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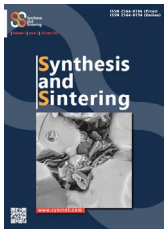
Synthesis and Sintering

ISSN 2564-0186 (Print), **ISSN** 2564-0194 (Online)



Research article

The effect of synthesized Cu_2O on the microbial corrosion inhibition of urban sewer systems

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A B S T R A C T

The microbial corrosion of reinforced concrete sewers was inhibited by synthesized cuprous oxide (Cu_2O) nanoparticles. The antibacterial characteristics of Cu_2O on Acidithiobacillus thiooxidans were investigated by temporal variation of pH, turbidity, and bacterial counting. Three reinforced concrete samples with different weight percentages of electrodeposited Cu_2O (0.06 wt%, 0.055 wt%, 0.05 wt%) were used. The bacterial counting showed that the number of bacteria in samples with 0.06, 0.055, and 0.05 wt% of Cu_2O was 4.82, 4.42, and 2.94 times lower than the blank sample (BS), respectively. After the bacterial growth, the optical density measurement showed that the percentage of turbidity enhancement for samples with 0.06, 0.055, and 0.05 wt% of Cu_2O were 108%, 118%, 165%, respectively, while it was 412% for the BS. Moreover, the pilot stage's pH monitoring revealed that the electrodeposited Cu_2O lowered the concentration of hydronium between 7 to 81 times compared to the BS. Experiments indicated that slight changes in the amount of electrodeposited Cu_2O lead to significant changes in samples' ability to hinder bacterial growth and microbial-induced corrosion.

KEYWORDS

Microbial corrosion
Acidithiobacillus thiooxidans
Concrete sewers
Synthesized cuprous oxide
Nanoparticles

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1. Introduction

Microbial induced corrosion (MIC) of reinforced concrete (RFC) sewer systems is a widespread and costly problem in many major cities [1, 2]. Under corrosive conditions, the structural integrity and the load-bearing capacity of the concrete decreases. Therefore, sewer pipes may crack and collapse [3, 4]. The subsequent repair and replacement of damaged sewer pipes are costly, and conventional corrosion preventing methods are too expensive [5, 6].

The corrosion in sewer systems is mainly caused by the biogenic sulfuric acid reactions with the concrete cementitious materials [7–10]. First, under the anaerobic condition of the slime layer (biofilm) at the bottom of the pipe wall, sulfate-reducing bacteria (SRB) such as Desulfovibrio sp. produces hydrogen sulfide (H_2S) from sulfur

Abbreviations

At	Acidithiobacillus thiooxidans
Blank	Reinforced concrete without Cu_2O
BS	Blank Sample
Cu_2O	Cuprous Oxide
I	Reinforced concrete with 0.06 wt% of Cu_2O
II	Reinforced concrete with 0.055 wt% of Cu_2O
III	Reinforced concrete with 0.05 wt% of Cu_2O
MIC	Microbial Induced Corrosion
OD	Optical Density
RFC	Reinforced Concrete
SOB	Sulfur Oxidizing Bacteria
SRB	Sulfate Reducing Bacteria

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Received 5 June 2021; Received in revised form 17 June 2021; Accepted 22 June 2021.

https://doi.org/10.53063/synsint.2021.1233

compounds present in the sewage [9, 10]. Then, H_2S diffuses into the wastewater flow and portions of its release into the pipe headspace [11, 12]. Lastly, under the aerobic condition that exists on the concrete surface above the waterline, sulfur-oxidizing bacteria (SOB) such as Acidithiobacillus thiooxidans (At) use H_2S and other sulfur compounds as a source of energy and oxidize them to biogenic sulfuric acid [13, 14].

Acidithiobacillus bacteria start colonization at the pH range of 6.5–8.5. During colonization, a harmful acidic condition (pH = 0.5–1) is formed on the concrete surface [2, 9, 15]. The reaction between the produced sulfuric acid and the main cement material ($Ca(OH)_2$) leads to the formation of gypsum ($CaSO_4.2H_2O$) and ettringite ($3CaO.Al_2O_3.(CaSO_4)_3.32H_2O$). These materials expand and cause internal cracking at the concrete interface and reduce the lifetime of the concrete. Therefore, controlling MIC is mainly related to the At activity [14, 16].

Recently, several methods have been used to protect concrete wastewater structures against MIC. Preventing microbial reproduction and metabolism in sewage, and inhibiting or reducing the formation of biological sulfuric acid are the two fundamental methods of controlling MIC [4, 13, 17–19]. There are two approaches: concrete modification and environmental treatment [1, 4, 19]. Concrete modification focuses on using more resistant materials against acidity, impermeability, and cracking, incorporating the antimicrobial agent into the concrete mix and coating systems [4, 17, 20, 21]. Controlling the pH and sulfate level with chemical agents are critical parameters in microbial activity protection. However, using chemical agents is not environmentally-friendly [8, 22, 23].

Nowadays, some different coating methods have been investigated. Spray lining is a widely used coating technique in which a high pH mixture with the aim of neutralization of acid and SOB deactivation is sprayed on the crown area of concrete [6, 17]. But poor adhesion between the coating material and concrete substrate was the main reason to consider other techniques. Electrodeposition is one of the recent concrete sewer pipe coating methods based on the precipitation of bio-toxic materials into the porous concrete substrate. This coating type is stable over time and is more effective on partially corroded concrete pipes [6, 24, 25].

Some of the previously reported suitable bactericide materials for concrete coating are iodopropargyl compounds (copper, zinc, lead, nickel, organic tin), metal oxides (copper, zinc, lead, manganese, silver), etc. [14, 18, 26, 27]. Recently, the bactericidal effects of nanoparticles, such as Ag, TiO_2, ZnO, Cu, and CuO in the prevention and control of concrete deterioration have been greatly reported [17, 18, 28]. Depending on the bacterial cell concentration, the leaching of nanoparticles from loosely adhered nanofilm, coated on concrete pipes' inner walls could significantly inhibit bacterial growth [17, 28].

Many studies demonstrated that copper oxides have high antimicrobial activity; they can absorb and denature proteins [17, 18, 28]. The bactericidal characteristics of cuprous oxide and functionalized zeolite coating against the At were reported [29, 30]. Also, the modification of electrodeposition of Cu_2O as an antimicrobial agent on the RFC substrate to control microbial corrosion in concrete sewers was studied in the authors' previous work [24]. Following that study, experimental evaluation of the bactericidal characteristics of the Cu_2O coated RFC pipes against the At is reported in this paper. To meet this end, temporal variation of pH, turbidity, and count of the aforementioned bacteria were measured.

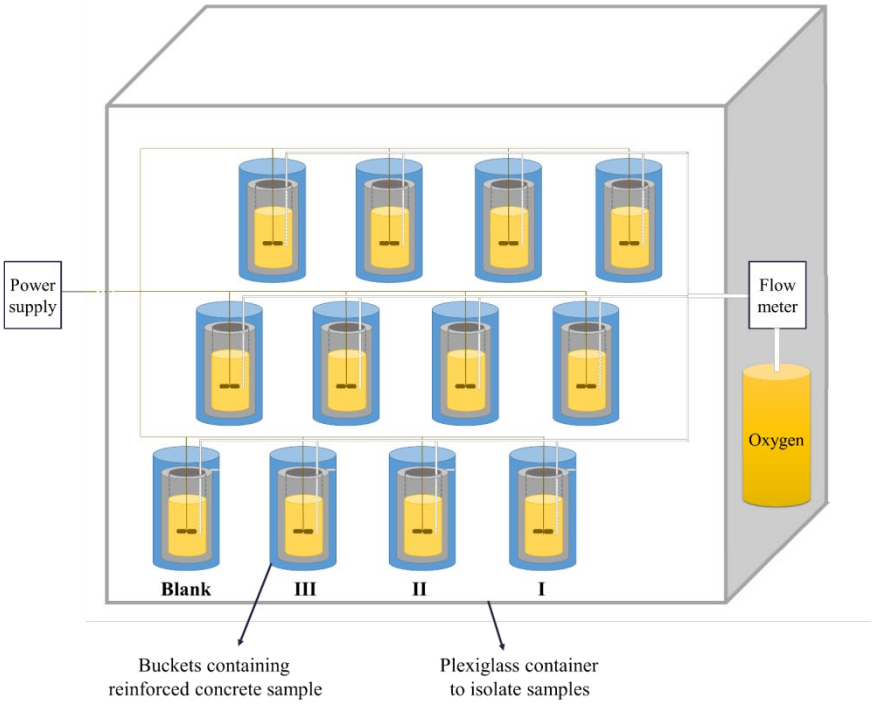

Fig. 1. The schema of the lab-scale pilot and reinforced concrete samples.

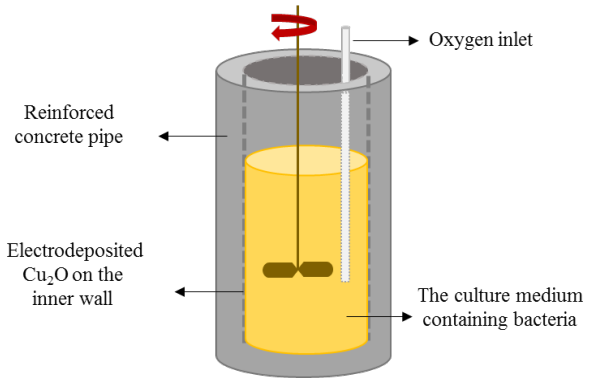

Fig. 2. The lab-scale semi-continuous reinforced concrete samples.

2. Materials and methods

2.1. Microorganism and preparation of the culture medium

Acidithiobacillus thiooxidans bacteria were prepared from the bacterial collection center of Sarcheshmeh Copper Complex. They were cultivated based on the iron-free 9k growth medium [26, 27]. A basal nutrient medium (BNM) containing (in g/L): $(NH_4)_2SO_4$ (0.2), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.25), and KH_2PO_4 (3.0) was dissolved in deionized water and autoclaved (Tazehyam company, Iran) at 121 °C for 20 min, to prepare the culture medium, sulfur (10 g/L) was autoclaved separately and added to the liquid culture medium. After autoclaving the culture medium and sulfur separately, sulfur was powdered (particles with an average diameter of 33.2 µm ± 5.4 µm) under semi-sterile conditions and then added to the culture medium. Sodium hydroxide (2 M) and sulfuric acid (0.5 M) were used to control the pH. Sulfur and all the salts used in the culture medium were prepared from Merck.

2.2. Bacterial growth conditions

For the inoculation stage, the culture media contained 15% (v/v) of the bacteria from a ten-day culture with a dry cell weight of 105 mg/L and pH of 3.8–4. The optimal pH for bacterial growth was adjusted to 2 by the sterile sulfuric acid (0.5 M). Then, the culture medium was incubated at 34 °C and 130 rpm for 144 hours (the aerosol incubator shaker KS4000icontrol, Tazehyam Company, Iran). Then, the bacteria were in the logarithmic growth stage, and the pH was about 1.2.

2.3. Preparation of concrete samples with different Cu_2O weight percentages

Concrete samples with different Cu_2O weight percentages were prepared based on our previous work [24]; a brief description of method is as follows. The inner part of synthesized RFC samples was poured by alkalizes Cu (II) lactate, as the solution of the electrodeposition process. Lactic acid was used as a ligand to stabilize Cu^{2+} in electrodeposition alkaline bath. The compositions of samples I, II, and III were as follow: Solution (I): the solution consisted of 0.6 M copper sulfate ($CuSO_4$) and 3 M lactic acid as a chelating agent to form the copper lactate complex (45 g of copper sulfate was dissolved in 75 mL of 88% lactic acid). The pH of the solution was adjusted to 9 by adding 5 M NaOH aqueous solution. Solution (II): it contained 0.4 M of $CuSO_4$ and 3 M of lactic acid, with the pH about 9 by adding 4 M NaOH. Solution (III): copper sulfate and lactic acid had a lower concentration in this solution, 0.1 M and 0.75 M, respectively and the bath pH was carefully adjusted to 9 by controlled addition of 2 M NaOH.

Cu_2O can only be synthesized in a limited potential range and current density. The stable potential ranges for deposition of Cu_2O in alkalize Cu (II) lactate solutions were from -0.15 to -0.1 V vs SCE and the current densities for galvanostatically deposition ranged from 0.05 to 3.0 mA/cm^2. Reference [24] contains all details of electrodeposition processes.

2.4. Experimental conditions in the pilot stage

Three RFC pipe samples, each one with three replicates in which Cu_2O as an antimicrobial agent was electrodeposited on their surface, were employed. Samples are labeled based on the concentration of Cu_2O: sample I with 0.06 wt% of Cu_2O; sample II with 0.055 wt% of Cu_2O, and sample III with 0.05 wt% of Cu_2O. The details of these sample electrodeposition process were explained in a previous study [24]. Each sample was considered a semi-continuous reactor exposed to synthetic sewage (culture medium and bacteria) to test the antibacterial activity of the Cu_2O coating. Fig. 1 demonstrates the schema of the lab-scale pilot.

Fig. 2 shows the lab-scale semi-continuous RFC samples. Each samples internal capacity was about 180 mL, of which 68% was filled with sterilized culture medium and the sulfur powder, 12% was filled with bacteria selected from the six-day cultivation, and the remaining space volume (the 20% volume) was empty. The empty volume was considered to simulate the laboratory samples conditions similar to the actual sewer pipes where the top part is empty. Because 20% (v/v) of the concrete volume was empty, the ratio of 12% bacteria infusion to 68% culture medium is identical to the percentage of 15% bacteria infusion to 85% culture medium. The experiments were run at the temperature of 25 ± 2 °C, a rotational speed of 90 rpm, and an oxygen flow rate of 1 L/min [28]. Every other day, eleven milliliters of the samples were taken for analysis and replaced by 11 mL of culture medium with 0.11 g sulfur at a pH of about 4. The culture medium was maintained at a constant level by injecting sterile deionized water.

The pH changes of the samples were measured to control bacterial growth. The pH of the culture medium in the electrodeposited concrete samples and BS were monitored every 48 hours over 45 days in the

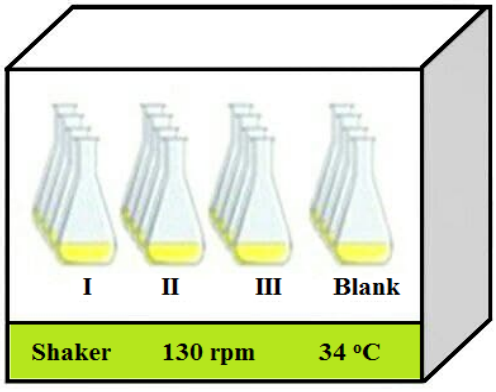

Fig. 3. The schematic of biofilm test stage (sample incubation).

pilot stage. Besides, the caps and the buckets' inner walls containing the samples were sterilized by 70% isopropyl alcohol (IPA) after each pH was measured.

2.5. Experimental conditions in the biofilm test stage

After completing the pilot phase, which lasted for 45 days, the culture medium was removed entirely from RFC samples. The biofilm had to be removed from the concrete surface immediately after removing the culture media, which was performed under sterile conditions near the flame and a sterilized laminar airflow hood. The internal surface of samples (2 g of each RFC sample) was crushed to equalize the conditions for all samples. Then the crushed concrete was dried for 12 hours in an incubator at 34 °C under sterile conditions. After drying, the specimens were grinded with sterilized mortar and pestle and then weighed.

Since three replicates were considered for each sample (I, II, III, and the blank), twelve concrete samples were studied in the pilot and biofilm tests. Twelve Erlenmeyers (500 mL) were prepared, and 100 mL of each culture medium was poured in each Erlenmeyer and then autoclaved. Following this, one gram of the grinded concrete was added to each Erlenmeyer flask. After inoculation, specimens were stored for 45 days in incubator shakers at 130 rpm and 34 °C. Fig. 3 shows the schemas of the biofilm test stage.

In the biofilm test, phase pH of the culture medium containing the grinded concrete was measured every 48 hours by a Behineh SAT-2000 pH meter. The optical density (OD) of samples was measured at 440 nm [31, 32] every five days by UV-Vis (ultra-wide spectrophotometer-visible S200) in the turbidity analysis. The bacterial growth was measured by Petrof-Hasser count slams every five days.

3. Results and discussion

3.1. pH monitoring results in the pilot stage

pH was monitored in the pilot stage to investigate the bacterial growth. The pH of the culture medium was around 4.5 by sulfur addition, and it was reduced to 4 by adding bacteria. Fig. 4 shows the pH variation of all samples. Over the primary days (4–6 days), the pH variation was similar in different samples, which shows that the electrodeposition of Cu_2O slightly affects the bacterial growth over the initial days. The pH of all samples was reduced during the first month. Then, the pH of the BS continued to drop, while the pH trend of the electrodeposited samples was upward. This shows that the electrodeposition of Cu_2O hinders bacterial growth in the long terms. Even though the effect of Cu_2O electrodeposition is more tangible in long terms, it was also effective during the first month, for example, in samples I, II, and III, the pH of the culture medium was reduced to 2.37, 2.37, and 2.09 in the 30^{th} day, respectively, while the pH of BS was 1.66. This shows that the concentration of hydronium in the BS was 2.18×10^{-2}, while the hydronium concentration in the electrodeposited samples I, II, and III were 4.26×10^{-3}, 4.26×10^{-3}, and 8.13×10^{-3}, respectively. In other words, the electrodeposited Cu_2O lowered the concentration of hydronium at least 2.7 times compared to the BS. The pH reduction can be either due to the bacterial activity or the release of hydroxide ions of the cement into the culture medium [2, 7].

According to the graph, samples I and II showed almost the same performance. The reasons were the preparedness of the growth conditions and the availability of the culture medium for the bacteria during this period. But the increasing trend or constancy of the pH are the signs of a pause of bacterial activity. This pause can be either due to the dissolution of the electrodeposited Cu_2O into the culture medium or the decrease in sulfur oxidation in the culture medium [26, 28, 33].

In all electrodeposited samples, the pH experienced an upward trend after passing a particular period. On the other hand, the BS did not show any increase in the pH. After 45 days, the BS pH was 1.43, while the pH of the electrodeposited samples I, II, and III were 3.34, 3.11, and 2.27, respectively. This shows that the concentration of hydronium in the BS was 3.71×10^{-2}, while the hydronium concentration in the electrodeposited samples I, II, and III were 4.57×10^{-4}, 7.76×10^{-4}, and 5.37×10^{-3}, respectively. In other words, the electrodeposited Cu_2O lowered the concentration of hydronium at most 81 times and at least 7 times compared to the BS in this period. Moreover, slight differences in the amount of electrodeposited Cu_2O resulted in a significant difference in samples' ability to hinder bacterial growth. The pH trend shows that the corrosion preventive effect of the electrodeposited Cu_2O occurs gradually, which is more tangible after one month.

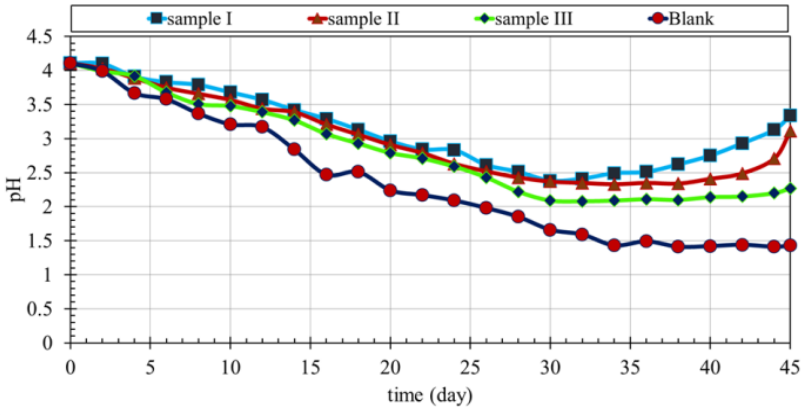

Fig. 4. Variation of the pH in the electrodeposited for samples I, II, III, and the blank in the pilot stage.

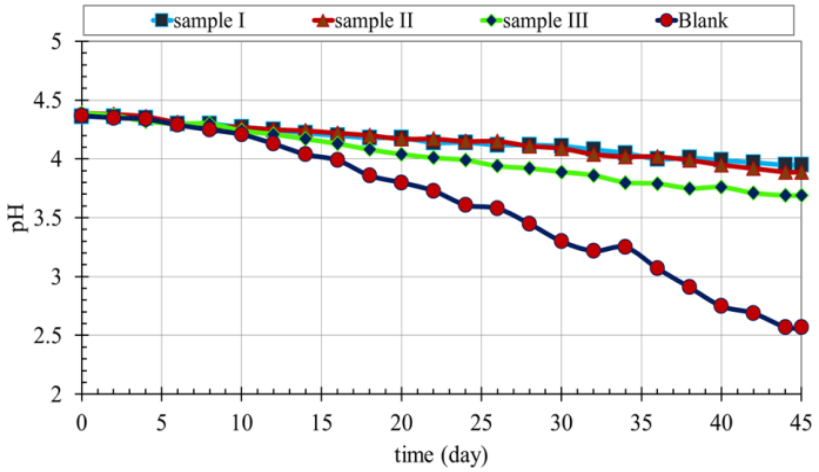

Fig. 5. Variation of the pH in the electrodeposited for samples I, II, III, and the blank in the biofilm test stage.

3.2. pH monitoring, turbidimetry and cell count results in the biofilm test stage

As mentioned in part 3.1, the pH of the culture medium was 4.5 after the sulfur addition. When the grinded concrete was added to the culture medium, in the biofilm test stage, the pH was about 4.35. Fig. 5 shows the pH changes in electrodeposited concrete samples I, II, III, and the BS during the biofilm test stage. As shown in this figure, pH was almost constant until the 7th day for all samples. Therefore, the bacterial growth delay happened during this period. But sudden changes were observed in the pH of the BS after the 12th day. This was a continuous change with an almost constant gradient that continued to the 45th day. Since the evolution of pH is an indicator of bacterial growth and biofilm layer formation, pH reduction improves bacteria growth [34, 35]. The pH changes trend in this figure indicates that the samples with electrodeposited Cu_2O show antibacterial performance. After 45 days, the BS pH was 2.57, while the pH of the electrodeposited samples I, II, and III were 3.95, 3.89, and 3.69, respectively. This shows that the concentration of hydronium in the BS was 2.69×10^{-3}, while the hydronium concentration in the electrodeposited samples I, II, and III were 1.12×10^{-4}, 1.29×10^{-4}, and 2.04×10^{-4}, respectively. In other words, the electrodeposited Cu_2O lowered the concentration of hydronium between 13 to 24 times compared to the BS. Therefore, the electrodeposition of Cu_2O considerably hinders bacterial growth in the biofilm test stage. As was expected, the sample performance with the lowest weight percentage of electrodeposited Cu_2O (sample III) was less intense than the other two samples. Therefore, there is a direct relationship between the antibacterial effect and the amount of electrodeposited Cu_2O.

Fig. 6 shows optical density (OD) results at 440 nm in the turbidity analysis for samples I, II, III, and the blank during the biofilm test stage. As the figure shows, all sample turbidity was almost constant up to the 5th day, and then increased during the test. The turbidity of the samples I, II, III, and the BS changes from 0.061, 0.063, 0.057, and 0.060 on the first day to 0.125, 0.131, 0.181, and 0.307 on the 45th day, respectively. The trend of turbidity in the BS increased with a more significant gradient after the 10th day. The turbidity and the microorganism growth are directly affected by the presence of electrodeposited Cu_2O. The turbidity enhancement factor (changes in turbidity/initial turbidity) after 45 days for samples I, II, and III were 1.08, 1.18, 1.65, respectively, while the turbidity enhancement factor for the blank was 4.12. This shows that the electrodeposition of Cu_2O significantly hinders bacterial growth in the biofilm test stage.

There is a logical relation between the turbidity analysis results and the

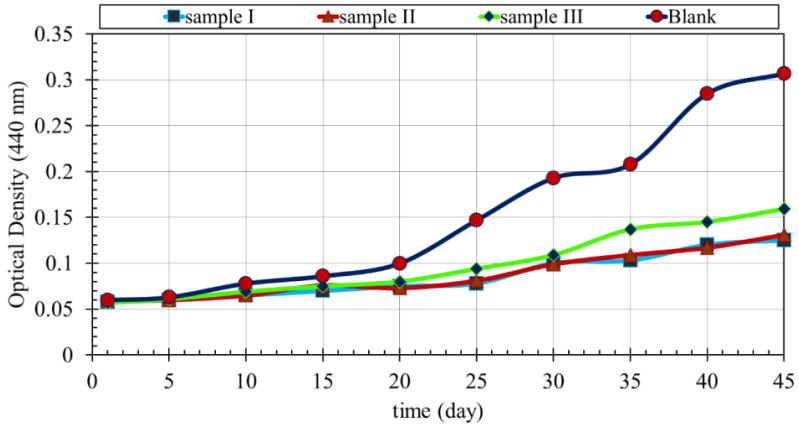

Fig. 6. Variations of optical density (OD) at 440 nm in the turbidity analysis during biofilm test stage.

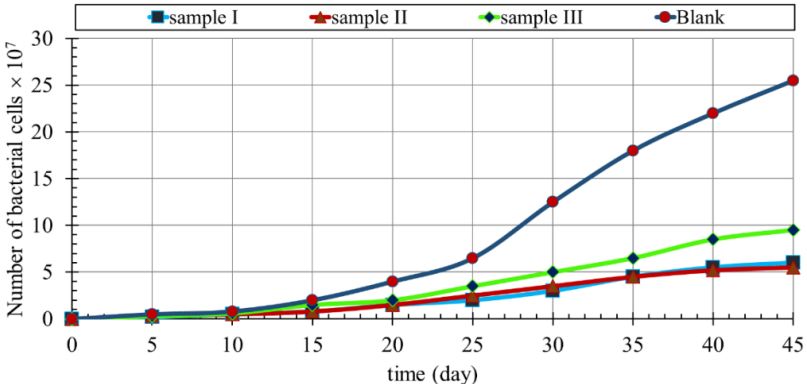

Fig. 7. Results of Acidithiobacillus thiooxidans bacteria counting for samples I, II, III, and the blank in the biofilm test stage.

pH variation in the biofilm test stage. The purpose of this research was to study the growth of At bacteria. These bacteria secrete some acidic byproducts, directly affecting the pH [30]. Therefore, a decrease in the pH values can indicate bacterial growth, which causes greater turbidity values [2, 26]. Besides, cell counting results can confirm the hypothesis that turbidity and pH are inversely proportional and both indicate bacterial growth.

Fig. 7 shows the results of At bacteria counting for the samples I, II, III, and the BS over 45 days of the biofilm test stage. As shown in this figure, no significant changes occurred in the trend of bacterial count over the first five days. The bacteria number slightly increased during the first ten days due to the adaptation of metabolites and enzymes to the new environment. According to the results, the number of bacteria did not change significantly in samples I, II, and III after the 40th day, and these samples almost reached the stationary phase. On the other hand, the number of bacteria in the BS increased significantly over the 45 days of the experiment, and the bacterial growth was still in the logarithmic phase.

The number of the bacteria on the first day were 0.2×107, 0.3×107, 0.3×107, 0.4×107 for samples I, II, III, and the BS, respectively. In comparison, the numbers were 5.5×107, 6×107, 9×107, 26.5×107 at the end of the biofilm test period. This shows that the number of bacteria in samples I, II, and III were 4.82, 4.42, and 2.94 times lower than the number of bacteria in the BS at the end of the biofilm test period.

4. Conclusions

Three concrete samples (I, II, and III) with different weight percentages of electrodeposited Cu_2O were used. In the biofilm test stage, bacterial growth nearly reached the stationary phase in samples (I, II, and III) after 40 days. But, for the BS, bacterial growth was still in the logarithmic phase even until the 45th day. The bacterial counting results showed that in samples I, II, and III, the number of bacteria was 4.82, 4.42, and 2.94 times lower than the BS. After 45 days for samples I, II, and III, the turbidity enhancement factor was 1.08, 1.18, and 1.65, respectively, while the turbidity enhancement factor for the BS was 4.12. This shows that the electrodeposition of Cu_2O significantly hinders bacterial growth.

Moreover, after 45 days, the BS pH was 2.57, while the pH of samples I, II, and III were 3.95, 3.89, and 3.69, respectively. In other words, the electrodeposited Cu_2O lowered the concentration of hydronium about 24, 21, and 13 times in samples I, II, and III compared to the BS, respectively. Therefore, the electrodeposition of Cu_2O considerably hinders bacterial growth in the biofilm test stage.

In the pilot stage, after 45 days, the BS pH was 1.43, while the pH of the samples I, II, and III were 3.34, 3.11, and 2.27, respectively. In other words, the electrodeposited Cu_2O lowered the concentration of hydronium about 81, 47, and 7 times in samples I, II, and III compared to the BS, respectively. Moreover, minor differences in the percentage of electrodeposited Cu_2O caused a significant change in samples' ability to hinder bacterial growth.

CRediT authorship contribution statement

Zahra Khademmodaresi: Conceptualization, Methodology, Writing – original draft.

Fereshteh Bakhtiari: Investigation, Resources, Writing – original draft.

Mohammadmehdi Azizi: Supervision, Project administration, Writing – review & editing.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of competing interest

The authors declare no competing interests.

Funding and acknowledgment

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit non-profitable sectors.

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