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Evaluating the efficacy of antimicrobial additives against biogenic acidification in simulated wastewater exposure solutions

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Abstract

Microbially Induced Corrosion of Concrete (MICC) is a progressive three-stage deterioration process that is primarily associated with sulfur-oxidizing bacteria (SOB). One strategy for mitigating MICC is the use of antimicrobial additives. It is hypothesized that the performance of antimicrobial products is, among other factors, heavily influenced by the pH of the environment, the bacterial population, and the level of bacterial activity. To test this hypothesis, three bacterial activity-population levels were tested in solutions with different pH levels to evaluate the efficacy of a typical antimicrobial product against planktonic SOB. The ability of the antimicrobial product to prevent or delay the biogenic acidification was considered as the criterion for its efficacy. The tested antimicrobial product was successful in delaying or preventing biogenic acidification with low and moderate bacterial populations and activity for all pH levels greater than 4. Lower pH levels were not tested in this investigation. Antimicrobial products were successful in delaying or preventing biogenic acidification with severe bacterial populations and activity for all pH levels tested greater than 6. The results support the main hypothesis of the research; therefore, the selection of whether to utilize an antimicrobial product requires an understanding of the operational pH of the environment as well as knowledge on the target bacterial population and activity.

Keywords: Microbially induced corrosion; Concrete corrosion; Biogenic sulfuric acid; Sulfur oxidizing bacteria; Antimicrobial additives

1 Introduction

The service life of concrete wastewater infrastructure such as sewer pipelines, manholes, wastewater digesters and septic tanks could be reduced significantly by microbially induced corrosion of concrete (MICC). MICC is a progressive deterioration process that is primarily associated with sulfur oxidizing bacteria (SOB) [1–3]. The process begins with the production of hydrogen sulfide (H_2S) under anaerobic conditions by reduction of sulfate ions (SO_4^{-2}) present in the wastewater by anaerobic sulfate reducing bacteria (SRB) residing below the waterline [4]. The formation of aqueous hydrogen sulfide (H_2S_{aq}) in wastewater is followed by its release into the headspace above the waterline as a gas, H_2S_g, which then dissolves in the moist layer on the surface of the inner walls of the concrete structure as H_2S_{aq} [5–8]. Hydrogen sulfide on concrete could either remain as it is or could be converted chemically to elemental sulfur, sulfite, and thiosulfate depending on the environmental chemical conditions. In the last cycle of the biological process, SOB consume the sulfur compounds as their nutrient source and oxidize them to sulfuric acid, which is the final product of the biogenic cycle [9]. Sulfuric acid attacks the cementitious paste of concrete and results in decalcification of calcium hydroxide ($Ca(OH)_2$) and decalcification of calcium silicate hydrates (C-S-H). The final products associated with the deterioration processes are expansive gypsum and ettringite [10–12].

The deterioration of concrete due to MICC has been described using a three-stage progressive deterioration process [1,3,13]. Initially the pH of the surface of the concrete is in the range of 12.5 to 14 [11]. Though the high alkalinity of concrete hinders bacterial survival initially, abiotic (chemical) pH reduction (pH > 9-10) of the surface of concrete (e.g., due to carbonation, calcium leaching, reaction between thiosulfuric and polythionic acids formed through atmospheric oxidation of H_2S) decreases concrete's natural ability to resist bacterial colonization (Stage I) [14,15]. This is followed by Stage II, which consists of the attachment of SOB on the concrete surface when the conditions become suitable for their growth. The first strains that attach on concrete surface, typically neutrophilic sulfur oxidizing bacteria (NSOB), start to consume H_2S and other preferred sulfur compounds and generate small amounts of sulfuric acid, which leads to further reduction in the surface pH ($\sim$4 < pH < 9-10) due to biogenic acidification (Stage II) [1,7,14,16]. Finally, at the lowered pH environment, acidophilic sulfur oxidizing bacteria (ASOB) can start to populate in the environment and produce

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concentrated sulfuric acid, which acidifies the environment further (pH < ~4), and cause severe concrete deterioration (Stage III) [16–19].

Several strategies have been proposed to prevent or slow down the degradation due to MICC. These strategies include improving the hydraulic design and ventilation of wastewater networks, enhancing concrete resistance to sulfuric acid, use of supplementary cementitious materials, geopolymers, calcium aluminate cements and colloidal silica, using polymeric liners and other coating techniques, biocide addition to wastewater to reduce the activities of SRB, and preventing the growth of SOB using antimicrobial additives for concrete as admixed or topical treatment [13,20–28]. Each prevention strategy has benefits and potential costs.

This paper studies the parameters that influence the efficacy of antimicrobial additives, which are mixed in concrete (admixed) or applied topically on concrete surface. The main objective is to study the factors affecting antibacterial efficacy without the confounding effects of concrete, and this paper is focused on studying biogenic acidification in solutions representing the exposure media, rather than concrete. As shown in Fig. 1, antimicrobial agents are used with the intention to prevent or delay the attachment and growth process of SOB (i.e., Stage II). It is important to note that mitigating the bacterial proliferation and growth may stop or slow down the onset or progression through Stage II, which is the essential stage that has to occur before Stage III can begin. As a result, it is anticipated that concrete may have a longer service life due to delayed or prevented biogenic sulfuric acid production. However, it has been shown that antimicrobial agents may not work after entering the Stage III or being subjected to pure acid immersion [29]. For example, Ding et.al. studied the antimicrobial efficacy against four strains of SOB and concluded that the antimicrobial product was effective when pH was high, but was not effective when pH was sufficiently low as to provide optimum growth [29]. Further, it has been hypothesized that when the bacterial population exceeds a certain threshold, or highly active, the antimicrobial additive may be less effective against the generation of biogenic sulfuric acid that is highly concentrated and continuous. Therefore, the key parameters that influence the efficacy of antimicrobial products are the pH of the environment, the bacterial population, and the level of bacterial activity.

2 Objective

The antimicrobial additives for concrete can be applied either as admixture or topically. Testing the performance of antimicrobial concretes using test setups designed to simulate MICC in the lab is not straightforward and requires long testing time. Therefore, the authors believe the antimicrobials for concrete must be tested with a proper and practical testing protocol before preparing antimicrobial concrete mixtures to be tested in complete MICC simulation setups. MICC simulation tests requires significant effort and time; therefore, eliminating some of the antimicrobial options before using them in concrete would help save time and resources.

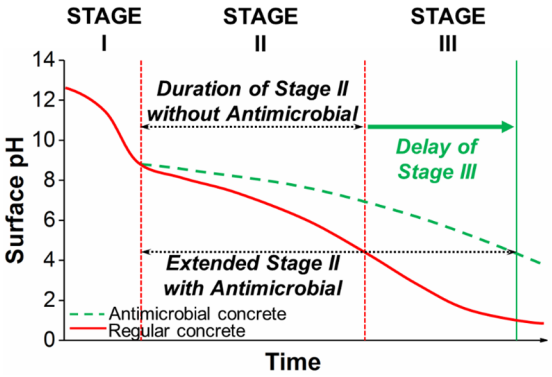

Figure 1. The hypothesized effect of antimicrobials on extending Stage II and delaying Stage III, resulting in increased service life of concrete structures (conceptual).

A practical and representative testing method is needed to evaluate the efficacy of antimicrobial products before they are used in concrete, mortar or paste [29]. In recent years, modified versions of ISO 22196 [30], ASTM D4783 [31], and ASTM G21 [32] have been used to demonstrate the effectiveness of the antimicrobial products used in concrete against SOB. However, these test methods do not reflect the complex biochemical process (e.g., biogenic acidification) of MICC in wastewater environments. Bacterial succession depends on many parameters including 1) pH of the environment, 2) bacterial population, and 3) bacterial activity. The primary objective of the study is to perform a fundamental investigation to determine limitations associated with the use of antimicrobial additives against planktonic (free-floating) SOB leading to biogenic acidification in bacterial suspensions where the pH and bacterial population and bacterial activity are varied.

3 Materials and Methods

3.1 Bacteria

Halothiobacillus Neapolitanus (ATCC 23641) and *Acidithiobacillus Thiooxidans* (ATCC 19703) were used in this study since these two species can consume almost all types of preferred sulfur substrates and can survive in a wide pH range between 0.5 and 8.0 when used together. The pH ranges of 3.5-8.0 and 0.5-3.5 are preferred by *H. Neapolitanus* and *A. Thiooxidans*, respectively [1,2,14]. Pure cultures of these two strains were obtained from the American Type Cultural Collection (ATCC). The bacteria strains were preserved and cultivated using appropriate microbiological preservation techniques such as agar plates, agar slants, and glycerol stock strains. *H. Neapolitanus* is a neutrophilic sulfur oxidizing bacteria (NSOB) that can reduce the pH of the environment from 7-8 to 3-4. *H. Neapolitanus* was cultivated in the ATCC medium 290 S6 as suggested by ATCC. *T. thiooxidans* is an acidophilic sulfur oxidizing bacteria (ASOB) that reduces pH from 3–4 to 0.5-1. *T. thiooxidans* was cultured in a modified version of ATCC medium 125, in which sublimed sulfur was replaced with equal amount of sodium thiosulfate. A total of 5% v/v of the culture of *H. neapolitanus* and a total of 5% v/v of the pure culture of *T. thiooxidans* at their exponential growth rate (e.g., after 1 week and 2 weeks of growth respectively) was used as the seed for the biogenic acidification.

3.2 Testing media

A simulated wastewater environment, a modified version of the nutrient media reported by Soleimani et al. [33], which promotes the growth of both SOB strains was prepared to be used as the nutrient source during the biogenic acidification. The recipe of the nutrient media (NM) was as follows: 10 g/L $Na_2S_2O_3$, 0.25 g/L $CaCl_2$, 3 g/L KH_2PO_4, 3 g/L K_2HPO_4, 0.8 g/L $MgCl_2 \times 6H_2O$, 0.1 g/L $(NH_4)_2SO_4$, 5 mg/L $FeSO_4$. In NM, sodium thiosulfate is the main nutrient source of SOB.

In order to test the effect of bacterial population-activity, three different acidification environment with various pH levels were created, which were designated as Severity Level I, II and III.

Severity Level 1 (SL1) is described as the environment with reduced bacterial population by dilution and low bacterial activity due to keeping them in nutrient-deficient media for an extended period. In these tests, fresh NM was acidified by actively growing bacteria until the pH dropped below 3. Then, the acidified media were maintained in static condition in closed bottles for 6 weeks. During this period, the bacterial population and activity were expected to decrease due to diminishing nutrient concentration. After 6 weeks of waiting period, the pH of the bacterial suspension was measured as 1.83. The suspension was then diluted using fresh NM to obtain several suspensions with specific pH values (i.e., 6.25, 6.00, 5.75, 5.10, 4.10, 3.00, and 2.00). Upon creating diluted suspensions with various pH, the antimicrobial product was injected into the SL1 exposure cells.

Severity Level 2 (SL2) is described as the environment with reduced bacterial population by dilution and medium bacterial activity due to keeping them in nutrient-deficient media for a period shorter than the waiting period specific for SL1. To prepare this environment, fresh NM was acidified with actively growing bacteria until the pH dropped below 3. The acidified media were kept in this condition for 3 weeks. During this period, the bacterial population and activity were expected to decrease due to diminishing nutrient concentration, but not as much as in the previous test conditions for which the waiting period after acidification was approximately 6 weeks. After 3 weeks of waiting period, the pH of the bacterial suspension was measured as 2.03. The suspension was then diluted by fresh NM to obtain several bacterial suspensions with specific pH values (i.e., 6.25, 6.00, 5.75, 5.10, 4.10, 3.00, and 2.00). As soon as the diluted suspensions with various pH were prepared, the antimicrobial product was injected into the SL2 exposure cells.

Severity Level 3 (SL3) is the environment which is expected to have high bacterial population and activity. These tests were performed on media acidified by highly active bacterial cultures in fresh NM. A total of 5% v/v of the culture of *H. neapolitanus* and a total of 5% v/v of the pure culture of *T. thiooxidans* at their exponential growth rate was injected to fresh NM having an initial pH of 6.55. The pH of the environment was not modified through dilution of already acidified media; the bacteria reduced the pH naturally by biogenic acidification. The antimicrobial product was injected into the exposure cells when target pH levels (ranging from 6.5 to 3) were reached. As a result, SL1 and SL2 media had reduced (diluted) bacterial population, and low bacterial activity as compared to freshly growing bacteria in a nutrient rich environment provided by SL3.

3.3 Antimicrobial product

A typical antimicrobial product that is used in concrete to mitigate MICC is used in this study. For this purpose, a silane quaternary ammonium chloride (SQA or Si-QAC) aqueous salt solution containing 3.6 % active ingredient (3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride) was used. The antimicrobial activity of SQA is related to its ability to be present chemically bonded to the various surfaces [34]. There are mainly two major hypotheses used to explain the antimicrobial working mechanism of SQA. First theory hypotheses that their long molecular carbon chain pierce the cellular membrane of the bacteria [35,36]. The antimicrobial effectiveness increases with longer molecular chain [37,38]. The other theory is that the ammonium cation (NH_4^+), which is a positively charged polyatomic ion, of SQA may lead ion exchange with cations within the cell membranes, which ultimately causes cell destruction [39,40]. In this study, the antimicrobial product was tested against planktonic SOB in testing media. Following the injection of the antimicrobial into the exposure cells, the mixture was shaken properly to obtain a homogeneous distribution of the antimicrobial.

The suggested dosage of the antimicrobial product is 1.5 % by mass of cementitious material, which equals to approximately 1% by mass of cement paste when water to cement ratio (w/c) is in the range of 0.42-0.50. Since these antimicrobial agents are effective in the cement paste portion of the concrete, the dosage used in the liquid solution was accepted and used as its dosage in cement paste, which is 1% by mass. Since the densities of the testing media and the antimicrobial product are almost the same, the dosage could also be expressed as 10 mg/mL.

3.4 Bacterial enumeration

Bacterial cell counts were performed in order to establish a quantitative bacterial population for the bacterial suspensions used in the experiments. The live colony forming units per milliliter (CFU/mL) of the SL1, SL2 and SL3 were determined by performing viable cell count, which is a traditional microbiological enumeration method. The samples were taken from SL1 and SL2 suspensions after they were diluted with NM to obtain specific pH values. For SL3, sampling was done when the actively growing bacteria acidified the testing media to the targeted pH values when the antimicrobial was added. A 10-fold serial dilution was performed up to 10^{-6} dilution tube. The bacterial suspensions in the dilution tubes were inoculated onto agar plates using the spread plate technique. The medium to prepare agar plates contained 10 g/L $Na_2S_2O_3$, 1.2 g/L Na_2HPO_4, 0.03 g/L $CaCl_2$, 1.8 g/L KH_2PO_4, 0.1 g/L $MgSO_4 \times 7H_2O$, 0.1 g/L $(NH_4)_2SO_4$, 0.02 g/L $MnSO_4$, 0.02 g/L $FeCl_3$, and 15 g/L agar. The agar plates were incubated at 30 °C for 1 week, followed by colony counting to determine the bacterial population.

3.5 Biogenic acidification and pH monitoring

Biogenic acidification is described as the acid production in the environment due to biological activities of microbes. In this study, biogenic acidification was performed by two SOB strains that generate sulfuric acid and lower the pH of the media. In order to evaluate the effectiveness of the antimicrobial product against the SOB, biogenic acidification was carried out in exposure cells with antimicrobial product and without the antimicrobial product (control exposure cells). The pH measurements, which serve as an indication of biological activity, show the SOB activity – the larger the pH reduction the higher the activity level. However, in the exposure cells with the antimicrobial product, no or delayed pH drop was expected. At least two replicate exposure cells were used for each case to verify the accuracy of the results. The exposure cells were placed on a rotary shaker at 100 rpm at a controlled temperature of 25±1 °C during the acidification. pH measurements were taken and recorded with a pH electrode and a benchtop multiparameter meter (VWR 89231-586 and VWR Symphony B40PCID, VWR International, Radnor, PA).

3.6 Sulfate concentration measurements

Presence of sulfate ions is a direct indication of sulfuric acid generation as a result of sulfur-oxidation. Separate exposure cells were created to analyze the sulfate concentration as a result of biogenic acidification by using the same approach used to create SL3 environment. The concentration of sulfate ions was determined using EPA Method 375.4, a standard method which is based on measurement of turbidity resulting from the precipitation of barium sulfate, which is an end product of the reaction between barium chloride and sulfate ion [41]. An Ultraviolet-Visible spectrophotometer (GENESYS 20, Thermo Fisher Scientific, Waltham, MA) was used at a wavelength of 420 nm to determine the turbidity of the samples.

4 Results and Discussion

4.1 Bacterial enumeration

The bacteria present in the environments created (SL1, SL2, and SL3) were quantitatively assessed. Both NSOB and ASOB were enumerated in this study. However, it should be noted that that NSOB are the dominant bacteria in the Stage II of MICC, which is the initiation of bacterial succession stage. The MICC process begins with the activity of NSOB, and if the antimicrobial is not effective during the early stages where NSOB is predominant, it would not be effective when conditions become more aggressive with lowered pH and high ASOB activity. Also, in order for ASOB to be active in deterioration, the environmental conditions must be suitable for their growth. Therefore, the activity of NSOB is essential for making the environment suitable for ASOB. Given that the antimicrobial additives are expected to prevent bacterial succession, they first need to combat with NSOB species. Although ASOB species are mainly responsible for the damage to concrete, NSOB species are responsible for initiating the process, and focusing on NSOB to investigate the effectiveness of antimicrobial additives is important since antimicrobial additives are expected to prevent the initiation of the bacterial succession process (Stage 2 of MICC), which begins with NSOB. Therefore, NSOB is the main bacteria focused in this paper even though both NSOB and ASOB were used in the experiments. Three replicates of agar plates were used in each case, and the average results of the enumeration were presented in Figure 2 and Figure 4. Error bars were not used since the values were sufficiently close to one another.

The bacterial suspension, (i.e. seed bacterial suspension) used to create the SL1 environment with different pH levels by dilution, had a pH of 1.83. NSOB population in that seed suspension was determined as 1.1×10^4 cfu/mL when the seed suspension of SL2 having a pH of 2.03 was 2.91×10^5 cfu/mL. These results support the fact that longer waiting period of SL1 compared to SL2 lead to decreased bacterial activity, thus decreased bacterial population. Figure 2 also shows that NSOB population decreases with increasing pH, which is a result of dilution. However, the trend of NSOB population for SL3 is different from SL1 and SL2 as the biogenic acidification is the main mechanism driving the pH change. The counting results present that NSOB population increased when the pH decreased from 6.5 to 5.1, then started to decrease with decreasing pH after that. It proves that NSOB has a preferred pH range and starts to leave its dominance to ASOB after some point [1,14,18].

Bacterial population was found to be directly proportional to the change in pH when it is adjusted by dilution , which was the case for SL1 and SL2. Furthermore, the waiting period and low pH of the SL1 and SL2 seed suspensions resulted in significantly smaller bacterial population than the SL3 cases. Actively growing bacteria in SL3 environment resulted in drastically greater population as expected.

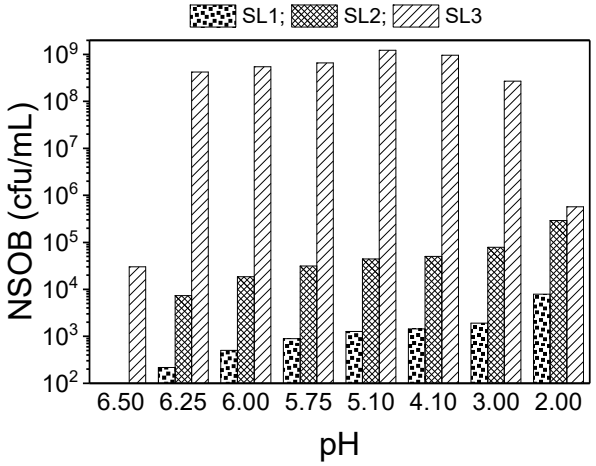

Figure 2. Bacterial population of NSOB in the SL1, SL2, and SL3 at different pH levels.

Figure 3 illustrates the effect of dilution on the population size of the bacteria with an image of the NSOB colonies grown on agar plates. It can be easily observed that the dilution made to obtain different pH levels in SL1 and SL2 caused a reduction in the observed colonies with proportional to dilution ratio.

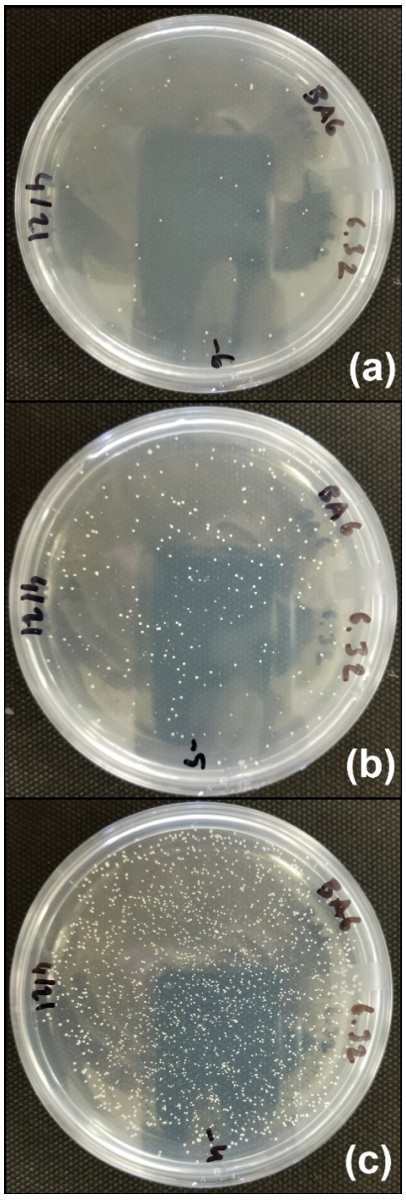

Figure 3. The effect of serial dilutions on NSOB colonies in the SL3 environment at pH of 6.32: (a) dilution of 10^{-7}, (b) dilution of 10^{-6}, (c) dilution of 10^{-5}.

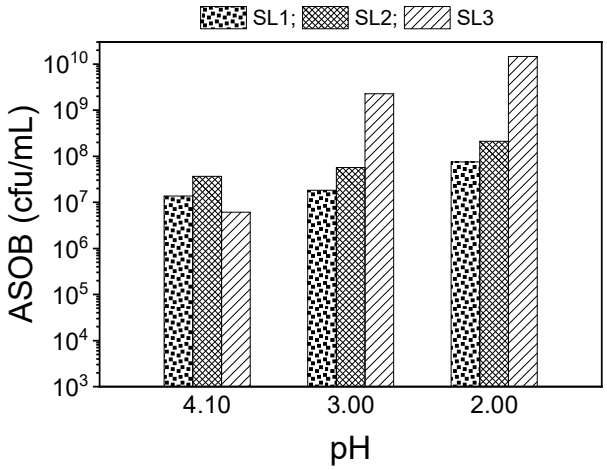

Figure 4. Bacterial population of ASOB in the SL1, SL2, and SL3 at different pH levels.

4.2 Biogenic acidification and pH monitoring

The pH reduction in the exposure cells can be achieved due to the biogenic sulfuric acid production by the SOB. The production of biogenic sulfuric acid is typically a result of oxidation of elemental sulfur or other sulfur compounds (i.e. hydrogen sulfide, thiosulfate) as described by Islander et.al. [1]. In this study, sulfuric acid was generated through oxidation of sodium thiosulfate since it is the main nutrient source for the SOB in the NM in presence of oxygen and water. Eq. (1) represents the possible chemical reaction [42,43].

$$Na_2S_2O_3 + 2O_2 + H_2O \rightarrow Na_2SO_4 + H_2SO_4 \quad (1)$$

Figure 5 presents the changes in pH and sulfate concentration due to biogenic acidification by SOB. NSOB and ASOB species used in the cells were able to reduce the pH of the nutrient media from 6.6 to 2 in 20 days. Sulfate ion concentration in the media increased as a result of biogenic sulfuric acid generation.

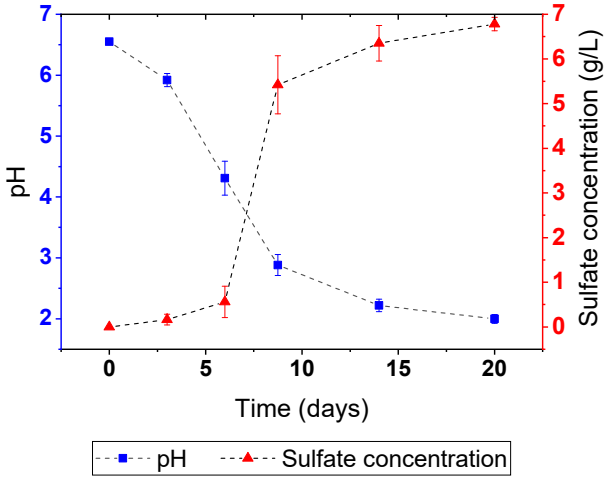

Figure 5. Sulfate concentration and pH change as a result of biogenic acidification process.

The SL1, SL2, and SL3 environments were obtained using the methods previously described (section 3.2). For the SL1 and SL2 environments, the testing media were created with a pH of 3.0, 4.1, 5.1, 6,0 and 6.5 through dilution of the already biogenically acidified bacterial media. The antimicrobial product was tested by injecting a dose of 1% by mass of media into the exposure cells containing 150 g of testing media. For brevity, only some of the pH measurement results are presented here. The media with pH of 3.1 for SL1 and SL2 did not show any bacterial activity in short term even in the control exposure cells. It is anticipated that the dilution made to obtain pH of 3.1 did not provide sufficient nutrients for the bacteria to grow. In Figures 6 and 7, the trend of pH measurements, which is an indication of biological activity, shows that the product was effective in terms of preventing pH reduction, thus bacterial activity. For SL1 and SL2 environments, the antimicrobial product prevented the pH reduction, while the continuous reduction of pH was observed in the control exposure cells. This implies that the antimicrobial limits the growth of the bacteria. The results obtained from the SL1 and SL2 were similar except for the

reaction kinetics. The exposure cells containing SL2 environment exhibited faster pH reduction. It is believed that the difference between the waiting periods was the main reason behind the bacterial activity and pH reduction rate initially.

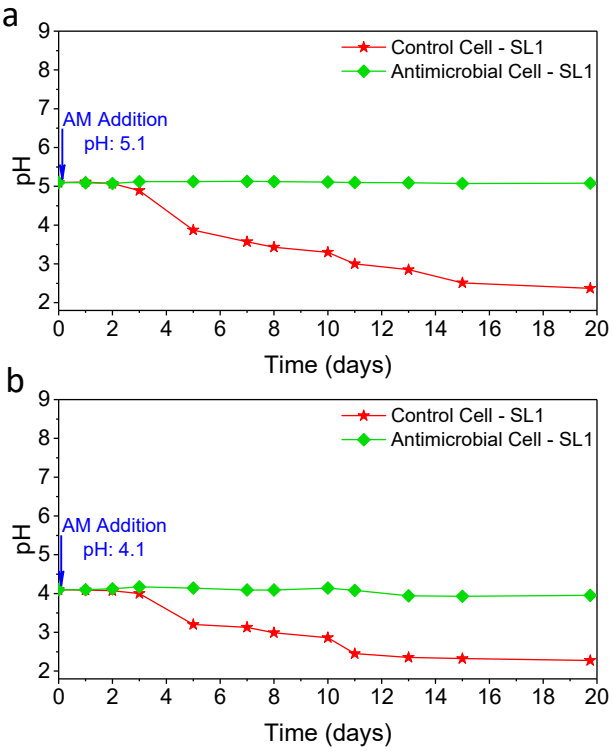

Figure 6. The pH trends of the testing media prepared using the SL1 bacterial suspension: (a) antimicrobial addition (AM) at pH: 5.1, NSOB population: 1.27×10^3 cfu/mL; (b) antimicrobial addition (AM) at pH: 4.1, NSOB population: 1.43×10^3 cfu/mL, ASOB population: 1.37×10^7.

For the SL3 environment, the antimicrobial was added to each exposure cell at different times since the targeted pH were achieved through pH reduction due to biogenic acidification instead of obtaining by dilution of existing already acidified (low pH) media. The pH of the exposure cells was monitored carefully and the antimicrobial was added when a target pH was reached. The antimicrobial was injected into different exposure cells when pH was 3.00, 4.10, 5.10, 5.75, 6.00 and 6.50. Selected results were presented in Figure 8. Control cases represent the exposure cells without antimicrobial product addition. It is shown in these figures that the antimicrobial product could prevent or delay the pH reduction when the pH of the media is larger than 6 when the media contained less bacterial population and activity. The product was ineffective in the exposure cells with pH of 5.75 and lower. The increase in the dosage of the product up to 10% by weight of testing media did not change the results when the pH is 5 and lower. The results of these high dosage experiments are not shown here for brevity.

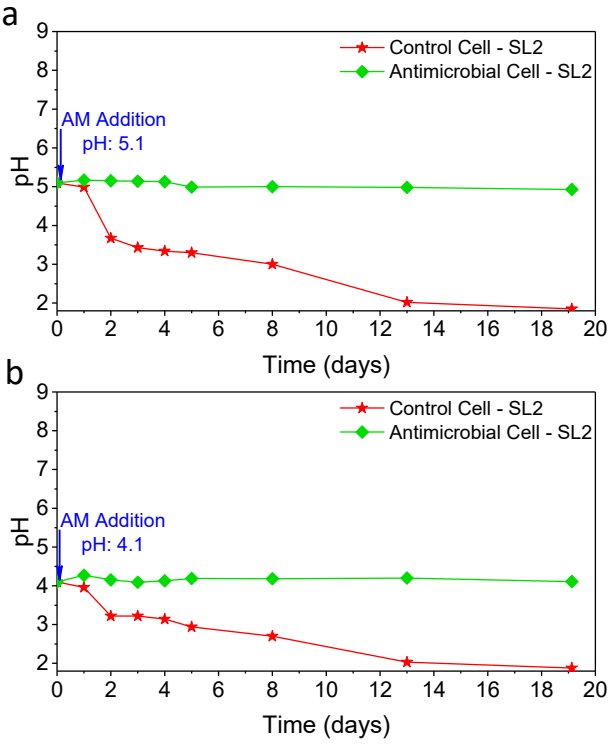

Figure 7. The pH trends of the testing media prepared using the SL2 bacterial suspension: (a) antimicrobial addition (AM) at pH: 5.1, NSOB population: 4.43×10^4 cfu/mL; (b) antimicrobial addition (AM) at pH: 4.1, NSOB population: 5.04×10^4 cfu/mL, ASOB population: 3.65×10^7.

As presented in Figure 8, the control cells showed that the overall trend of pH in pure biogenic acidification case without dilution, SL3 media, was not a steady reduction. The pH decreased from 6.5 to approximately 5.7 initially, then it increased back to 6.5. After that time, the pH suddenly decreased to more acidic levels (to around 3.5) followed by a slower and more steady decrease in pH. The initial 5 days appears to have served as a preparation period for NSOB to adapt the environment and maximize their population, and the available nutrient was rapidly consumed between day 5 and 7 since the activity and population increased significantly. After 7 days, ASOB started to become the active species, but a pH reduction at a slower rate was observed due to limited nutrient and high biomass in the media. The pH increase between day 3 and day 5 might have been caused by the reduction of thiosulfate ($S_2O_3^{-2}$), tetrathionate ($S_4O_6^{-2}$), and pentathionate ($S_5O_6^{-2}$) to elemental sulfur as a result of abiotic and biotic, most probably, reactions. However, this pH increase was even more rampant in the antimicrobial cells when the antimicrobial was added to SL3 media at pH of 6.25 and 6.00. No pH change was observed when the antimicrobial was added at pH of 6.50 since the bacteria was probably completely deactivated by the antimicrobial. The excessive increase in pH up to 7-8 in antimicrobial cells as shown in Figure 8 (b) and (c) may be a result of bacterial resistance observed in those cells.

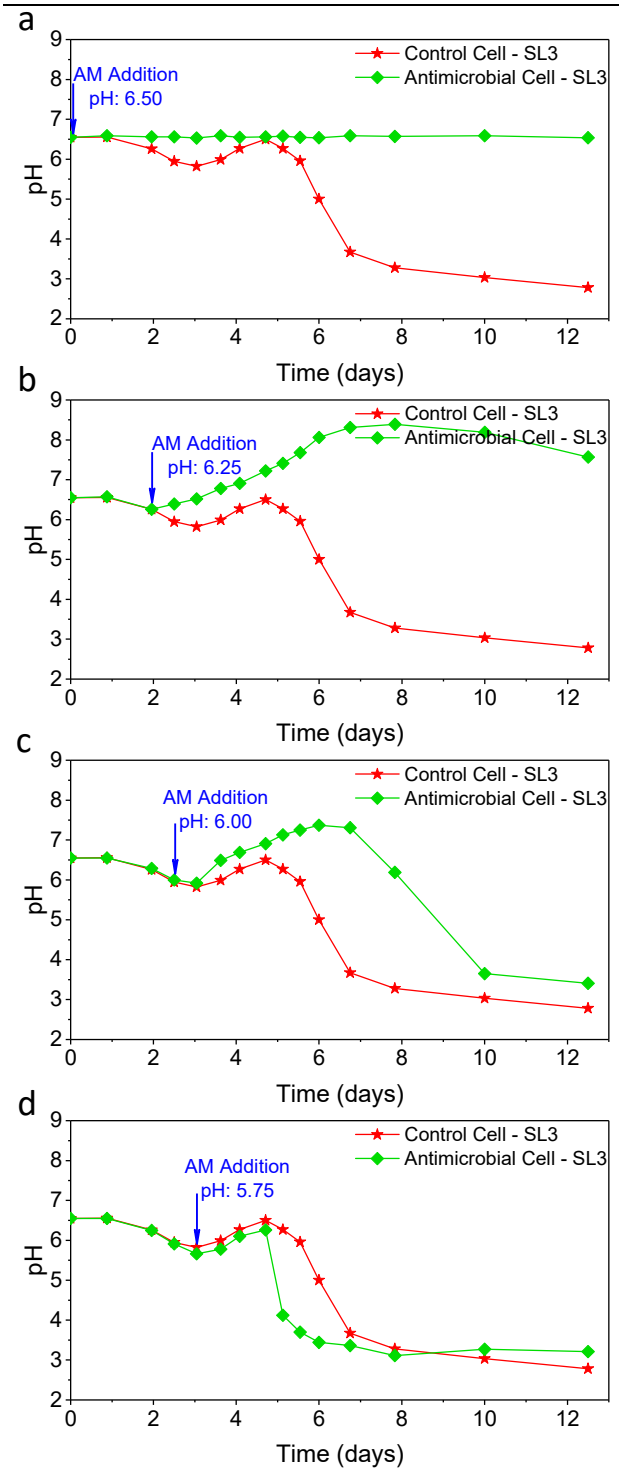

Figure 8. The pH trends of the testing media prepared using the SL3 bacterial suspension: (a) antimicrobial addition (AM) at pH: 6.5, NSOB population: 3×10^4 cfu/mL; (b) antimicrobial addition (AM) at pH: 6.25, NSOB population: 4.2×10^8 cfu/mL; (c) antimicrobial addition (AM) at pH: 6, NSOB population: 5.5×10^8 cfu/mL; (d) antimicrobial addition (AM) at pH: 5.75, NSOB population: 6.6×10^8 cfu/mL.

The antimicrobial at a dosage rate of 10 mg/mL was shown to be effective for environments with a pH of 4.1 and greater in SL1 and SL2 environments; however lower pH environments were not tested in this paper. The antimicrobial at a dosage rate of 10 mg/mL was shown to be effective for environments with a pH of 6.25 for SL3 environment; while delaying the acidification when it was added at the pH of 6.25 and 6, and ineffective for a pH of 5.75. Bacterial population-to-antimicrobial product concentration ratio appears to play a key role in the effectiveness of the antimicrobial. This conclusion has been drawn based on the fact that differing levels of effectiveness have been observed for exposure at the same pH such as 5.1 in adjusted pH approach (SL1 and SL2) and pure biogenic acidification (SL3) approach. It could be concluded that pH of the environment is not the only indication of severity of the environment that is required to describe the potential efficacy of the antimicrobial. Product efficacy depends indirectly on pH, but directly related to bacterial population size and activity.

5 Summary

The primary goal of this study was to determine limitations associated with the use of antimicrobial additives against planktonic SOB in bacterial suspensions where the pH, bacterial population, and bacterial activity are controlled. The following conclusions can be made:

- The antimicrobial product efficacy is not only related to pH of the environment, but also to the bacterial population and bacterial activity level.
- A successful testing procedure for testing the efficacy of antimicrobial products should consider the variations in pH, bacterial population, and bacterial activity.
- The antimicrobial product has been shown to be effective in an environment with lower bacterial population and activity (SL1 and SL2). This effectiveness occurs when the pH is greater than 4 (Nothing was tested below 4).
- The antimicrobial product has been shown to be less effective in an environment with very high bacterial population and high bacterial activity (SL3).
- Typically, the antimicrobial product is used either by mixing it with concrete (admixture) and/or applying topically.
- The efficacy of the product in practice may differ from the bacterial suspension tests that are presented here.
- Antimicrobial products are expected to work in delaying or preventing Stage II of MICC.
- The testing procedure presented in this paper may be a good candidate to be standardized for Stage II testing of MICC, particularly to evaluate the antimicrobial efficacy.

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