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Effects of bacterial cells and two types of extracellular polymers on bioclogging of sand columns

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SUMMARY

Microbially induced reductions in the saturated hydraulic conductivity, K_s, of natural porous media, conventionally called bioclogging, occurs frequently in natural and engineered subsurface systems. Bioclogging can affect artificial groundwater recharge, *in situ* bioremediation of contaminated aquifers, or permeable reactive barriers. In this study, we designed a series of percolation experiments to simulate the growth and metabolism of bacteria in sand columns. The experimental results showed that the bacterial cell amount gradually increased to a maximum of 8.91 $\log_{10}$ CFU/g sand at 144 h during the bioclogging process, followed by a decrease to 7.89 $\log_{10}$ CFU/g sand until 336 h. The same variation pattern was found for the concentration of tightly bound extracellular polymeric substances (TB-EPS), which had a peak value of 220.76 μg/g sand at 144 h. In the same experiments, the concentration of loosely bound extracellular polymeric substances (LB-EPS) increased sharply from 54.45 to 575.57 μg/g sand in 192 h, followed by a slight decline to 505.04 μg/g sand. The increase of the bacterial cell amount along with the other two concentrations could reduce the K_s of porous media, but their relative contributions varied to a large degree during different percolation stages. At the beginning of the tests (e.g., 48 h before), bacterial cells were likely responsible for the K_s reduction of porous media because no increase was found for the other two concentrations. With the accumulation of cells and EPS production from 48 to 144 h, both were important for the reduction of K_s. However, in the late period of percolation tests from 144 to 192 h, LB-EPS was probably responsible for the further reduction of K_s, as the bacterial cell amount and TB-EPS concentration decreased. Quantitative contributions of bacterial cell amount and the two types of extracellular polymers to K_s reductions were also evaluated.

1. Introduction

Microorganisms are considered to have a crucial effect on the removal of contaminants in subsurface environments (Cajthaml et al., 2009; Stasinakis, 2012; Yang et al., 2013a,b). At the same time, the accumulation of microbial cells and the associated metabolites that they produce in natural porous media can directly affect the pathway of fluid flow in the subsurface (Arnon et al., 2005; Seifert and Engesgaard, 2007). In the presence of high nutrient loading, biomass (cells and exopolymers) accumulation can reduce the saturated hydraulic conductivity, K_s, and porosity of porous media due to a clogged pore space. The process is also known as bioclogging (Baveye et al., 1998; Vandevivere and Baveye, 1992a,b; Vandevivere et al., 1995). This phenomenon is widely found in aquifer storage and recovery (Pavelic et al., 2007), permeable reactive barriers (Liang et al., 2000), drip irrigation (Puig-Bargués et al., 2005), and *in situ* bioremediation of organic contaminants in subsurface environments (Calderer et al., 2014). Particularly, in the artificial recharge of aquifers, bioclogging may markedly decrease the efficiency of injection wells due to a drastic reduction in the K_s of aquifer materials (Oberdorfer and Peterson, 1985).

Many researchers have related the large reduction in the K_s of porous media to plugs that are formed by the accumulation of bacterial cells in pore throats (Vandevivere and Baveye, 1992a,b,c,d). Vandevivere and Baveye (1992b) reported a significant reduction in K_s at biomass densities of more than 4 mg/cm^3 by using sand columns inoculated with *Arthrobacter* sp. A few studies also

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reported that exopolymers excreted by prokaryotic organisms served as key clogging agents in porous media under some conditions (Cullimore and Mansuy, 1987; Ehlinger et al., 1987; Hoyle, 1994) because they cause a high frictional resistance (Characklis, 1971). Gardner (1972) noted that exopolymers led to reductions in the volume and size of fluid-conducting pores because of their highly viscous gel structure. We also conducted an extensive series of percolation column experiments, and the results showed that the maximum production of exopolymers in the column resulted in the largest reduction of K_s of porous media (Xia et al., 2014a). The accumulation of gas bubbles can also cause bioclogging if these gas bubbles are sufficiently large, such that they become trapped in the network of pores (Battersby et al., 1985; Harremoës et al., 1980). For example, K_s reduction was found to be associated with the production of N_2 (Ronen et al., 1989; Tollner et al., 1983; Wood and Bassett, 1975) and CH_4 (De Lozada et al., 1994; Reynolds et al., 1992). Some experiments have shown that FeS precipitates (Davis, 1967; Van Beek, 1984) and other organisms, such as fungi (Okubo and Matsumoto, 1983; Ragusa et al., 1994; Ripley and Saleem, 1973; Seki et al., 1996) and protozoans (DeLeo and Baveye, 1997; Mattison et al., 2002; Okubo and Matsumoto, 1983), can be observed in clogged porous materials. However, their effects, especially in the case of protozoans, are still somewhat poorly understood. The occurrence of protozoans in clogged layers implied that they could contribute to the reduction of K_s (Calaway, 1957; Hilton and Whitehall, 1979; Okubo and Matsumoto, 1983), while other authors argued there was an inhibition effect of protozoa via bacteria predation (DeLeo and Baveye, 1997; Sinclair et al., 1993).

Exopolymers, known as extracellular polymeric substances (EPS), have a dynamic double-layer structure including tightly and loosely bound layers (Li and Yang, 2007; Poxon and Darby, 1997; Ramesh et al., 2006). A few researchers have referred to the tightly bound EPS (TB-EPS) as "capsules" and the loosely bound EPS (LB-EPS) as "slime layers" (Vandevivere and Baveye, 1992a). Flemming and Wingender (2010) further defined a "capsule" as a discrete polysaccharide (sometimes also protein) layer that is firmly attached to the surface of bacterial cells, while the "slime layer" is less compact, amorphous and shed into the more distant extracellular environment. In the wastewater treatment industry, LB-EPS are considered to have a significant influence on the characteristics of sludge and biofilms (Flemming and Wingender, 2010) because they are the primary surface for cell attachment and contribute more to biofilm flocculation, sedimentation and dewaterability than TB-EPS (Li and Yang, 2007; Ramesh et al., 2006). In terms of the bioclogging process, Vandevivere and Baveye (1992b) noted that bacterial cells were able to withstand convective transport by forming large, loosely packed slimes and adhering to pore walls by means of exopolymeric linkages. These loose slimes could be carried down-stream by the percolating liquid before being trapped at pore constrictions. This implied that the loose slimes, or LB-EPS, might serve as important agents in the reduction of the K_s of porous media. The impact of different types of bacterial exopolymers on bioclogging is worthy of investigation.

In this study, a series of percolation experiments was carried out with a flowing nutrient solution, to stimulate the growth and metabolism of bacteria. The objectives of this work were: (1) to examine the correlation between the bacterial cell amount and the K_s of porous media; (2) to investigate the relationships between different bacterial extracellular polymers (LB-EPS and TB-EPS) at different concentrations and the reduction of K_s; and (3) to determine the distributions of bacterial cells and EPS in the pore space via scanning electron microscopy (SEM) and to evaluate relative contributions of related factors to the reduction of K_s during different percolation stages.

2. Materials and methods

2.1. Experimental setup

Percolation experiments were carried out in 22-cm length, 5-cm inside diameter columns constructed from cast acrylic tubing. Both the inlet and outlet of the columns were covered by fine stainless steel meshes to prevent sand grains from creeping out. Piezometers were installed at 0, 2, 6, 12, and 18 cm from the inlet to monitor the hydraulic gradient. Before packing sand, the columns and connecting pipes were carefully sterilized by exposure to ultraviolet light. Each column was packed with sand (d_{50} = 0.539 mm) from a natural aquifer near the Dagu River (N 36.38024°, E 120.12089°) in Jiaozhou city, Shandong province of China. Before being packed, the sand was cleaned by soaking in 0.25 M HCl for 24 h to remove trace metals and then treated with 0.25 M NaOH and deionized Milli-Q water until the seepage water reached neutral pH (Yang et al., 2013b). Finally, the sand was baked at 550 °C for 2 h to remove organic matter. The particle size distribution of the sand grains is presented in Fig. 1.

2.2. Bacterial inoculum and nutrient solution

A water sample contained mixed bacterium was collected from a recharge well near the Dagu River (N 36.38024°, E 120.12089°) and used as an inoculum in this study. Using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), the predominant strains in the sample were identified to be *Methylobacterium*, *Janthinobacterium*, *Yersinia*, *Staphylococcus*, and *Acidovorax*, most of which were verified to be viscous EPS producers (Xia et al., 2014a). In order to stimulate the growth and EPS production of bacteria, a synthetic nutrient solution was introduced to the sand columns. Like in Xia et al. (2014a), the nutrient solution contained 58 mg/L glucose, 5 g/L NaCl, 1.91 mg/L NH_4Cl, 0.92 mg/L K_2HPO_4, 45 mg/L $MgSO_4{\cdot}7H_2O$, 20 mg/L $CaCl_2{\cdot}2H_2O$, and 1 mL of a trace elements solution, which contained 2 mg/L $FeSO_4{\cdot}7H_2O$, 0.4 mg/L $CuSO_4{\cdot}5H_2O$, and 2 mg/L H_3BO_3. The solution had a pH value of 7.0–7.2. Before the percolation experiments, the nutrient medium was autoclaved at 121 °C for 15 min.

2.3. Experimental design

The sand columns were slowly saturated with 8 pore volumes of sterilized Milli-Q water. Then, 5 pore volumes of bacterial inoculum were injected into the columns. Following that, the

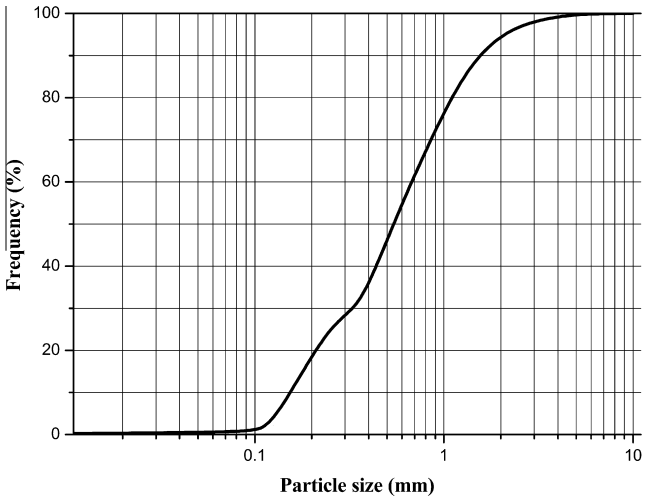

Fig. 1. The particle size distribution curve of the sand sample.