Journal of Hydrology 519 (2014) 3153–3162

Contents lists available at ScienceDirect

Journal of Hydrology

journal homepage: www.elsevier.com/locate/jhydrol

Influences of environmental factors on bacterial extracellular polymeric substances production in porous media

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ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 29 September 2014
Accepted 18 October 2014
Available online 1 November 2014
This manuscript was handled by Laurent Charlet, Editor-in-Chief, with the assistance of Christophe Tournassat, Associate Editor

Keywords:
Bioclogging
Porous media
EPS
Saturated hydraulic conductivity

SUMMARY

Bioclogging of natural porous media occurs frequently under a wide range of conditions. It may influence the performance of permeable reactive barrier and constructed wetland. It is also one of the factors that determine the effect of artificial groundwater recharge and *in situ* bioremediation process. In this study, a series of percolation column experiments were conducted to simulate bioclogging process in porous media. The predominant bacteria in porous media which induced clogging were identified to be *Methylobacterium*, *Janthinobacterium*, *Yersinia*, *Staphylococcus* and *Acidovorax*, most of which had been shown to effectively produce viscous extracellular polymeric substances (EPS). The column in which EPS production was maximized also coincided with the largest reduction in saturated hydraulic conductivity of porous media. In addition, carbon concentration was the most significant factor to affect polysaccharide, protein and EPS secretion, followed by phosphorus concentration and temperature. The coupled effect of carbon and phosphorus concentration was also very important to stimulate polysaccharide and EPS production.

1. Introduction

Microorganisms play a crucial role in the degradation of organic contaminants in subsurface environments. This biodegradation process involves microbes that utilize organic compounds as their growth substrates to keep cells growing, which leads to the removal of contaminants. Besides these metabolism activities (Cajthaml et al., 2009; Stasinakis, 2012; Yang et al., 2013a; Yang et al., 2013b), microbes also become significant for their carrier function in solute transport in subsurface environments, which directly affects the spreading and decay of contaminant plumes in the subsurface. In the presence of high nutrient loading, biomass accumulation can cause drastic reduction in saturated hydraulic conductivity of porous media due to clogged pores, known as bioclogging (Baveye et al., 1998; Vandevivere and Baveye, 1992a,b; Vandevivere et al., 1995). Bioclogging is widely found in permeable reactive barrier (Liang et al., 2000), aquifer storage and recovery (Pavelic et al., 2007), microbial enhanced oil recovery (Cai et al., 2013; Jiménez et al., 2012), drip irrigation (Puig-Bargues et al., 2005), bioremediation of organic contaminants in subsurface environments (Calderer et al., 2014), and landfill leachate collection systems (Beaven et al., 2013; Singhal and Islam, 2008). Bioclogging could trigger deleterious consequences such as the reduction of artificial recharge efficiency and increasing operating cost; therefore, its mechanisms are worthy of great value.

Bioclogging in porous media due to bacterial cells was first observed by McCalla (1951). Following his work, researchers found that the microbial exopolymers contributed more to bioclogging than cells (Mitchell and Nevo, 1964; Vandevivere and Baveye, 1992b). Exopolymers, also known as extracellular polymeric substances (EPS) (Ras et al., 2011), were slimy or gummy materials excreted by microorganisms. Vandevivere and Baveye (1992b) performed sand column experiments, with both EPS producing and non-producing bacteria strains, and identified the EPS as main contributor to bioclogging.

As EPS are considered to be one of the major factors in bioclogging process (Vandevivere and Baveye, 1992b), exploring the impacts of various environmental factors on EPS production would pave the way for bioclogging prediction and control. Numerous environmental factors have been documented to influence EPS contents. Some researchers studied the influences of aerobic and anaerobic conditions on EPS yields (Nielsen and Frølund, 1996). Miqueleto et al. (2010) discussed the effects of different carbon sources and C/N radio on EPS production in an anaerobic

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http://dx.doi.org/10.1016/j.jhydrol.2014.10.045
0022-1694/

sequencing batch biofilm reactor. Fang et al. (2002) studied the influence of Zn on the EPS production by sulfate-reducing bacteria. A similar work done by Yang et al. (2013) revealed the coupled effect between heavy metals and biofilm EPS in porous media. For most of these studies, the researchers focused on a single factor once at a time without considering the effects of the interactions among different factors. Single-factor experiments were generally time and energy consuming. In this work, we handle this issue by applying the Response Surface Methodology (RSM), which is an effective statistical technique to solve multivariate scenarios. RSM has been widely used in many fields, such as the manufacturing of biochemical and biotechnological products (Aybastıer and Demir, 2010; Chatterjee et al., 2012).

In our study, based on sand and inoculum sampled from a natural aquifer, we conducted molecular biology identification, saturated hydraulic conductivity measurement and EPS production experiments. The specific objectives of this work were to: (1) isolate and identify the dominant bacteria in porous media using polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE), and analyze their characteristics; (2) build correlation between microbial EPS yield and relative saturated hydraulic conductivities of porous media and (3) investigate the influences of key environmental factors and their combined effect on the yield of microbial polysaccharide, protein and EPS, by applying both the single-factor experiment method and RSM analysis. This work will contribute to the precise prediction and efficient control of bioclogging in porous media in the future.

2. Materials and methods

2.1. Sample collection

Sand samples were taken from a natural aquifer near Dagu river (N36.38024, E120.12089°) in Jiaozhou city, Shandong province of China. The sand samples were sieved according to the Specification of Soil Test (Ministry of Water Resources, 1999) and the average particle size were measured to be 0.539 mm. Before repacked in the columns, the sand samples were soaked in HCl solution (0.25 M) for 24 h, followed by NaOH solution (0.25 M) for 24 h. Then they were washed by Milli-Q water and incinerated at 550 °C for 2 h to remove any organic matter on the sand surface.

2.2. Inoculum

A mixture of microbial consortium and water collected from a recharge well near Dagu river was used as inoculum. After the water sample was taken from the site, it was inoculated and fully aerated for 2 h. Then, the sample was settled for 30 min to form a uniform suspension before introduced into the sand columns.

2.3. Synthetic nutrient solution

After the water sample was taken from the field, the aqueous profile of the sampled groundwater was measured in the lab. This included concentrations of K^+, Na^+, Ca^{2+}, Mg^{2+}, NH_4^+, Cl^- and SO_4^{2-}. In order to stimulate bacteria growth and EPS production, synthetic nutrient solution according to the water sample profile was introduced, which contained glucose (variable), 5 g/L NaCl, 1.91 mg/L NH_4Cl, K_2HPO_4 (variable), 45 mg/L $MgSO_4{\cdot}7H_2O$, 20 mg/L $CaCl_2{\cdot}2H_2O$, and 1 mL of a trace elements solution, containing 2 mg/L $FeSO_4{\cdot}7H_2O$, 0.4 mg/L $CuSO_4{\cdot}5H_2O$, 0.04 mg/L $MnSO_4{\cdot}6H_2O$, 0.04 mg/L $Na_2MoO_4{\cdot}2H_2O$, 2 mg/L H_3BO_3 and pH 7.0–7.2. Before column experiments, the nutrient medium was autoclaved at 121 °C for 15 min.

2.4. Operation of the column

The sand column experiments were conducted in columns (22 cm in length and with an inside diameter of 5 cm) constructed from cast acrylic tubing. The inlet and outlet of the columns were covered by 80-mesh stainless steel screens. In addition, a 2-cm-thick glass bead (diameter 1 cm) layer was placed on the top of the inlet screen to make an even influent passing through the porous media surface. Piezometers were installed at the column inlet and outlet to monitor the hydraulic head difference gradient. Before packing, the columns and connections were sterilized by ultraviolet carefully. The columns were packed with wet sand in an increment of 2 cm to avoid air entrapment and homogenize every layer. The packed columns had an average porosity of 0.385.

The columns were saturated slowly with 12 pore volumes of sterilized Milli-Q water and then 8 pore volumes of the suspended bacteria prepared in Section 2.2. Peristaltic pumps (BT100-2J, Longerpump, Baoding, China) were used to inject the sterilized nutrient medium into the columns in an upward-flow mode. The pumping rate at inlet was kept at 5 mL/min. After 9 days' incubation, the columns were dismantled for bacterial strains identification and EPS quantification.

2.5. Saturated hydraulic conductivity evaluation

A constant flow rate test was used to measure the saturated hydraulic conductivity K_s [L/T], according to Darcy's law:

$$Q = A \cdot K_S \frac{\Delta H}{L} \quad (1)$$

where Q is the flow rate [L^3/T], A is the cross sectional area of the column (19.625 cm^2 in this work), ΔH is the hydraulic head difference between the column inlet and outlet, and L is the distance between the two points where ΔH is measured (18 cm in this work).

The calculated K_s value was normalized by K_{s0}, the initial saturated hydraulic conductivity of the sand column. The ratio K_s/K_{s0} was used to describe the degree of clogging in different columns.

2.6. Microbial community analysis

2.6.1. Genomic DNA extraction

After a 9-days' incubation, the column was dismantled and sand samples of 2 g each were transferred into a sterile plastic tube containing 10 mL aseptic phosphate buffered (PB, 10 mM, pH 7.4) solution and 1 g of sterile glass beads. The genomic DNA was extracted using a bead beating protocol by means of the commercial Power Soil™ isolation kit (MO-BIO Laboratories, Inc., Carlsbad, CA, USA), following manufacturer's instructions.

2.6.2. PCR-DGGE

The extracted genomic DNA was used as the template for PCR. Amplifications were performed using the universal primers BA101F (5′-TGGCGGACGGGTGAGTAA-3′, Invitrogen, Shanghai) and BA534R (5′-ATTACCGCGGCTGCTGG-3′, Invitrogen, Shanghai). A 40 bp GC-clamp was linked to the forward primer BA534R. PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystems, USA) and the products were analyzed by DGGE using a Bio-Rad™ universal mutation detection (Bio-Rad Laboratories, USA), according to the procedure described by EI Sheikha (2010). The gel was electrophoresed at 150 V for 9 h. After that, the gel was stained by silver staining and gel images were photographed.

2.6.3. Sequencing of DGGE bands and homology search

DNA sequencing was performed using a DNA sequenator (ABI377 DNA Analyzer, Applied biosystems, USA). The obtained