Water Research 89 (2016) 321–329

Contents lists available at ScienceDirect

Water Research

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

Monitoring sulfide-oxidizing biofilm activity on cement surfaces using non-invasive self-referencing microsensors

Liqiu Cheng [a, *], Mitch W. House [b], W. Jason Weiss [b, c], M. Katherine Banks [a]

[a] Zachry Department of Civil Engineering, Texas A&M University, 3136 TAMU, College Station, TX 77843-3136, USA
[b] Lyles School of Civil Engineering, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907-2051, USA
[c] Bindley Bioscience Center, Physiological Sensing Facility, Discovery Park, Purdue University, 1203 W. State Street, West Lafayette, IN 47907-2057, USA

ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form
15 August 2015
Accepted 28 November 2015
Available online 14 December 2015

Keywords:
Self-referring microsensors
Microbially influenced corrosion
Biofilm
Sulfide-oxidizing bacteria

ABSTRACT

Microbially influenced corrosion (MIC) in concrete results in significant cost for infrastructure maintenance. Prior studies have employed molecular techniques to identify microbial community species in corroded concrete, but failed to explore bacterial activity and functionality during deterioration. In this study, biofilms of different sulfur-oxidizing bacteria compositions were developed on the surface of cement paste samples to simulate the natural ecological succession of microbial communities during MIC processes. Noninvasive, self-referencing (SR) microsensors were used to quantify real time changes of oxygen, hydrogen ion and calcium ion flux for the biofilm to provide more information about bacterial behavior during deterioration. Results showed higher transport rates in oxygen consumption, and hydrogen ion at 4 weeks than 2 weeks, indicating increased bacterial activity over time. Samples with five species biofilm had the highest hydrogen ion and calcium ion transport rates, confirming attribution of acidophilic sulfur-oxidizing microorganisms (ASOM). Differences in transport rates between three species samples and two species samples confirmed the diversity between *Thiomonas intermedia* and *Starkeya novella*. The limitations of SR sensors in corrosion application could be improved in future studies when combined with molecular techniques to identify the roles of major bacterial species in the deterioration process.

1. Introduction

Concrete has been widely used as a construction material for centuries due to its advantages in strength, durability, plasticity and low cost. However, application of concrete is facing enhanced deterioration caused by carbonation (Lin et al., 2013), chloride corrosion (Abd El Haleem et al., 2013; Hartt, 2013) and biological reactions which lead to high financial costs for operation and maintenance (Beider et al., 2002). Microbially influenced corrosion (MIC) usually occurs in harsh environments, such as water distribution systems, sewage structures and wastewater treatment plants, and significantly reduces the service time of the infrastructure and endangers water quality (Coleman and Gaudet, 1993; Marleni et al., 2012; O'Connell et al., 2012; Zhang et al., 2008). Previous studies (Herisson et al., 2013; Islander et al., 1991; Parker, 1951) established MIC mechanism in sewer systems involving a sulfur cycle beginning with anaerobic production of hydrogen sulfide (H_2S) by sulfate-reducing microorganisms (SRM) in submerged part of sewer. Released H_2S in the pipe atmosphere (Jiang et al., 2014; Joseph et al., 2012; Lahav et al., 2006; Yongsiri et al., 2003) is absorbed by the moist concrete wall (original pH 12–13) and then converted to elemental sulfur or partially oxidized sulfur species and eventually to sulfuric acid which corrodes the concrete, with the assistance of sulfide-oxidizing microorganisms (SOM).

To better understand the corrosion processes and the interaction between concrete structure and attached biofilm, various studies have been conducted to identify the microbial species involved in concrete degradation. *Acidihiobacillus* spp. (Milde et al., 1983; Parker, 1951) were identified using conventional culture-dependent methods in corroded sewer pipes. With the development of molecular techniques like polymerase chain reaction (PCR) and cloning of 16S rRNA gene fragments, microbial communities in the corroded area were characterized and more SOM species were identified such as *Thiothrix*, *Thiomicrospira*, and *Beggiatoa*, along with other coexisting heterotrophic bacteria as α-, β- and γ-*Proteobacteria*, and *Acidobacteria* (Bielefeldt et al., 2010; Little et al.,

* Corresponding author.
E-mail address: liqiucheng@tamu.edu (L. Cheng).

http://dx.doi.org/10.1016/j.watres.2015.11.066
0043-1354/

2000; Nica et al., 2000; Vincke et al., 2001). In recent years universal small-subunit (SSU) rRNA gene amplicon pyrosequencing was used to character microbial community is concrete sewer system (Cayford et al., 2012; Gomez-Alvarez et al., 2012; Ling et al., 2014, 2015; Santo Domingo et al., 2011). While some field studies still confirmed *Acidithiobacillus* spp. dominating the microbial community (>50%) (Ling et al., 2014), other studies showed extremely low abundance (<3%) of *Acidithiobacillus* spp., suggesting that they may not be as important as people used to believe (Cayford et al., 2012). Succession of microbial community was also explored as results showed high variation and abundances of heterotrophic types under neutral pH while low diversity biofilms dominated by acidophilic species were discovered in severe corrosion sites (Islander et al., 1991; Okabe et al., 2007). Although molecular techniques provided detailed information about microbial community composition, the link between the species and their real-time function in the corrosion process is still not well understood. Furthermore, sensitivity and detection range of these techniques is challenged by the environment as high calcium and heavy metals inhibit nucleic acid amplification (Vincke et al., 2001; Wilson, 1997).

Non-invasive self-referencing (SR) microsensors have been successfully used for biochemical and agricultural studies (McLamore et al., 2010b; Porterfield and Smith, 2000) and also in the environmental field, focusing on characterization and quantification of real time biofilm behavior (McLamore et al., 2011; 2010c). SR sensors are capable of detecting change of various analytes, including inorganic ions (H^+, Ca^{2+}, K^+, NH_4^+, Cl^-) (McLamore et al., 2009; 2011; 2010c; Sun et al., 2012), organic compounds (indole-3-acetic acid and glucose) (McLamore et al., 2010a; Shi et al., 2011) and metabolic oxygen (McLamore and Porterfield, 2011; McLamore et al., 2010c) in the biofilm boundary layer. The background noise is eliminated by sensor oscillation, hence the descriptor of "self-referencing". With the advantages of being non-invasive and non-destructive, SR sensors are powerful tools in detecting real time changes in electrochemical signals related to biological activity, while maintaining biofilm functionality. In this study, biofilms of five well-documented and closely related *Acidithiobacillus* species were developed to explore the application of SR sensors in MIC detection. With real time measurements of key elements, including oxygen, hydrogen ion (H^+) and calcium ion (Ca^{2+}) in the biofilm, information about bacterial metabolism and their impact on cement deterioration was collected to better understand the corrosion process.

2. Material and methods

2.1. Cement paste samples

The samples were prepared at water to cementitious materials ratios (w/c) of 0.42 with ordinary Type I Portland Cement (OPC, BuzziUnicem USA), containing (weight %) 64.4% of CaO, 20.9% of SiO_2, 5.2% Al_2O_3, and 0.3% Fe_2O_3. The size of the sample was 1.91 cm (3/4 inch) in diameter and 0.32 cm (1/8 inch) in thickness. After setting for 24 h, samples were sealed in plastic bags and placed at 25 °C in 100% humidity chamber for 60 days. Samples were then glued with epoxy onto microscope glass slides and used for surface biofilm development.

2.2. Biofilm development

Acidithiobacillus thiooxidans (ATCC 19377), *Halothiobacillus neapolitanus* (ATCC 23638), *Thiobacillus thioparus* (ATCC 23646), *Thiomonas intermedia* (ATCC 15466) and *Starkeya novella* (ATCC 8093, formerly *Thiobacillus novellus*) were used in the experiment and were obtained from American Type Culture Collection (ATCC, Manassas, VA). Each of these five species was grown individually in specific medium following ATCC instructions. Nutrient solution was developed from ATCC Medium 290 S6 for *Thiobacilli* and ATCC Medium 152 for *Thiobacillus* containing (in g/L): 1.2 Na_2HPO_4, 1.8 KH_2PO_4, 0.02 $FeCl_3$, 0.02 $MnSO_4$, 0.5 $MgCl_2$, 1.0 NH_4Cl, 1.0 Yeast Extract, and 10.0 $Na_2S_2O_3 \cdot 7H_2O$. Each species was harvested, concentrated and transferred into one or more up-flow aerated bioreactors (volume of 3 L) following species composition listed in Table 1. Cell numbers were counted to insure that in every bioreactor, each species added had a similar number of cells around 1×10^8. The bioreactors were placed in a biological controlled room at 30 °C. Cement paste samples affixed to glass slides were suspended in growth media in bioreactors when cell numbers in the bioreactors reached steady state. Biofilms after 2 weeks and 4 weeks of growth on the cement surface were considered as immature and mature biofilm respectively (Huang et al., 2013; McLamore et al., 2009) and were used in the following analysis. Cement samples placed in nutrient solution without bacterial inoculation were used as controls, and they were stored in a biosafety cabinet with constant air flow and UV disinfection 3–5 times a day to make sure the solution was not contaminated.

2.3. Sensor structure

Fiber optic oxygen microsensors (optrodes), with 5–7 μm tip diameter, were constructed following published procedures (Chatni et al., 2009; Chatni and Porterfield, 2009; McLamore and Porterfield, 2011) with platinum tetrakispentafluorophenyl porphyrin (PtPFPP) as fluorophore. For each chosen fluorophore, there is a constant relationship between oxygen concentration and the measuring phase angle. Calibration of the optrodes were conducted at both air purged O_2 saturation (21%) and 0% O_2 saturation (nitrogen purged) solutions. Measured phase angle was transduced to a current using a lock-in amplifier and no reference electrodes were needed. Micro ion-selective electrodes (μISE) were used to measure proton and calcium transport of the biofilm based on the recognition-transduction mechanism. Both types of electrodes used the borosilicate glass capillaries with 2–5 μm tip diameter and were constructed following standard methods (McLamore et al., 2009; 2010c; Porterfield et al., 2009). The specific liquid ion exchange membranes (LIX) (hydrogen ionophore I and calcium Ionophore IV, Sigma–Aldrich, St. Louis, MO) filled in the capillary's tips were used to recognize the target ions (H^+ and Ca^{2+}). The real-time changes of the voltage signals between the μISE and the Ag/AgCl reference electrode were recorded.

2.4. Experimental section

The diagram of the self-referencing sensor system for biofilm flux measurement is presented in Fig. 1. Three linear stepper motors placed at the X, Y, and Z-axis control sensor movement. Each flux reading was collected after measurements were completed at both initial position (ΔX from sample surface) and sample position (at sample surface). The values of data acquisition frequency and ΔX were set to maintain the concentration gradient in the boundary layer during sensor movement, and to eliminate significant sensor drift and background noise (Kuhtreiber and Jaffe, 1990; McLamore et al., 2009). Before measurements, samples were taken out of the bioreactors and placed in specifically designed sample holders filled with sterile nutrient solution to keep samples in the vertical plane which is perpendicular to the movement direction of the microsensor tip for 60 min to allow for stabilization. Final values of flux were calculated as average of readings recorded at 3–5 different locations along sample surface.