"Engineered Concrete Sealants: Cutting Edge Technology Against MICC"

Abstract

Microbially induced corrosion of concrete (MICC) significantly reduces the service life of concrete infrastructure, leading to economic and environmental concerns. The following study evaluates the antimicrobial activity of Kismet Antimicrobial Technologies (AgCNP and X-CNP) against *Acidithiobacillus thiooxidans,* a key sulfur-oxidizing bacterium responsible for MICC. The minimum inhibitory concentration (or MIC) of the nanoparticle formulations (AgCNP and X-CNP) was determined using a well diffusion assay, with results indicating that both AgCNP and X-CNP exhibited antimicrobial activity at a minimum inhibitory concentration (bacteriostatic) of 0.2 mg/mL. Further pH analysis of the liquid cultures demonstrated that the treated samples maintained a higher pH, indicating suppression of bacterial growth. Due to its superior solubility, AgCNP in mineral spirits (AgCNP-MS) was incorporated into a concrete sealant and tested against *Staphylococcus* spp. (modified ASTM E2197-11 protocol). Increasing AgCNP-MS concentrations correlated with enhanced bacterial reduction, achieving a 4-log reduction in both 5% and 10% concentrations of the solvent mixture, which is approximately a maximum concentration of 1.7 mg/mL or 0.2% by weight of AgCNP-MS within the final product of the concrete sealant. These findings suggest that AgCNP-MS is a promising antimicrobial additive for mitigating MICC in concrete infrastructure. A final study tested the two different concentrations of AgCNP-MS against *Acidithiobacillus thiooxidans* to verify the efficacy of the final product. All concentrations resulted in the complete eradication of *A. thiooxidans,* reflecting a saturation point of the silicone-based sealant with 5% AgCNP-MS, or approximately a maximum of 0.1% by weight of AgCNP, or 0.85 mg/mL, needed for efficacy against *A. thiooxidans* under the following testing conditions.

1. Introduction

Concrete structures in wastewater infrastructure and in acidic environments where sulfur or thiosulphate is available are susceptible to a biodeterioration process called microbially induced corrosion of concrete (MICC). MICC can occur when sulfate-reducing and sulfur-oxidizing bacteria, which generate sulfuric acid, aggressively degrade concrete surfaces, reducing the expected service life of concrete from 100 years to 30 to 50 years (Fomina et al., 2020). The premature deterioration of infrastructure can lead to operational disruptions, safety concerns, and environmental risks, underscoring the need for effective mitigation strategies to minimize economic losses. Sewage infrastructure restorations alone are estimated to cost in the US range up to USD 1.6 trillion in total (Wang et al 2023).

Biogenic acidification of concrete is primarily achieved by sulfur-oxidizing bacteria (SOB), such as *Acidithiobacillus thiooxidans* (Pramanik et al., 2024). Mitigation strategies to decrease the effects of MICC include the use of concrete admixtures and surface-applied antimicrobial sealers, which can reduce the impact of bacterial

colonization. Kismet Technologies has developed a range of ceria-based antimicrobial technologies designed to inhibit the MICC process, with a particular focus on applications in concrete sealants. The two technologies are silver-mediated cerium oxide nanoparticles, abbreviated as AgCNPs, and chitosan-mediated cerium oxide nanoparticles, abbreviated as X-CNPs.

The study was designed to determine the minimum inhibitory concentration (MIC) of several Kismet technologies in *Acidithiobacillus thiooxidans* and to evaluate which technology presented the higher antimicrobial activity. The study also measures pH throughout the cultivation of *A. thiooxidans* when antimicrobial technologies are added to the broth media to understand the inhibition of sulfur-oxidizing bacteria. Based on the MIC and liquid culture results, a formulation was created using a silicone-based sealant with Kismet Technologies nanoparticles. The final formulations were then tested for efficacy against both gram-positive and gram-negative bacteria.

2. Methodology

2.1 Agar well diffusion test to determine the MIC of *Acidithiobacillus thiooxidans*

The minimum inhibitory concentration (MIC) of *Acidithiobacillus thiooxidans* was determined using a modified well diffusion test protocol. While the Kirby-Bauer disk diffusion method is the standard for MIC determination, the conventional medium, Mueller-Hinton Agar, does not support the optimal growth conditions required for *A. thiooxidans* (Hudzicki, J., 2009). To ensure accurate antimicrobial assessment, the well diffusion method was adapted in accordance with established guidelines for sulfur-oxidizing bacteria, maintaining conditions that are conducive to their growth and metabolic activity (ATCC, 2024). This method evaluated the antimicrobial activity of Kismet's nanoparticle technologies (AgCNP and X-CNP), wherein the antimicrobial agents diffuse through the agar medium and inhibit bacterial growth (Balouiri et al., 2016). The thiobacillus agar was further modified with bromocresol green to enhance colony visualization, allowing for clear differentiation of growing colonies (Starosvetsky et al., 2013).

A bacteria suspension was made using a sterile loop. Four bacteria colonies were added to 3.5 mL of 1X Tris Buffer Saline (TBS), also known as 1X solution containing Tris/Tris-HCl 25 mM, NaCl 0.13M & KCl 0.0027M solution. The turbidity of the bacterial suspension was compared with the 0.5 McFarland standard, and the turbidity was adjusted by adding more microorganisms if the suspension was too light or by diluting it with 1X TBS if the suspension was too dense (Hudzicki, J., 2009).

The bacteria suspension was streaked three times on the agar by using a cotton swab. The lid of the inoculated plates was slightly open to allow the surface of the agar plates to dry for no longer than 15 minutes (Hudzicki, 2009). The treatments (AgCNP and

X-CNP) were added to each well of the agar, with several concentrations tested, including 0.1, 0.2, 0.3, and 0.4 mg/mL of each treatment. Additionally, each test was compared to a positive control (without any antimicrobial product) and a negative control, which was a sterility control using Thiobacillus Agar. The plates were incubated at 30°C for 12 days, during which time growth was observed. The inhibition zone diameters were measured after the incubation period.

2.2 pH and bacteria viability in *A. thiooxidans* Liquid Culture with Kismet Antimicrobial Technologies

A liquid culture with AgCNPs and X-CNPs was performed to determine the delay in acidification of the media when the nanoparticles were added. Antimicrobials technologies were added to the Thiobacillus broth at several concentrations, including 0.2, 0.3, 0.4, and 0.5 mg/mL, respectively. The different treatment variants were diluted according to ATCC Medium 125 (ATCC, 2024). The initial concentration of the microbe was determined by hemocytometer counting (cells/mL) for an initial inoculum of 8.58e+04 cells/mL of *A. thiooxidans* in each liquid solution at time point 0 minutes, and pH was measured. The experiment included a positive control (without an antimicrobial product) and a negative control (without bacterial inoculation). At the end of the incubation period (22 days), the pH was measured to obtain the final results.

2.3 Efficacy test of sealant product Methodology

Following MIC and liquid culture testing, AgCNP was selected for incorporation into a concrete sealant due to its antimicrobial efficacy. Different concentrations of AgCNP were tested to determine the best concentration of bactericidal activity. The initial test was completed against a *Staphylococcus spp.* inoculating a 1-inch by 1-inch area of the concrete surface. A 10 **µL** suspension of *Staphylococcus* was applied to each area and spread evenly, followed by a 24-hour inoculation challenge time. After the challenge time, sterile swabs were used to collect any bacteria remaining on the surface of each inoculated square and transferred into 2.5 mL of 1X Phosphate Buffered Saline (PBS), also known as a 1X solution concentration of NaCl (0.137 M), KCl (0.0027 M), and, most importantly, for the nanoparticles a concentration of 0.0119 M of Phosphates, which is a known neutralizer for the AgCNP (Singh et al., 2011). A 1 mL aliquot was then plated on 3M Petrifilm pads and incubated for 24 hours before reading colonies/bacterial enumeration.

2.4 Efficacy test of Sealant product Methodology (against *A. thiooxidans)*

Each paver was coated with one of the following treatments: silicone-based sealant (SBS), SBS+5% AgCNP in solvent, or SBS+10%AgCNP in solvent. The solvent used was mineral spirits (abbreviated to AgCNP-MS). Once the concrete pavers were coated with

treatments and dried, they were then inoculated with 100 **µL** of *A. thiooxidans* and subjected to a 2-hour challenge time to evaluate the antimicrobial efficacy of each treatment. Given that *A. thiooxidans* thrives in a wet environment, the inoculated pavers were placed in a Nalgene jar, partially submerged in 40 mL of thiobacillus broth, sealed with parafilm, and incubated in an environmental chamber at 22°C with 85% relative humidity.

After the 2-hour challenge, the pavers were removed and submerged in a neutralizing solution inside a Nalgene jar (Picture 1). The jar was then shaken to dislodge any surviving bacteria into suspension. The resulting suspension was processed through a membrane filtration system, and the filter was subsequently placed on thiobacillus agar. The plates were incubated at 30°C for 12 days to assess bacterial survival and determine the efficacy of each treatment compared to the controls.

Picture 1. Paver with Sealant testing for efficacy

3. Results

3.1 Agar well diffusion test to determine the MIC of *Acidithiobacillus thiooxidans*

The zone of inhibition of *A. thiooxidans* increases when the concentration of nanoparticles increases from 0.2 to 0.5 mg/mL of AgCNP and X-CNPs (Figure 1). Previous testing did not show any antimicrobial efficacy at 0.1mg/mL, but it did at 0.2 mg/mL (data not shown). The concentration that showed the highest antimicrobial activity was 0.5 mg/mL, and the minimum concentration that showed antimicrobial activity (bacteriostatic) was 0.2 mg/mL (Figure 1). The positive control didn't show any inhibition of growth,

proving that the inhibition of growth was caused only by the antimicrobial agents diffused in the agar (Figure 2). The negative control showed no growth, and the agar media remained blue, indicating the absence of growth. The agar was supplemented with bromocresol green (BCG), which under acidic conditions turns yellow and at pH above 3.8 turns blue (Figure 2). *A. thiooxidans* absorbs BCG, and colonies appear bright yellow instead of their usual transparent pigmentation. The absence of growth at the negative control ensured there was no cross-contamination when performing the test.

X-CNPs exhibited the highest antimicrobial activity against *A. thiooxidans*. However, when incorporated into silicone-based sealant, X-CNPs demonstrated limited solubility capabilities. Therefore, AgCNPs were chosen to be incorporated into the silicone-based sealant, as it also demonstrated antimicrobial activity against the microbe while addressing the solubility issue.

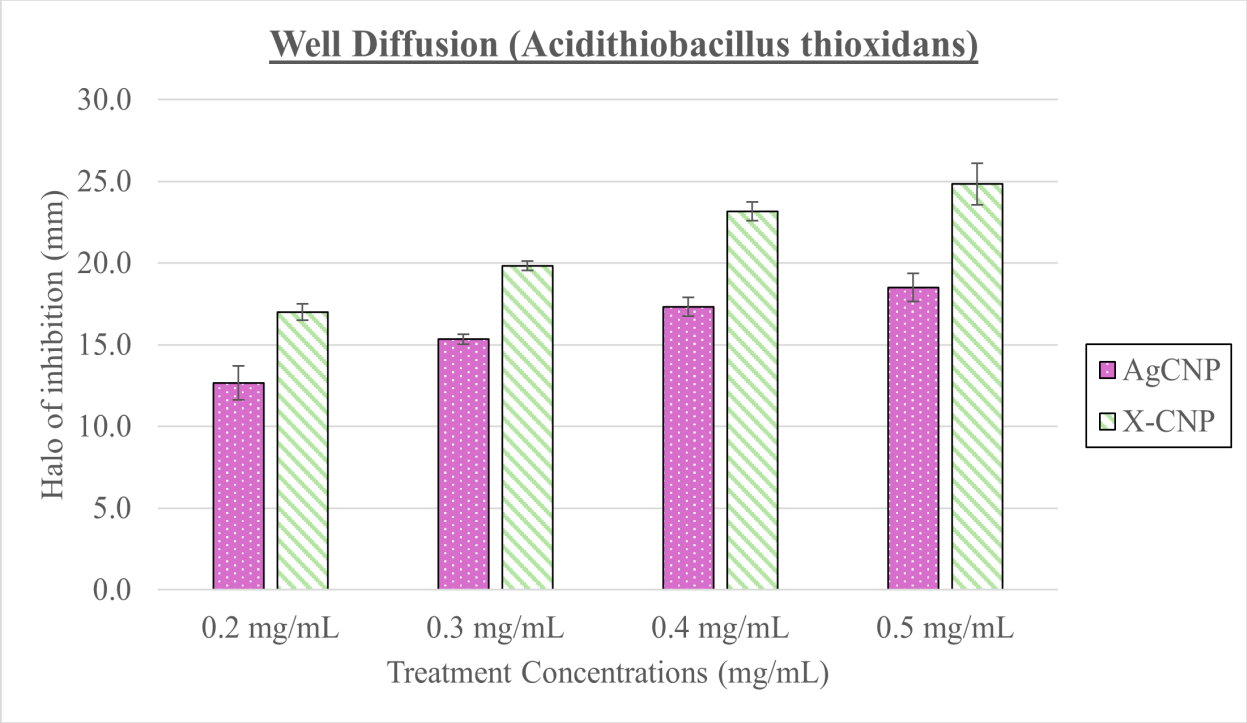

Figure 1. Zone of inhibition diameters (mm) of *A. thiooxidans* using AgCNPs and X-CNPs as an antimicrobial agent.

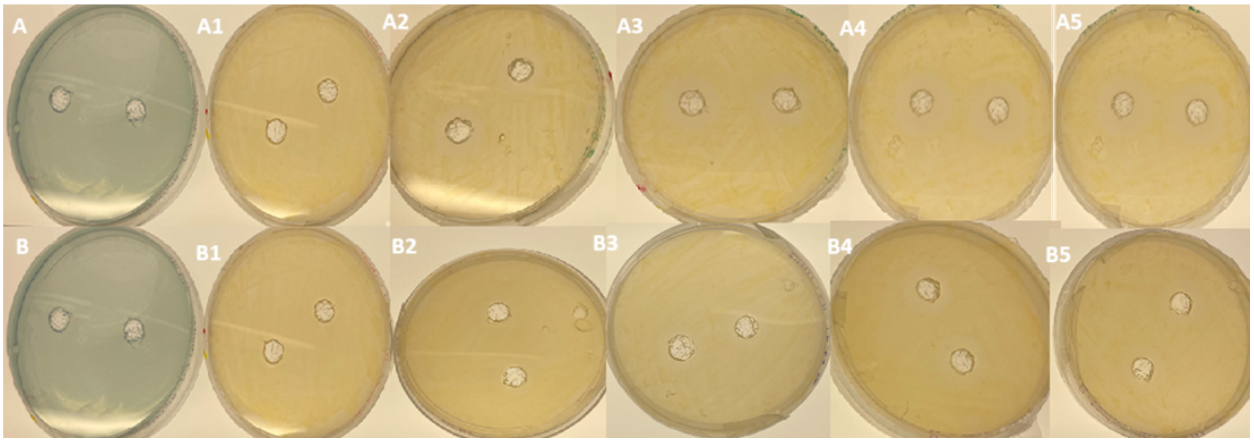

Figure 2. Agar well diffusion test of AgCNPs and X-CNPs using *A. thiooxidans* as a test microorganism. Row A corresponds to the results of X-CNPs, and row B to AgCNPs. A/B: negative control, A1/B1: positive control, A2/B2: 0.2 mg/mL, A3/B3: 0.3mg/mL, A4/B4: 0.4 mg/mL, and A5/B5: 0.5 mg/mL.

3.2 pH and bacteria viability changes at liquid culture of *A. thiooxidans* supplemented with Kismet Antimicrobial Technologies

As shown in Figure 3, the pH of positive control was lower than that of the treated cultures. The decrease in pH resulted from the bacteria's oxidation of thiosulfate to sulfuric acid, a primary indicator of the bacteria's viability. The antimicrobial agents (AgCNPs and X-CNPs) inhibited bacterial growth and mitigated the reduction in pH, which is a good indicator of MICC reduction. Among the treatments, X-CNPs exhibited the highest pH values, which could indicate less viable bacteria or suppression of bacteria growth. These results align with the results obtained by the well diffusion test. As mentioned earlier, despite the high antimicrobial performance of X-CNPs, AgCNPs were selected for incorporation into the silicone-based sealant due to their superior solubility in the mineral spirits solvent.

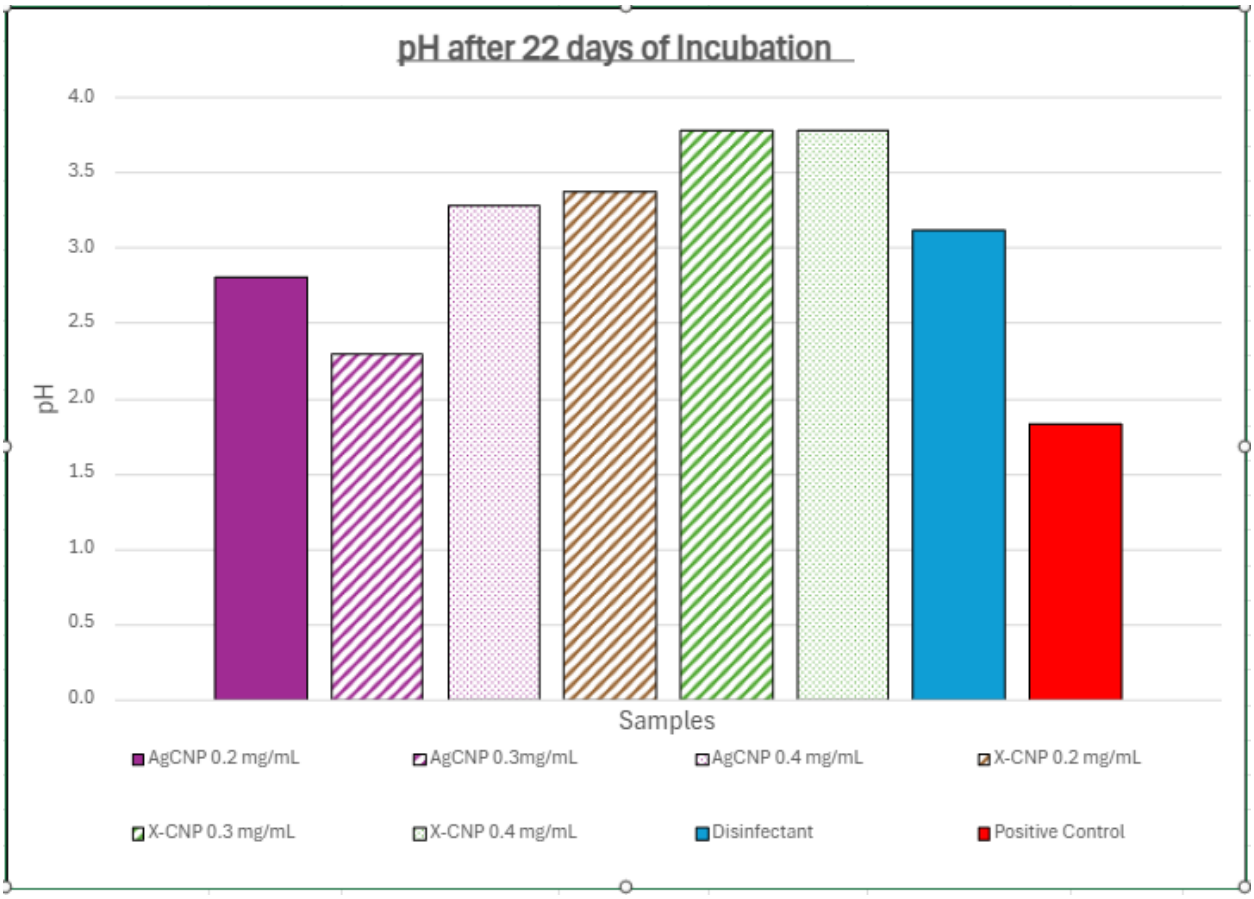

Figure 3. pH at the end of the incubation time of *A. thiooxidans* when exposed to Kismet antimicrobial technologies – AgCNPs and X-CNPs.

3.3 Dry coupon efficacy test

Silver-mediated cerium oxide nanoparticles dispersed in mineral spirits (AgCNP-MS) were incorporated into silicone-based sealant at different concentrations: 1% AgCNP-MS into the silicone-based sealant, 2% AgCNP-MS into the silicone-based sealant, 5% AgCNP-MS into the silicone-based sealant, and 10% AgCNP-MS into the silicone-based sealant. A positive and negative control was also used, which consisted of the silicone-based sealant without any AgCNP-MS. In addition, both positive and negative controls were included. The positive control consisted of untreated silicone-based sealant inoculated with *Staphylococcus* spp., while the negative control was left uninoculated to confirm the absence of cross-contamination during testing. Results from the 24-hour challenge assay demonstrated a dose-dependent increase in bacterial log reduction with increasing concentrations of AgCNP-MS. Incorporating AgCNPs into mineral spirits effectively enabled their solubility and uniform dispersion within the sealant. These results confirm that the modified sealant formulation exhibits antimicrobial activity against *Staphylococcus* spp., demonstrating the bactericidal efficacy of the AgCNP-MS system. (Table 1).

Sample	Bacterial Recovery after 24 Hours	Log Reduction
Silicone Sealant- 24hrs (Positive Control)	4.31E+03	
Silicone Sealant with 1% AgCNP-MS	1.78E+03	0.385207
Silicone Sealant with 2% AgCNP-MS	1.34E+02	1.580249
Silicone Sealant with 5% AgCNP-MS	1.33E+00	3.509874
Silicone Sealant with 10% AgCNP-MS	0.00E+00	3.634813 (i.e.. complete reduction)

Table 1. Dry coupon efficacy test when *Staphylococcus* spp. is exposed to Silicone Sealant at several concentrations of AgCNP.

The highest levels of antimicrobial efficacy were observed in formulations containing 5% and 10% AgCNP-MS, corresponding to approximately a maximum concentration of 0.1% and 0.2% AgCNPs by weight, respectively. These two concentrations demonstrated the greatest log reduction in bacterial recovery over 24 hours, with the 10% formulation achieving complete eradication of *Staphylococcus* spp. Given their superior performance in the dry coupon efficacy test, the 5% and 10% AgCNP-MS formulations were selected for subsequent antimicrobial testing against *Acidithiobacillus thiooxidans.*

3.4 Efficacy test of AgCNP-modified Sealant against *A. thiooxidans*

The antimicrobial efficacy of modified Silicone-based sealant (SBS) formulations against *Acidithiobacillus thiooxidans* was assessed through a 2-hour wet contact assay (modified from the generic ASTM protocol E2197). Both formulations tested – SBS with 5% and 10% AgCNPs dispersed in mineral spirits (AgCNP-MS) demonstrated identical bactericidal performance against the Gram-negative bacteria. Both achieved a log reduction of 4.11 (Table 2). These results indicate a high level of efficacy under both formulations, suggesting a potential saturation point in antimicrobial performance under the tested conditions. This has led to the final formulation being selected as 5% AgCNP-MS in silicone-based sealant.

Carrier	Rep	CFU/carrier	Log Density per carrier	Mean LD
Positive Control	1	1.32E+04	4.12	4.11
	2	1.28E+04	4.11	
	3	1.26E+04	4.10	
Negative Control	1	0.00E+00	0	0
	2	0.00E+00	0	
	3	0.00E+00	0	
Sealant with 5% AgCNP in solvent	1	0.00E+00	0	0
	2	0.00E+00	0	
	3	0.00E+00	0	
Sealant with 10% AgCNP in solvent	1	0.00E+00	0	0
	2	0.00E+00	0	
	3	0.00E+00	0	

Carrier	Rep	CFU/carrier	Log Density per carrier	Mean LD	Log Reduction
Positive Control	1	1.32E+04	4.12	4.11	-
	2	1.28E+04	4.11		
	3	1.26E+04	4.10		
Negative Control	1	0	0	0	No bacteria
	2	0	0		
	3	0	0		
Sealant with 5% AgCNPin solvent	1	0	0	0	4.11
	2	0	0		
	3	0	0		
Sealant with 10% AgCNP in solvent	1	0	0	0	4.11
	2	0	0		
	3	0	0		

Table 2. Log reduction of A. thiooxidans after 2-hour exposure to silicone-based sealant and silicone-based sealant with AgCNP. Note that both positive and negative controls were run on surfaces treated with the unmodified sealant demonstrating the effect of the AgCNP.

4. Conclusion

The study presented promising outcomes to mitigate the MICC caused by *A. thiooxidans*. The incorporation of Kismet Antimicrobial Technologies into the concrete sealants represents an attractive outcome for the construction and water system sectors. AgCNP and X-CNP showed inhibition of the bacteria growth at a minimum concentration of 0.2 mg/mL. There was a positive correlation between the concentration of the microbial agent and the diameter of the zone of inhibition; as the concentration increased, the inhibition also increased (from 0.2 to 0.5 mg/mL). These results were confirmed by the pH values obtained in the liquid culture, where AgCNP and X-CNP exhibited the highest pH values compared to the positive control.

Silver-mediated cerium oxide nanoparticles (AgCNPs) were chosen for incorporation into the silicone-based sealant due to their antimicrobial activity against sulfur-oxidizing bacteria and their high solubility when added to the formulation. The incorporation of AgCNP into the sealant resulted in a significant reduction in bacterial survivability on the concrete surface. The higher the concentration of AgCNP, correlated with increased bacterial reduction, with the 10% AgCNP-MS concentration achieving complete eradication of *Staphylococcus spp.* Log reductions indicate that AgCNPs effectively enhance the antimicrobial properties of the concrete sealant, reducing bacteria degradation of the concrete. Additionally, the AgCNP-MS formulations were later tested against *A. thiooxidans*, a key contributor to MICC. Both treatments resulted in a 4.11 log reduction, a complete eradication of *A. thiooxidans*. Log reductions indicate that AgCNP effectively enhances the antimicrobial properties of the concrete sealant, reducing bacteria degradation of the concrete.

5. References

[1] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, *6*(2), 71-79.

[2] Starosvetsky, J., Zukerman, U., & Armon, R. H. (2013). A simple medium modification for isolation, growth and enumeration of Acidithiobacillus thiooxidans (syn. Thiobacillus thiooxidans) from water samples. *Journal of Microbiological Methods*, *92*(2), 178-182.

[3] Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology*, *15*(1), 1-23.

[4] Pramanik, S. K., Bhuiyan, M., Robert, D., Roychand, R., Gao, L., Cole, I., & Pramanik, B. K. (2024). Bio-corrosion in concrete sewer systems: Mechanisms and mitigation strategies. *Science of The Total Environment*, 171231.

[5] Fomina, E. V., Sverguzova, S. V., Goncharova, E. N., & Flores-Vivián, I. (2020). Simulation of accelerated concrete destruction by inoculation of an association of bacteria of the genus Thiobacillus. In *IOP Conference Series: Materials Science and Engineering* (Vol. 945, No. 1, p. 012008). IOP Publishing.

[6] Wang, D., Guan, F., Feng, C., Mathivanan, K., Zhang, R., & Sand, W. (2023). Review on microbially influenced concrete corrosion. *Microorganisms*, *11*(8), 2076.

[7] ATCC, "*Acidithiobacillus thiooxidans* (Waksman and Joffe) Kelly and Wood 8085" , 8 March 2024. Acidithiobacillus thiooxidans (Waksman and Joffe) Kelly and Wood - 8085 | ATCC

[8] Singh, S., Dosani , T., Karakoti , A., Kumar, A., Seal, S., & Self, W. (2011, June 24). *A phosphate-dependent shift in redox state of cerium oxide nanoparticles and its effects on catalytic properties*. Biomaterials. https://www.sciencedirect.com/science/article/abs/pii/S0142961211006442?via%3Dihub