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ORIGINAL PAPER

The inhibitory effect of antimicrobial zeolite on the biofilm of *Acidithiobacillus thiooxidans*

Tesfaalem Haile · George Nakhla

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Abstract The inhibitory effect of antimicrobial zeolite coated concrete specimens (Z2) against *Acidithiobacillus thiooxidans* was studied by measuring biomass dry cell weight (DCW), biological sulphate generation, and oxygen uptake rates (OURs). Uncoated (UC), and blank zeolite coated without antimicrobial agent (ZC) concrete specimens were used as controls. The study was undertaken by exposing inoculated basal nutrient medium (BNM) to the various specimens. The coating material was prepared by mixing zeolite, epoxy and cure with ratios, by weight of 2:2:1. Concrete specimens were characterized before and after exposure to inoculated or sterile BNM by field emission-scanning electron microscopy (FE-SEM). Gypsum, which was absent in the other test concrete specimens, was detected in uncoated specimens exposed to the bacterium. In UC and ZC, the growth of the bacteria increased throughout the duration of the experiment. However, significant biomass inhibition was observed in experiments where Z2 was used. The overall biomass growth rate in suspension before the specimens were placed ranged from 3.18 to 3.5 mg DCW day^{-1}. After the bacterium was exposed to UC and ZC, growth continued with a corresponding value of 4 ± 0.4 and 5.5 ± 0.6 mg DCW day^{-1}, respectively. No biomass growth was observed upon exposure of the bacterium to Z2. Similarly, while biological sulphur oxidation rates in UC and ZC were 88 ± 13 and 238 ± 25 mg SO_4^{2-} day^{-1}, respectively, no sulphate production was observed in experiments where Z2 concrete specimens were used. Peak OURs for UC and ZC ranged from 2.6 to 5.2 mg l^{-1} h^{-1}, and there was no oxygen uptake in those experiments where Z2 was used. The present study revealed that the antimicrobial zeolite inhibits the growth of both planktonic as well as biofilm populations of *Acidithiobacillus thiooxidans*.

Keywords Biofilm · Antibacterial agents · Zeolites · Scanning electron microscopy · Concrete · Corrosion inhibition

T. Haile (✉)
Department of Civil and Environmental Engineering, University of Western Ontario, 1151 Richmond Street, London, ON N6A 5B8, Canada
e-mail: thaile@uwo.ca

G. Nakhla
Department of Chemical and Biochemical Engineering, University of Western Ontario, 1151 Richmond Street, London, ON N6A 5B8, Canada
e-mail: gnakhla@eng.uwo.ca

Introduction

Bacterial-induced corrosion (BIC) of sewer concrete pipe, wastewater collection systems, and treatment plants has been reported in many places of the world. According to the report of US Federal Highway Administration, the industrial cost of corrosion in the

US is about $138 billion/year, 36 billion of which is attributed to corrosion in drinking water pipe lines and sewer systems (http://events.nace.org/publicaffairs/images_cocorr/ccsupp.pdf). In Los Angeles County, ~10% of the sewer pipes are subject to significant corrosion due to biological sulphuric acid attack, and the costs for the rehabilitation of these pipelines are roughly estimated at $600 million (Sydney et al. 1996). In Germany, the restoration of the overall damaged sewer systems is estimated to cost about $150 billion per year (Kaempfer and Berndt 1998). In Flanders (Belgium), BIC of sewers is approximated at $8 million per year, representing about 10% of total cost for wastewater collection and treatment (Vincke et al. 2002).

BIC in sewer systems takes place in a sequential fashion under anaerobic and aerobic conditions. Sulphate Reducing Bacteria (SRB) from the genera *Desulfovibrio* are the primary culprits for initiation of corrosion under anaerobic conditions. SRB that exist in the slime layer in the submerged sections of the concrete sewers convert naturally occurring sulphate in the wastewater to hydrogen sulphide (H_2S) (Parande et al. 2006). H_2S is a weak acid which dissolves in wastewater under neutral or alkaline conditions. However, under turbulent and/or acidic conditions, H_2S is emitted to the sewer atmosphere, where it reacts with oxygen above the water line at the concrete surface to form elemental sulphur (Sand 1997). Elemental sulphur is a substrate which many *Thiobacilli* species metabolize to sulphuric acid under aerobic conditions (Atlas and Bartha 1998; Vincke et al. 2002). *Thiobacilli* species are acidophilic and grow at pH as low as 0.5 (Konishi et al. 1995).

BIC is initiated at the concrete surfaces exposed to the sewer atmosphere upon oxidization of hydrogen sulphide and sulphur by autotrophic *Thiobacillus* sp. growing on the moist surface to sulphuric acid (Gadekar et al. 2006), which reacts with the free lime ($Ca(OH)_2$) of concrete to form gypsum ($CaSO_4{\cdot}2H_2O$) (Mori et al. 1992). The formation of ettringite ($3CaO{\cdot}Al_2O_3{\cdot}3CaSO_4{\cdot}32H_2O$) during the reaction of sulphate with concrete causes another facet of the problem (Redner et al. 1994). Ettringite is expansive, and causes internal cracking and pitting, thus providing a larger surface area for the chemical reaction to occur and additional sites for acid penetration into the concrete (Zhang et al. 2008). The formation of the aforementioned calcium-containing corrosion byproducts weakens the structural integrity of concrete (Yamanaka et al. 2002), leading to a decrease in the load-bearing capacity and eventual collapse of the sewer system.

In sewers and wastewater collection systems, mitigation of BIC has mainly been by inhibition of H_2S generation or elimination of H_2S formed, which includes specific prevention of SRB activity by elevating the pH of the wastewater with the addition of NaOH, or $Ca(OH)_2$, application of biocides, utilization of alternative electron acceptors such as O_2, NO_3^- or NO_2^- to increase the redox potential of the system, chemical oxidation of H_2S generated using H_2O_2, Cl_2, NaClO, $KMnO_4$, CaO, MgO and chemical precipitation by Fe^{2+} (Garcia De Lomas et al. 2005; Zhang et al. 2008).

To reduce the high cost of chemical addition, the use of acid-resistant high performance coating materials, such as fibre glass reinforced linings, special mortar linings and brick or ceramic linings, and polyurethane, acrylic, and epoxy resin coatings has been adopted as alternative for MIC protection strategies (Saricimen et al. 2003; Beeldens et al. 2001; Almusallam et al. 2001; Liu and Vipulanandan 2003; De Belie et al. 2004). The aforementioned protective materials, however, do not stop the proliferation of corrosive microorganisms, such as *Acidithiobacillus thiooxidans* and *Desulfovibrio desulfricans*, but rather hinder the penetration of corrosive chemicals into concrete. More recently, the use of metals as antimicrobial agents to protect concrete pipes from bacterially induced corrosion has been investigated. Hewayde et al. (2007) studied coating of concrete pipes with copper oxide and silver oxide to mitigate corrosion by inhibiting *D. desulfricans.*

In our previous studies we have demonstrated that the antimicrobial zeolite coating inhibits the growth of *A. thiooxidans* in suspension (Haile and Nakhla 2008). Furthermore, we showed that the leaching of toxic metals from the zeolite coating does not adversely impact biological wastewater treatment (Haile and Nakhla 2008).

In this study we present the resistance of zeolite coated concrete specimens to biogenic sulphate attack, and the antibacterial characteristics of silver-loaded zeolite coating to planktonic as well as biofilm population of *A. thiooxidans.* Evidence of scanning electron microscopy (SEM) micrographs, bacterial

growth kinetics, and leaching pattern of metals from the concrete specimens and coating films are presented.

Experimental procedures

Preparation of concrete specimens

Unless specified, all chemicals were reagent grade and obtained from VWR Scientific (VWR Canada, Mississauga, ON, Canada). Ready mix concrete was obtained from Lafarge Canada which is known as Agilia Mix in the market (Lafarge Canada Inc., London, ON, Canada). Mixing, casting, and curing of the concrete cubes were performed according to standard specifications (ASTM C192-90, C172-90 2000). Cubic concrete specimens measuring $35 \times 35 \times 35$ mm^3 were cut using a masonry diamond saw from $75 \times 75 \times 280$ mm^3 concretes (Husqvarna, Target Guardmatic TS 510 Masonry saw, Las Vegas, Nevada, USA).

Preparation of zeolite coatings

The zeolite coating was applied at zeolite to epoxy to cure ratio (by weight) of 2:2:1 (Haile and Nakhla 2008). The epoxy resin and cure used were the ones commonly utilized by the industry for the rehabilitation of pipes and manholes (Ameron International, High performance Amercoat 90HS epoxy resin and Amercoat 90HS cure, Alpharetta, GA, USA). The zeolite powder was purchased from AgION Technologies Inc. (Boston, Massachusetts, USA). The amount of silver-loaded in the treated zeolite was 5% by weight with 14% zinc as a stabilizer. To increase the workability of the coating mixture, epoxy was dissolved in a thinner (Ameron International, Amercoat 65/T5, Alpharetta, GA, USA) before mixing with unloaded or silver-loaded zeolites. Coating was performed by immersing the concrete specimens in $50 \times 50 \times 50$ mm^3 stainless steel molds containing the coating mixtures. Uncoated (UC), blank zeolite (ZC, without silver) coated, and antimicrobial silver-loaded zeolite (Z2) coated concrete specimens were tested. The epoxy resin was used as wetting agent as well as carrier of the zeolite powder. A detail of the zeolite coating preparation is reported by Haile and Nakhla (2008).

Microorganism and culture condition

Acidithiobacillus thiooxidans strain ATCC 19703 was purchased from the American Type Culture Collection. The bacterium was grown in a basal nutrient medium (BNM) containing the following ingredients (in g l^{-1}): $(NH_4)_2SO_4$ (0.2), $MgSO_4 \cdot 7H_2O$ (0.5), $CaCl_2$ (0.25), KH_2PO_4 (3.0), $FeSO_4 \cdot 7H_2O$ (0.005), and precipitated sulphur powder (10.0) (http://www.atcc.org/Attachments/2509.pdf).

Biofilm inhibition experiments

The experimental setup of the biofilm inhibition experiment is illustrated in Fig. 1. To study the inhibitory impact of the antimicrobial zeolite coated concrete specimens on *A. thiooxidans* biofilm, the bacterium was first cultivated in separate batch experiments containing 500 ml BNM which were agitated using a Standard Shaker (New Brunswick Scientific, Model Classic C10, New Brunswick, New Jersey, USA) at 100 rpm at room temperature. On day 10 (during the exponential growth phase), the UC, ZC, and Z2 specimens were placed in the beakers containing the *A. thiooxidans* culture. UC and ZC were used as control specimens, while Z2 was the test concrete specimen. To ensure reproducibility, experiments were performed in duplicates from two different cultures. Sterile BNM containing UC and ZC concrete specimens were run parallel to the inoculated BNM

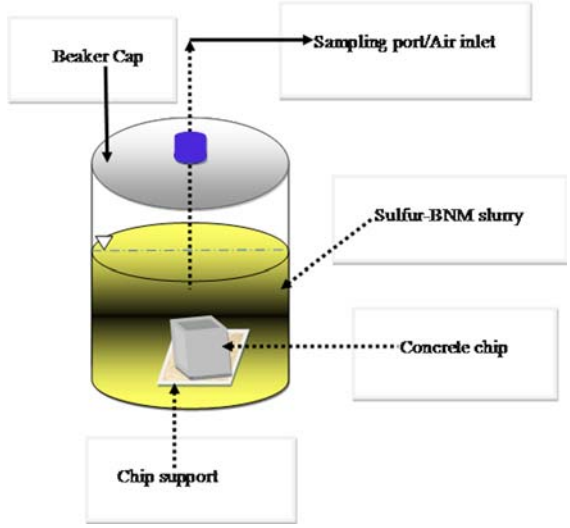

Fig. 1 Schematic diagram of the biofilm inhibition experiment

containing the different test concrete specimens to standardize the analytical results as well as test for possible cross contamination. Biofilm dry cell weight (DCW) analysis and scanning electron microscope evaluation was performed on daily basis. Likewise, DCW, pH, sulphate, and metals were monitored routinely in suspension (sulphur-BNM slurry) to determine planktonic biomass before, as well as, after the placement of specimens. A total of 30 concrete specimens were tested, 10 for each group, i.e. uncoated, blank zeolite coated and silver-loaded zeolite coated concrete specimens. Biofilm were detached from concrete specimens using a water bath ultrasonicator (ETL Testing laboratories Inc., Model 75HT AQUASONICTH, Cortland, New York, USA) by placing them in separate beakers filled with 100 ml sterile BNM (McNamara et al. 2006). The concrete specimens were sonicated for 5 min (McNamara et al. 2006).

Respiration test

To investigate the viability of *A. thiooxidans* cells exposed to the different specimens, it was imperative to study the respiratory activity of the bacterium. A picture of the respirometer (Challenger Environmental Systems, Inc, Challenge AER-200, Spingdale, Arkansas, USA) used for measurement of cumulative oxygen uptake (COU) and oxygen uptake rate (OUR) is shown in Fig. 2. After the biofilm inhibition experiment was terminated, biofilm from each concrete specimen was detached separately. The respiration test was conducted by inoculating BNM with *A. thiooxidans* biomass obtained from the detached biofilm and suspension of each concrete specimen. Summary of abbreviations and codes are described in Table 1. The experimental design of the respiration test is illustrated in Table 2. A total of eight bottles were used to test the respiratory activity of the bacterium exposed to the different concrete specimens: suspended biomass obtained from the inoculated BNM exposed to uncoated concrete specimens (UC-F), detached biomass from the inoculated BNM exposed to uncoated concrete specimens (UC-A), suspended biomass from the inoculated BNM exposed to blank zeolite coated concrete specimens (ZC-F), detached biomass from inoculated BNM exposed to blank zeolite coated concrete specimens (ZC-A),

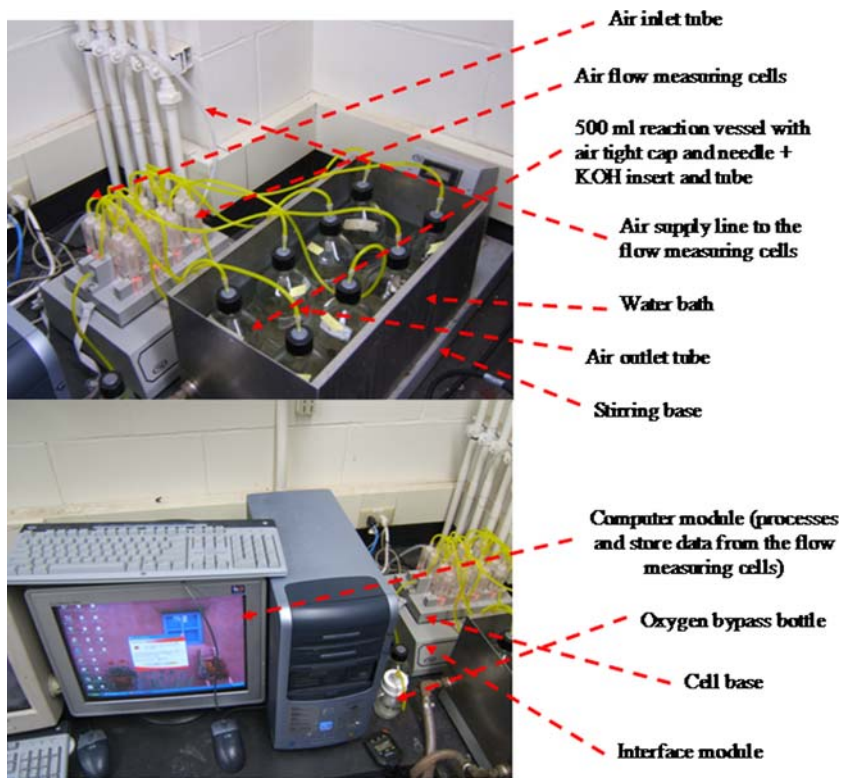

Fig. 2 Experimental set up of the aerobic respiration test

Table 1 Summary of abbreviations and codes

UC-F, Reaction vessels inoculated with planktonic biomass obtained from uncoated concrete specimens
UC-A, Reaction vessel inoculated with detached biomass obtained from uncoated concrete specimens
ZC-F, Reaction vessel inoculated with planktonic biomass obtained from blank zeolite (ZC) coated concrete specimens
ZC-A, Reaction vessel inoculated with detached biomass obtained from blank zeolite (ZC) coated concrete specimens
Z2-F, Reaction vessel inoculated with planktonic biomass obtained from silver-loaded zeolite (Z2) coated concrete specimens
Z2-A, Reaction vessel inoculated with detached biomass obtained from silver-loaded zeolite (Z2) coated concrete specimens
UC-INCL, Uncoated specimens exposed to *A. thiooxidans* inoculated BNM
ZC-INCL, Blank zeolite coated specimens exposed to *A. thiooxidans* inoculated BNM
Z2-INCL, Silver-loaded zeolite coated specimens exposed to *A. thiooxidans* inoculated BNM
UC-STRL, Uncoated specimens exposed to *A. thiooxidans* sterile BNM
ZC-STRL, Blank zeolite coated specimens exposed to *A. thiooxidans* sterile BNM

Table 2 The experimental design of *A. thiooxidans* respiration test

Cell number	UC-F 1	UC-A 2	ZC-F 3	ZC-A 4	Z2-F 5	Z2-A 6	UC-F 7	ZC-F 8
BNM (ml)	450	450	450	450	450	450	450	450
Non-sterilized inoculum (ml)	50	50	50	50	50	50	–	–
Sterilized inoculum (ml)	–	–	–	–	–	–	50	50

suspended biomass from inoculated BNM exposed to antimicrobial zeolite coated concrete specimens (Z2-F), detached biomass obtained from inoculated BNM exposed to antimicrobial zeolite coated concrete specimens (Z2-A). Two additional bottles each were inoculated with sterile BNM exposed to uncoated (UC) and blank zeolite coated (ZC), to account for non-biological oxygen demand. The oxygen uptake of the first 6 test experiments was standardized, taking into account the non-biological oxygen demand.

In each growth inhibition experiments uncoated and blank zeolite coated concrete specimens were exposed to sterile BNM, to investigate the presence of indigenous *Acidithiobacilli* species and abiotic corrosion.

Concrete specimens characterization

Concrete specimens were characterized by a Field Emission-Scanning Electron Microscope, FE-SEM, (Hitachi High-Technologies Canada Inc., S-4500, Toronto, ON, Canada) to analyze concrete textures before and after exposure.

Analytical procedures

Biomass (dry cell weight, DCW, mg) was measured as reported elsewhere (Haile and Nakhla 2008). Analysis of soluble metals, i.e. Ca, Si, Ag, Zn, and sulphate, were carried out after filtering the BNM through a 0.45 μm membrane filter (Pall Corporation, Michigan, USA). Metal analysis was conducted using inductively coupled plasma (ICP) (Varian Inc., Vista-Pro Axial, Palo Alto, California, USA). The detection limit of ICP was 0.001 mg l^{-1} for all the metals analysed. Sulphate concentration was determined by Ion Chromatograph, AS9 column (Dionex Corporation, Model 640, Sunnyvale, California, USA), using Na_2CO_3 as an eluent and at flow rate of 1 ml/min.

Results and discussion

Concrete characterization

The thickness of the zeolite coating film on the concrete was measured at nine different locations in each specimens and the average thickness of the

zeolite coating was found to be 200 ± 20 μm, emphasizing the coating uniformity.

Figure 3 illustrates FE-SEM micrographs of all the tested concrete specimens before exposure to BNM, after immersion in a sterile BNM or *A. thiooxidans* inoculated BNM. Uncoated concrete specimens exposed to sterile BNM (Fig. 3b) suffered surface deterioration due to dissolution of concrete paste as demonstrated by the increase in pH and concentration of leached calcium and silicon of the BNM containing the aforementioned concrete specimens. The susceptibility of UC specimens to bacterial induced corrosion was confirmed by the formation of corrosion crystals, mainly gypsum, on the concrete specimens exposed to *A. thiooxidans* inoculated BNM (Fig. 3c) which were absent in uncoated concrete specimens

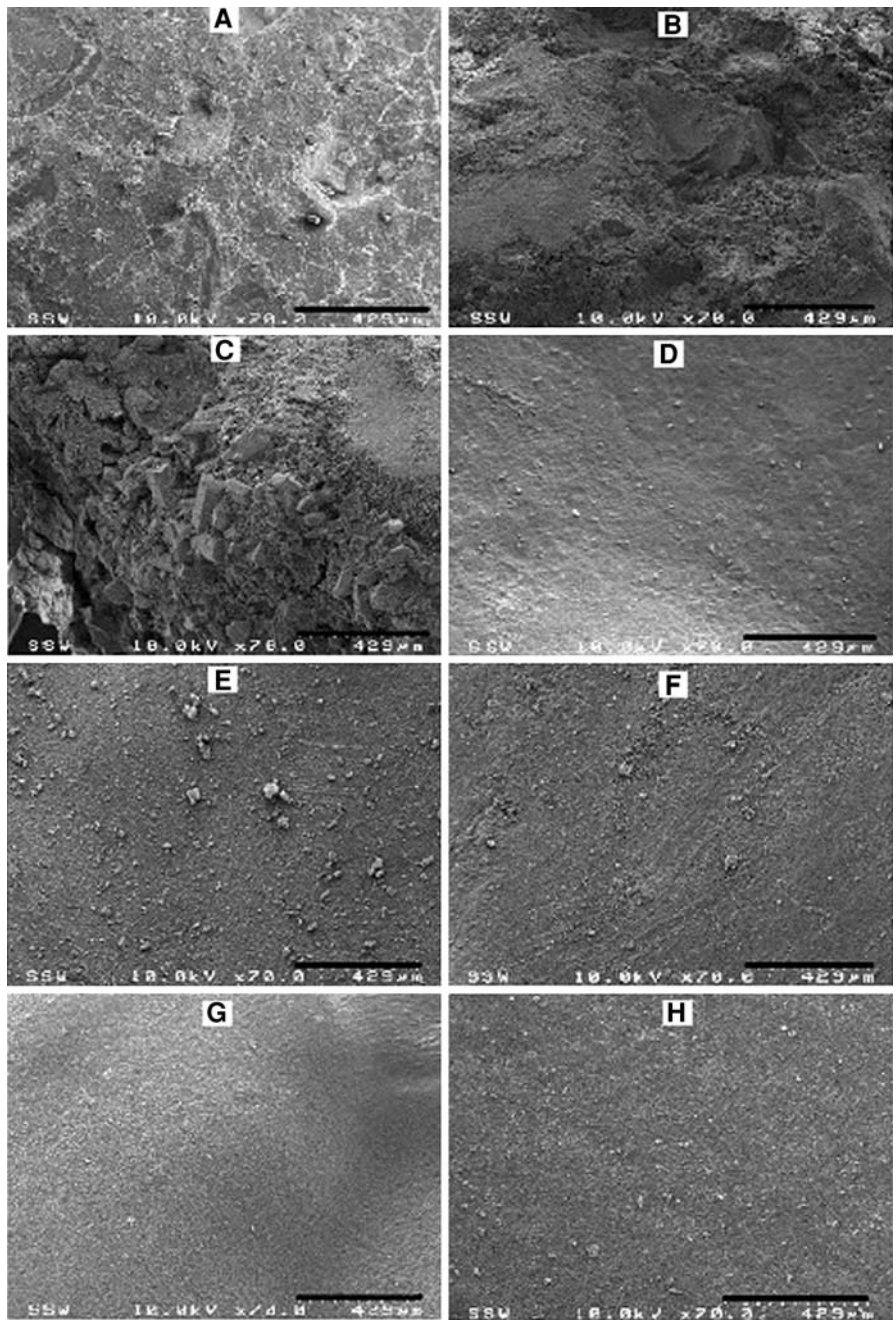

Fig. 3 FE-SEM Image of concrete specimens before and after exposure to *A. thiooxidans* inoculated or sterile BNM: **a** uncoated specimen before exposure, **b** uncoated specimen after exposure to sterile medium, **c** uncoated specimen after exposure to inoculated medium, **d** ZC before exposure to inoculated media, **e** ZC after exposure to sterile medium, **f** ZC after exposure to inoculated medium, **g** Z2 before exposure, **h** Z2 after exposure to inoculated medium. Scale bars are 429 μm

not exposed to BNM (Fig. 3a) and exposed to the sterile BNM (Fig. 3b). The absence of corrosion byproducts in ZC and Z2 coupled with the lack of significant surface deterioration demonstrated the resistance of the zeolite coating to bacterial induced corrosion, as was also reported by Haile and Nakhla (2008). No discernible differences were encountered between concrete specimens coated with zeolite, but not immersed in BNM (Fig. 3d) and those immersed in sterile BNM (Fig. 3e, g). However, there was slight surface modification on the blank zoolite coated concrete specimens immersed in *A. thiooxidans* inoculated BNM (Fig. 3f). The significance of anti-microbial zeolite coating in protecting concrete structures from bacterial induced corrosion was justified by the absence of any surface modification on Z2 concrete specimens exposed to *A. thiooxidans* inoculated BNM (Fig. 3h).

Biomass growth kinetics

DCW before and after specimens were placed in test beakers is shown in Fig. 4 while the mass of biofilm detached from specimens is illustrated in Fig. 5a and b. Bacterial growth kinetics are presented after subtraction of the initial biomass which varied from 40 to 43 mg DCW from the measured biomass. In UC and ZC, the growth of the suspended bacteria increased throughout the duration of the experiment after a 2-day lag phase (Fig. 4). There was no growth in the sterile BNM samples before and after the placement of UC and ZC specimens. Prior to the addition of the Z2 specimens, bacterial growth rates were comparable to both UC and ZC were observed. However, in Z2, a significant reduction in biomass was observed upon specimen placement. The overall biomass generation rate in suspension before the specimens were placed were 3.2 ± 0.4, 3.4 ± 0.7 and 3.5 ± 0.3 mg DCW d^{-1} for UC, ZC and Z2 with pH values ranging from 2 to 2.3 (Fig. 6). Upon specimens placement, growth in the UC and ZC continued with corresponding overall growth rates of 4 ± 0.4 and 5.5 ± 0.6 mg DCW d^{-1}, respectively. In Z2 growth ceased and declines in planktonic DCW were observed after the specimens were placed (Fig. 4). As demonstrated in Fig. 5b, the total DCW in Z2 did not change, thus confirming that the decrease in planktonic DCW was due to attachment of biomass on the surface of the Z2. The overall rates of biofilm formation on specimens surfaces, calculated from the slopes of Fig. 5a, were 12.4 ± 1.5, 6.5 ± 1.2 and 8.6 ± 0.3 mg DCW d^{-1} for the UC, ZC and Z2 specimens, respectively. Biofilm formation in UC specimens was twice ZC due to the surface roughness as well as higher relative porosity compared to the zeolite coated specimens.

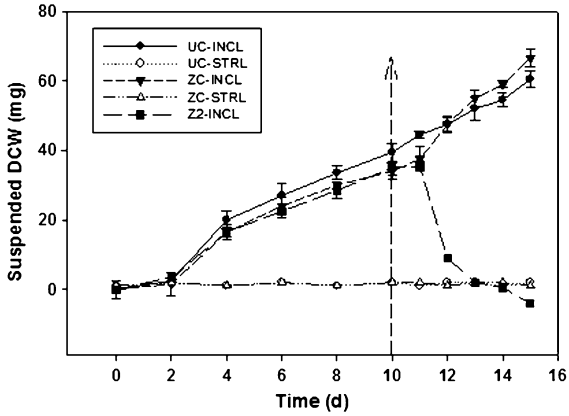

Fig. 4 Profile of planktonic *A. thiooxidans* biomass (DCW, mg) before and after the specimens were placed into the test beakers. *Symbols*: •, uncoated concrete specimens exposed to inoculated BNM; *o*, uncoated concrete specimens exposed to sterile BNM; ▼, ZC concrete specimens exposed to inoculated BNM; Δ, ZC concrete specimens exposed to sterile BNM; ■, Z2 concrete specimens exposed to inoculated BNM. *Dashed arrow* indicates the day on which specimens were placed into the test beakers. *Note*: Total biomass DCW was determined by multiplying the concentration (mg/l) with that of the working volume (l)

The increase in pH of the sterile BNM containing UC specimens is attributed to the leaching of OH ions from the concrete matrix, and the relatively unchanging pH in ZC and Z2 is due to the protective role of zeolite coatings from deterioration by cement paste dissolution (Fig. 6). Even though, the leaching of calcium was the highest in *A. thiooxidans* inoculated BNM containing the uncoated concrete specimen (Table 3), the pH of the medium did not change significantly due to the continuous biological sulphuric acid production which neutralized the alkalinity contributed by the leaching of $Ca(OH)_2$.

Biological sulphate production

Figure 7a and b illustrate the temporal variation of biological sulphate production by *A. thiooxidans* for all the specimens tested. It must be asserted that in the experiments where uncoated concrete specimens were

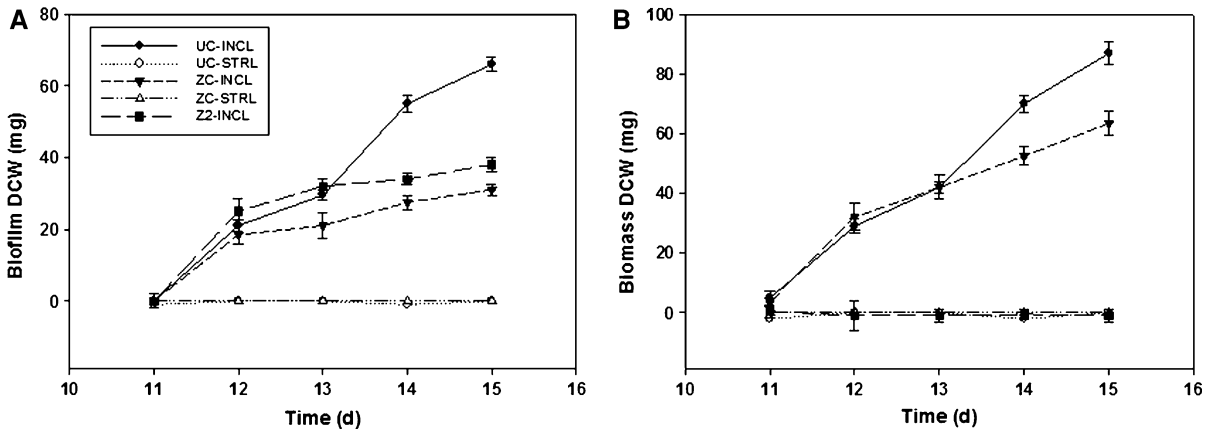

Fig. 5 Profile of *A. thiooxidans* biofilm DCW (**a**) and Total *A. thiooxidans* biomass DCW, planktonic plus biofilm, (**b**). *Symbols*: •, uncoated concrete specimens exposed to inoculated BNM; *o*, uncoated concrete specimens exposed to sterile BNM; ▼, ZC concrete specimens exposed to inoculated BNM; Δ, ZC concrete specimens exposed to sterile BNM; ■, Z2 concrete specimens exposed to inoculated BNM

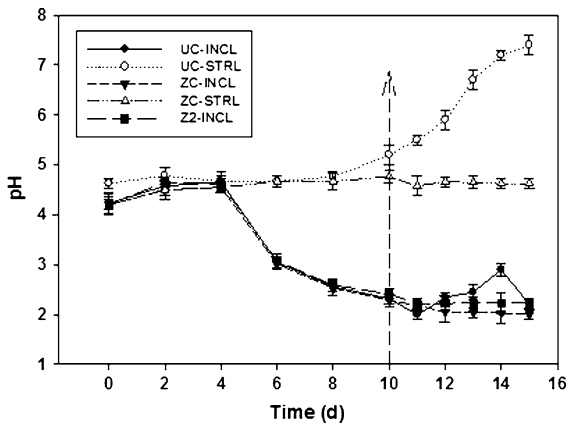

Fig. 6 pH as indicator of *A. thiooxidans* activity before and after the specimens were placed. *Symbols*: •, uncoated concrete specimens exposed to inoculated BNM; *o*, uncoated concrete specimens exposed to sterile BNM; ▼, ZC concrete specimens exposed to inoculated BNM; Δ, ZC concrete specimens exposed to sterile BNM; ■, Z2 concrete specimens exposed to inoculated BNM

exposed to the sterile BNM, there was no production of sulphate, indicating that sulphur oxidation in the inoculated experiments was biologically mediated. As apparent from Fig. 4, biological sulphur oxidation after specimens placement was highest in the ZC and uncoated specimens, where the sulphate production in UC and ZC were 238 ± 25 mg d^{-1} and 88 ± 13 mg d^{-1} SO_4^{2-}, respectively. Biological sulphate production was lower in inoculated BNM containing the uncoated concrete specimens due to its consumption for the formation of bio-corrosion byproduct, mainly gypsum (Fig. 3c). No sulphate production was observed after Z2 concrete specimens were placed in the BNM indicating its antimicrobial characteristics.

Respirometric studies

Figure 8a and b, illustrate OUR and CUO for all the test conditions. Consistent with the DCW concentrations,

Table 3 Summary of leaching rate of metals from concrete specimens and zeolite coatings

Specimens	Leaching from concrete (mg d^{-1})		Leaching from zeolite coating (mg per g coating material d^{-1})	
	Ca	Si	Ag	Zn
UC-INCL	18.7 ± 1.5	3.7 ± 0.89	N/A	N/A
ZC-INCL	1.3 ± 0.3	0.5 ± 0.2	N/A	N/A
Z2-INCL	0	0	0	0.01 ± 0.001
UC-STRL	2.6 ± 0.9	0	N/A	N/A
ZC-STRL	0	0	N/A	N/A

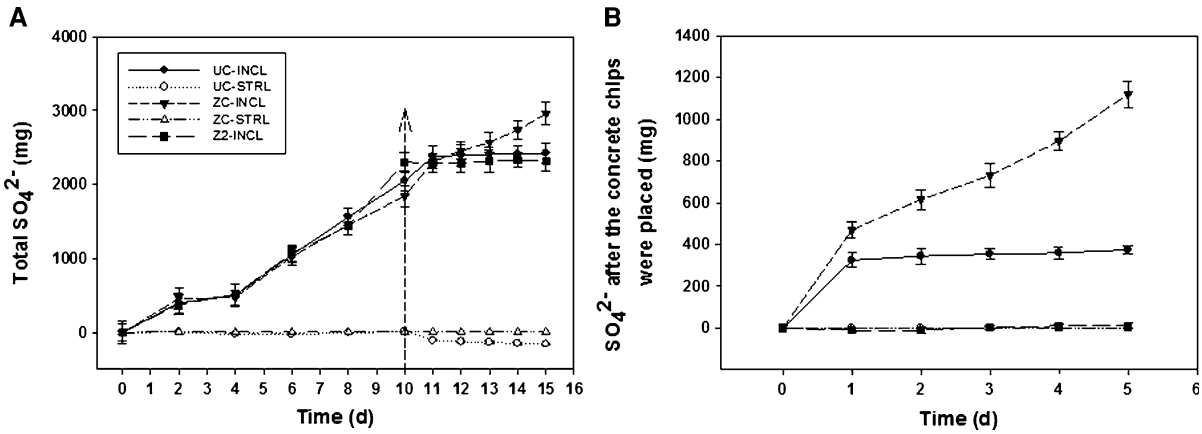

Fig. 7 Total biogenic sulphate produced throughout the duration of the inhibition experiment (**a**) and after the specimens were placed in the test beaker (**b**). *Symbols*: •, uncoated concrete specimens exposed to inoculated BNM; *o*, uncoated concrete specimens exposed to sterile BNM; ▼, ZC concrete specimens exposed to inoculated BNM; Δ, ZC concrete specimens exposed to sterile BNM; ■, Z2 concrete specimens exposed to inoculated BNM. *Note*: Total sulphate is determined by multiplying the concentration (mg/l) with that of the working volume (l)

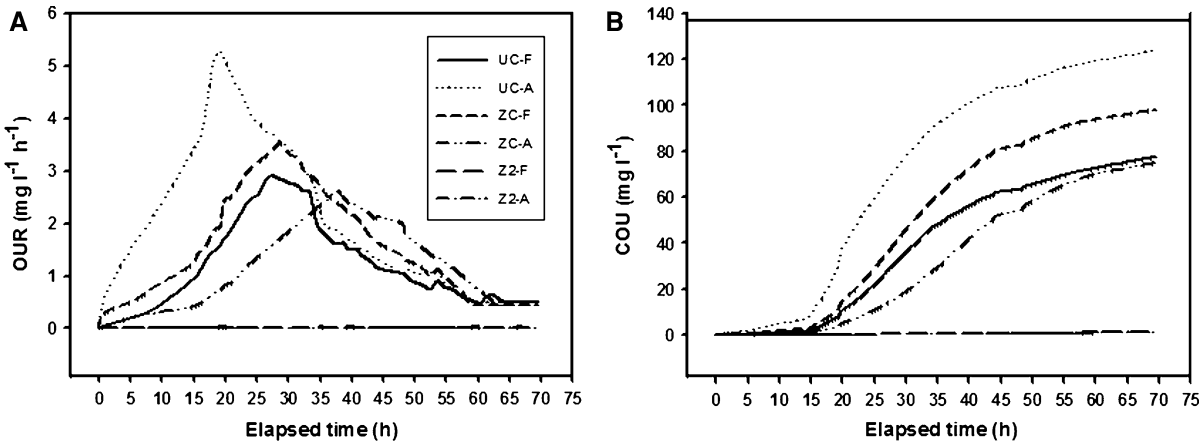

Fig. 8 Profile of oxygen uptake rate, OUR (**a**) and cumulative oxygen uptake, CUO (**b**)

the activity of the bacterium in terms of OUR was higher for the biofilm detached from uncoated concrete specimens compared to that of ZC. The peak OURs were 2.8, 5.2, 3.5, 2.6 mg l^{-1} h^{-1} for UC-F, UC-A, ZC-F and ZC-A peaking at 34, 20, 29, and 38 h, respectively. The COUs at the aforementioned peak time ranged from 36 to 48 mg l^{-1} for UC-F, UC-A, ZC-F and ZC-A. All the indication is that zeolite coating inhibits biofilm formation as confirmed by lower OUR values in respirometric studies where inoculums detached from ZC was used which is an indication of low biomass growth on the aforementioned specimens surface.

There was no oxygen uptake in those experiments where Z2 was used. Our results demonstrated that biofilm formation was higher in the uncoated specimens due to surface roughness as well as increased porosity compared to the ZC specimens as confirmed from FE-SEM (Fig. 3).

To further investigate the kinetics *A. thiooxidans* activity, b_H (decay rate) was calculated by plotting ln(OUR) versus time and calculating the slope according to Eq. 1, and maximum specific growth rate (μ_{max}, h^{-1}) was calculated by plotting $\ln(OUR/OUR_{initial})$ versus time and calculating the slope according to Eq. 2 developed by Orhon (Orhon et al. 1995):

$$\ln \text{OUR} = [\ln(1 - \text{fe}) b_H X_{Ho}] - b_H t \quad (1)$$

$$\ln(\text{OUR}/\text{OUR}_{\text{initial}}) = (\mu_{\max} - b_H) t \quad (2)$$

where, OUR is oxygen uptake rate at time t, $\text{OUR}_{\text{initial}}$, is oxygen uptake rate at time zero, fe is endogenous respiration, X_{H0} is concentration of heterotrophic biomass. b_H was calculated to be 0.06 h^{-1} for UC as well as ZC with R^2 value of 0.97 for both. The maximum specific growth rates were 0.26, 0.35 0.20, 0.22 h^{-1} for UC-F, UC-A, ZC-F and ZC-A, with corresponding R^2 value of 0.98, 0.97, 0.99, 0.97. For Z2-F and Z2-A, the aforementioned parameters were not determined, because there was no oxygen consumption in the respiratory tests where inoculums obtained from Z2 concrete specimens were used.

Leaching of metals

Biological induced concrete corrosion manifests itself through the formation of gypsum ($CaSO_4 \cdot 2H_2O$), ettringite ($3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O$) or thaumasite ($Ca_3Si(CO_3)(SO_4)(OH)_6 \cdot 12(H_2O)$) (Hansen 1963; Bensted 1999; Hartshorn et al. 1999; Tian and Cohen 2000). The aforementioned corrosion byproducts are the result of the reaction of biologically formed sulphates with calcium or silicon, and hence the need to investigate the kinetics of calcium and silicon leaching pattern. The leaching rates of metals from concrete as well as zeolite coating are reported in Table 3. The leaching rates of calcium and silicon for uncoated specimens exposed to inoculated BNM were 14.4 and 7.4 folds higher than those of ZC concrete specimens exposed to inoculated BNM, respectively, reflecting the protective role zeolite plays (Aviam et al. 2004). Abiotic leaching of calcium from uncoated concrete specimens exposed to sterile BNM was 86% lower than those exposed to inoculated BNM. There was no detectable leaching of the aforementioned metals in Z2 concrete specimens exposed to inoculated BNM and ZC concrete specimens exposed to sterile BNM. The reduced leaching rate of metals in ZC compared to uncoated concrete specimens in sterile BNM is attributed to the protective role of zeolite and the absence of calcium and silicon leaching from Z2 is attributed to its antimicrobial activity.

Although, no detectable silver leached from the antimicrobial zeolite coating (Z2 concrete specimen), zinc leached at a rate of 1.6 mg $m^{-2}d^{-1}$ surface coating (Table 3). This is attributed to the selectivity of zeolites to silver relative to other exchangeable ions i.e. H^+, NH_4^+, Ca^{2+}, Zn^{2+} (Top and Ulku 2004).

By conducting parallel growth tests of silver-loaded zeolite alone, combined completely mixed silver-loaded zeolite and biomass, and silver-loaded zeolite separated from biomass by a dialysis membrane, Matsumura et al. (2003) confirmed not only that the presence of bacteria is necessary for leaching of silver from zeolite but also that the antimicrobial action of silver is contingent upon contact between the zeolite and the biomass. The simultaneous lack of biomass growth and silver leaching in the Z2 specimens, consistent with the observations of Matsumura et al. (2003), suggests that the death of *A. thiooxidans* due to the antimicrobial characteristics of our coating materials has played a negative role in the release of silver ions from the zeolite matrix.

In the present work, the antimicrobial characteristics of silver-loaded zeolite to planktonic as well as biofilm of *Acidithiobacillus thiooxidans* is demonstrated experimentally. Future works on the use of zeolite as antimicrobial agents should include, optimization experiments to increase silver-loading capacity of zeolite matrix by employing ion-exchange trails at various zeolite particles size; mechanistic studies on the mode of inhibition of silver-loaded zeolite; and antimicrobial studies using *Thiobacillus* species isolated from sewer systems employing molecular microbiology techniques.

Conclusions

- Zeolite coating is resistant to bacterial induced corrosion at *A. thiooxidans* biomass concentration as high as 114 ± 11 mg DCW l^{-1} and biological sulphate concentration of 7100 ± 256 mg l^{-1}.
- Zeolite (5% Ag by weight) is inhibitory to planktonic as well as biofilm of *A. thiooxidans*, as confirmed by respirometric studies.
- The OUR of planktonic biomass was higher in respirometric studies where ZC concrete specimens were used (3.5 mg l^{-1} h^{-1}) compared to that of uncoated concrete specimens (2.8 mg l^{-1} h^{-1}).
- The OUR of detached biomass for uncoated concrete specimens (5.2 mg l^{-1} h^{-1}) were higher than that of ZC (2.6 mg l^{-1} h^{-1}), confirming that ZC inhibits biofilm formation.

- While there was leaching of calcium and silicon in both UC (Ca = 18.7 mg d^{-1}; Si = 3.7 mg d^{-1}) and ZC (Ca = 1.3 mg d^{-1}; Si = 0.5 mg d^{-1}) exposed to inoculated BNM, the absence the aforementioned metals in the leachates of Z2 concomitant with the lack of microbial activity demonstrated that the leaching of the aforementioned metals was biologically mediated.

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