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Coupled interactions between metals and bacterial biofilms in porous media: Implications for biofilm stability, fluid flow and metal transport

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ABSTRACT

The ability to predict the impact of metal–microbe interactions on biofilm stability, hydrodynamics and contaminant transport is a key goal in hydrogeology, membrane bioreactors and geomicrobiology. Yet, we have not been able to find experimental studies reporting the coupling of these three elements in a systematic way. In this study sand column experiments were carried out to investigate the coupled effects of aqueous zinc (Zn^{2+}) on biofilm stability and of biofilm on Zn^{2+} transport in saturated sand columns. A series of aqueous Zn concentrations ranging from 0 to 100 ppm were pumped through biofilm-colonised columns and a set of related parameters (hydraulic conductivity, cell numbers, EPS, metal breakthrough curve and metal distribution) was measured. Our results show that biofilm formation prior to introduction of Zn resulted in a significant decrease of hydraulic conductivities. After 10 days of metal exposure, Zn showed concentration-dependent toxicity on bacterial cells in the biofilm. However different Zn concentrations produced distinct non-linear effects on EPS production, which resulted in either recovery or further decrease of hydraulic conductivity in the porous matrix. This non-linear response of biofilm to metal concentration could lead to different metal transport patterns in the long term. The concentration of metal contaminants plays a critical role in regulating the effect of metal–biofilm interaction. Our phenomenological study establishes linkages between chemical, microbial and physical processes of metal–biofilm interaction, and is an essential precursor to the development of models for this complex system. Specifically, these interactions are shown to be unpredictable, suggesting that more work needs to be done to constrain flow and transport parameters in biofilm-colonised porous media.

1. Introduction

Over 99% of microorganisms on Earth live in the form of biofilms (Vu et al., 2009), which are populations of cells surrounded by a matrix of extracellular polymeric substances (EPS) (Denkhaus et al., 2007). Typical constituents of EPS are polysaccharide and protein, often accompanied by lesser amount of nucleic acids, lipids or humic substances (Flemming, 1995; Flemming and Wingender, 2001). EPS plays a key role in the formation of biofilm by stabilisation and protection of the biofilm structure through increasing resistance to dehydration and biocides. EPS also facilitates cell adhesion to surfaces (Omoike et al., 2004; Perry et al., 2005) and promotes cell aggregation (Cammarota and Sant'Anna, 1998). Numerous studies have shown that the growth of biofilms in porous media results in a decrease of porosity, flow velocity and hydraulic conductivity (Taylor et al., 1990; Vandevivere and Baveye, 1992a, 1992b; Kim, 2004). These changes of flow properties, in conjunction with chemical interactions at bacterial and biofilm surfaces, may retard metallic contaminant transport in aquifers, membrane bioreactors and engineered bio-barriers.

Biofilms are dynamic and responsive to their environment. Cells may detach from biofilms individually or in clumps with EPS and become mobile (Hunt et al., 2004). Since bacterial surfaces and EPS components are negatively charged in natural environments (e.g. Harden and Harris, 1952; Mittleman and Geesey, 1985), they display a considerable sorption capacity to most positively-charged metal cations (e.g. Texier et al., 1999; Liu et al., 2001; Yee and Fein, 2001; Ngwenya et al., 2003), leading to reduced metal transport. However, the transport of metal adsorbed onto bacteria and EPS components may be facilitated when bacteria and EPS components are mobilised. Yee and Fein (2002) documented that the transport velocity of metals adsorbed to bacteria can be either enhanced or inhibited, depending on the behaviour of the bacteria to which they are attached. Pang et al. (2005) reported a significant increase in total cadmium (Cd) effluent concentration after injection of bacteria and concluded that aqueous Cd migrated 17 to 20 times faster when it travelled with mobile bacteria in aquifer gravel media, compared with control experiments. Numerical models have been developed and verified to address this bacteria-facilitated solute transport phenomenon (Pang and Simunek, 2006; Bekhit et al., 2009). However, attempts to understand the role of EPS in metal transport have led to conflicting observations. Sand column experiments performed with Cd in the presence and absence of selected extracellular polymer showed that EPS in solution could complex metal ions and increase metal

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transport rates in porous media (Chen et al., 1995). Similarly, EPS was shown to enhance transport of Cr(III) in soil columns, in the form of Cr-EPS complexes (Kantar et al., 2011). However, Liu et al. (2007) demonstrated that the presence of EPS increased bacterial adhesion to glass beads in column experiments, a property which might be expected to limit the mobility of adsorbed contaminants.

With exposure of biofilms to aqueous metallic contaminants the metal–biofilm interaction, and in particular the influence of metals on biofilm growth and EPS production, is expected to further complicate the pattern of fluid flow and associated metal mobility and fate. On one hand, at toxic concentrations metals tend to inhibit and kill microbial cells and increase the detachment of bacteria and EPS from biofilms (Harrison et al., 2004). This could lead to enhancement of metal mobility by co-transport with bacterial cells and/or EPS. The tolerance of bacterial cells and biofilm matrix to metal cations will depend upon the metals, their concentration and speciation, the media composition and microbial species present. On the other hand, it has been documented that at certain metal concentrations EPS production may be enhanced (Fang et al., 2002; García-Meza et al., 2005; Aguilera et al., 2008) as a protective mechanism against toxicity. The increased production of EPS may lead to retardation of metal transport directly by adsorption and indirectly by decreasing hydraulic conductivity, or to increased transport due to metal-EPS complexation.

Overall, the complex metal–biofilm interactions make understanding and predicting the transport and fate of metals difficult. Previous studies of biofilm-metal interactions have focused mainly on the bioremediation of soil, sediment and wastewater through metal immobilization by biofilms (Lion et al., 1988; Ferris et al., 1989; van Hullebusch et al., 2003), and biocorrosion (Beech and Sunner, 2004). The potential effects of metal–biofilm interactions on biofilm stability and metal transport in porous media are currently unclear. The objectives of this study were (i) to investigate the effects of different metal concentrations on the potentially coupled processes of biofilm growth, EPS production and corresponding hydraulic conductivity changes; and (ii) to investigate the effects of biofilm on metal transport and immobilization in porous media. To the best of our knowledge, it is the first attempt to document the existence of coupled hydrogeological–biogeochemical processes of metal–biofilm interaction in an experimental system. Such information is an essential first step before developing approaches to modelling the transport and fate of metal contaminants in a range of applications including hydrogeology, membrane bioreactors and geomicrobiology.

2. Material and methods

2.1. Bacteria strain, growth medium and reagents

The gram-negative, metal-resistant (can survive in 1 mM Cu or Zn) bacterium *Pantoea agglomerans* was used throughout the study because its surface chemistry and metal adsorption behaviour have been well characterized previously in our laboratory (Ngwenya et al., 2003; Ngwenya, 2007; Ngwenya et al., 2010; Kapetas et al., 2011). Commercially available standard medium nutrient broth No 3 (Sigma-Aldrich Inc) containing meat extract 1 g l^{-1}, yeast extract 2 g l^{-1}, peptone 5 g l^{-1} and sodium chloride 5 g l^{-1} was used to cultivate the culture in flasks and to develop biofilm in columns. The final pH of the medium was adjusted to 7. All reagents were of analytical grade and solutions were made in 18.2 MΩ cm water.

2.2. Column set-up

The experimental columns (26 mm I.D × 250 mm) were constructed from polycarbonate and capped at both ends with o-ring sealed polyetheretherketone (PEEK) end-pieces. Two 30 μm pore diameter polyethylene frits in each end-piece were used to ensure even flow of the medium over the packing surface. General purpose grade sand (Fisher Scientific) used to fill the columns was first sieved to a narrow grain size range from 120 μm up to 350 μm. The sand was washed three times with hydrogen peroxide (6%) in 80 °C water bath to remove organic matter; followed by washing in 1 M HNO_3 to remove trace metals; then with 1 M NaOH and deionized water to adjust pH to 7. Finally, the sand was autoclaved at 125 °C for 1 h. All the columns, tubing and other parts in contact with the flowing solution were sterilized by soaking in 70% ethanol overnight. Packing was performed aseptically in a laminar flow hood using the wet-packing method to avoid trapping of air (Deshpande and Shonnard, 1999). The packed columns had an average porosity of 44 ± 2.5% and the initial pore volume was approximately 47 ml. A peristaltic pump (Master Flex, Cole-Parmer) was used to introduce the sterilized medium through a 0.22 μm in-line filter into the sand columns to prevent potential contamination reaching the column and preventing biomass from the column flowing back into the medium bottle. Flow was run from the bottom to the top of the column to remove any trapped air and to minimize the possibility of preferential flow.

2.3. Column flow experiments

Nutrient broth medium was pumped first into the sand columns for at least 24 h to allow the system to stabilise before bacterial inoculation. The flow rate was set to 0.3 ml min^{-1}. After this stabilisation period, 20 ml of *P. agglomerans* culture grown to stationary phase with a cell density of 5.4×10^7 cells ml^{-1} was injected into columns using a sterile syringe needle inserted 5 cm above the bottom of the sand pack. Immediately after inoculation, the flow was stopped for 3–4 h to foster cell attachment and the columns were put horizontally in the mean time to avoid the potential cell deposition onto the bottom of the column due to gravity.

Zinc was chosen for this study as a representative of metal contaminants for two reasons. Firstly, zinc has no redox chemistry which might add further complication to understanding its interaction with biofilm in the experiments. Secondly, thermodynamic parameters for Zn adsorption onto bacteria have been previously characterized (Ngwenya et al., 2003). Aqueous Zn concentrations ranging from 0 to 100 ppm were used and these concentrations fall within the range of Zn concentrations encountered in contaminated aquatic environments, 3.25 ppb–99.45 ppm Zn (Toner et al., 2005).

Each flow experiment consisted of the following three stages: in the first stage following inoculation and attachment, the biofilm was allowed to develop for 7 days with flowing full-strength nutrient medium (13 g l^{-1}). The use of full-strength nutrient medium was to accelerate biofilm formation and shorten the total experiment time. In the second stage full-strength nutrient medium was diluted 10-fold to minimize the medium complexation of Zn (NaCl was added to maintain the same ionic strength). It has been reported that biofilm structure and morphology were re-established within 2 days when high nutrient concentration medium was changed to lower one (Stoodley et al., 1999). Therefore in our study the diluted medium was run for two days to rebalance the effluent cell concentration and stabilise the biofilm. In the third stage different concentrations of Zn were added to the 10-fold diluted medium from a 1000 ppm stock standard to obtain final Zn concentrations of 5, 10, 50 and 100 ppm. Immediately following the introduction of Zn, the effluent solution from these columns was collected at 15 minute intervals in the first day to determine Zn breakthrough. Then the Zn containing medium was pumped for 9 more days. During these three stages, hydraulic head and effluent culturable cell numbers were quantified regularly. At the end of experiments, columns were dismantled and sand samples from each column were divided into 10 × 2 cm layers. Adsorbed Zn along the sand columns and biofilm including culturable cell numbers and EPS were extracted and analyzed (See Section 2.4).

Two columns were used as controls: the first was a column with bacteria inoculation but without Zn introduction; the second was a