Sulzberger et al., 1990), such that microbial metabolisms have been differentiated based on iron utilization, i.e. ferrous iron-oxidizing bacteria (e.g. *Gallionella ferruginea*, *Leptothrix ochracea*) and ferric iron-reducing bacteria (e.g. *Shewanella* sp., *Geobacter* sp.). The uptake of iron by bacterial cells is due to i) passive interactions, like biosorption, that lead to metal fixation on the membranes, cell walls or capsids of microorganisms, and ii) active interactions that lead to metal uptake by bacteria (*via* membrane ion pumps) and transformation into the mineral phase (nanoparticles of magnetite $-Fe_3O_4$, Thomas-Keprta et al., 2000). Some bacteria (e.g. *Pseudomonas* species) mobilize iron from hydrous ferric oxide (HFO) or organic complexes by producing highly-active iron-chelating molecules called siderophores (Banin et al., 2005) or organic acids (i.e. oxalate or citrate).

Many biogeochemists and geomicrobiologists have shown that microbial activity has significant influence on iron cycling (Konhauser, 2007; Haese, 2006). However, iron acquisition poses a problem for many aerobic organisms. Indeed, iron has a redox chemistry that is an important aspect of its speciation. In oxic freshwaters the +3 oxidation state is the thermodynamically stable form (Davison, 1993). In the majority of surface waters (pH ~ 6–8), the concentration of free and complexed dissolved ferric iron tends to be very low due to extensive hydrolysis and precipitation forming insoluble (particulate/colloidal) oxides and hydroxides (Stumm and Morgan, 1981). The presence of dissolved iron oxides has been attributed to dissolved organic complexes of ferric iron (Perdue et al., 1976; Koenings, 1976) or the presence of small HFO particles coated with natural organic matter (Shapiro, 1966).

Organic matter also plays an important role in iron speciation in water. Dissolved or colloidal organic matter may stabilise and delay the oxidation of ferrous iron, the other form of iron in water. Free ferrous iron is stable in a reduced state, whereas it is rapidly oxidized to insoluble HFO in the presence of O_2. No universal iron speciation is possible since iron speciation in natural waters depends on redox and light conditions, pH, as well as the amount and type of organic matter. Studies (Nagai et al., 2004, 2008; Xing and Liu, 2011) have shown that the percentage of organic iron (i.e. associated with organic ligands) varies considerably in freshwater lakes (35–99%), while Rue and Bruland (1995) found that more than 99% of the dissolved ferric iron in central North Pacific surface waters is chelated by natural organic ligands.

The uptake of iron by natural microbial consortium is not well documented (compared to specific bacteria), indicating there is a need to understand how biofilms and iron interact due to the essential nature of iron for organisms. An understanding of bacterial global regulatory networks that govern biofilm formation may offer practical solutions to favour or control biofilm formation (e.g. biotechnology, water treatment). The objective of the present work was to study the uptake of iron by natural biofilms. Using biofilms formed from water samples, the amount of iron and its localization were explored, while other experiments examined the mechanism of iron uptake by iron–organic complexes.

2. Materiel and methods

2.1. Production of biofilms

We used a lab-scale pilot to produce biofilms in controlled conditions. The pilot is composed of several glass columns filled with glass beads and fed with natural water (details are given in Supplementary Data). A complementary peristaltic pump is used to supply the feedwater with a chosen solution of iron. Experiments were performed night and day in a thermostatic room (25 or 35 °C). Two columns by conditions were used for duplicate experiments.

All the experiments were performed with natural freshwaters from the Vienne River (sampling location: N46°40′57″/E00°34′17″). Main physico–chemical parameters of this river are presented in Supplementary Data section. Six series of experiments were conducted between October 2009 and August 2010. For one run, about 1 m^3 of water was sampled and stored at 4 °C in 20 L polypropylene tins before using. To avoid the decantation of suspended particles inside columns, all the waters used for biofilm production were pre-filtered at 10 μm (PVDF membranes).

Experiments lasted three weeks to have enough biofilm inside the column. At the end of colonization period, each column was rinsed out with demineralised water before being emptied. Glass beads supporting biofilms were collected in a 250 mL-beaker and covered with demineralised water. Biofilms were recovered by sonication at 80 Watts for 3 × 5 minutes (Bioblock Scientific 86480). Preliminary tests validated that the recovery of biofilm was reproducible (about 95% +/−5% of total carbon).

2.2. Iron supply

For experiments needing an iron supply, columns were continuously fed with an iron solution in addition to the normal feedwater flow. In all experiments, iron was supplied until a final iron concentration of 1.2 mg Fe/L in the feedwater. This value was chosen to favour the effect of the "iron provided by the experiments" compared to the "endogenous iron". Iron was supplied with different ligand: oxalate, nitrilotriacetate, citrate, or ethylene diamine tetra acetate (Log $K_{Fe\text{-}Ligand}$ are 6.8, 8.5, 13.1 and 16.9, respectively – Sillen and Martell, 1964).

2.3. Characterization of biofilm

2.3.1. Determination of total element concentrations

Total element concentrations were determined after an acid digestion of the biofilms. Briefly 2 mL of nitric acid (69%, Fisher