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Investigation of biocorrosion caused by *Arthrobacter sulfureus* in brass

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ABSTRACT

Copper and its alloys are commonly used in water distribution lines. *Arthrobacter sulfureus* is an aerobic bacteria commonly found in soil. These are found to be responsible for blue green water problem and increased copper concentration in water. An attempt to study the brass corrosion by *Arthrobacter sulfureus* in neutral medium was made. Maximum corrosion rate of 0.33 mm/y was observed in brass for a period of 28 days of exposure to the *Arthrobacter sulfureus* as against the corrosion rate of 0.2 mm/y for the control. Maximum corrosion rate of 0.35 mm/y was observed at second week of immersion in the presence of *Arthrobacter sulfureus*. The optical density studies for the bacterial culture was found to show attainment of stationary phase in 48 h. Scanning electron microscopy analysis of the samples shows the presence of pitting corrosion. The energy dispersive X-ray analysis of the samples showed increased oxygen and phosphorus content in the sample due to bacterial activity. The EDAX studies also showed a drop in the copper content and increase in the oxygen content. Blue green deposits indicated the formation of malachite as a result of the bacterial activity. Growth test for *Arthrobacter sulfureus* in the presence of neem leaf extract showed better control over bacterial population.

Keywords: *Arthrobacter sulfureus*, biocorrosion, brass, neutral medium.

1 INTRODUCTION

Corrosion is an important problem studied in all industries from petroleum to food processing units [1]. Out of many factors that lead to corrosion, microorganisms play a significant role. Corrosion caused by microorganisms accounts for 20% of the degradation costs and cannot be ignored [1]. Material loss due to the bacterial activity is commonly referred to as microbially induced corrosion (MIC) or biocorrosion [1,2]. The accumulation of charges on the metal surface which has appreciable roughness facilitates the formation of biofilm which is a layer formed out of combination of bacterial cells and extracellular polymeric substances and dirt [3]. There are various theories for the MIC separately explaining the corrosive action of aerobic and anaerobic bacterial strains. The metabolic product of the bacteria which includes various organic and inorganic acids results in corrosion of the material [4]. Brass and copper are commonly used material for transportation of water in industrial cooling systems [5]. When copper or its alloys such as brass are used for water transportation, blue green water problem or the formation of blue green deposits leading to material damage has been observed frequently [5]. *Arthrobacter sulfureus* is aerobic bacteria commonly found in soil which finds its way to the water carrying lines made of copper or its alloys. They have been mentioned to cause blue green water problem due to corrosion of the copper and its alloys. It has also been found that, due to their corrosive activity, sudden increase in the copper content has been observed [6]. The biocorrosion can be controlled by using biocides. Biocides are chemicals that can be added in small quantities to stop the growth of the microbes. The biocides are believed to work on two principles i) electrostatic interaction between negatively charged centers on the cell membrane and the positive groups on the biocide molecules ii) hydrophobic nature of the biocide molecules disrupts the cell membrane and causes physical damage to the bacterial cells [7]. The commercially used biocide chemicals are toxic and non-biodegradable [8,9]. Hence, there is a search for environmental friendly corrosion inhibitors out of which plant extracts are important. The present work focuses on the corrosive effect of the *Arthrobacter sulfureus* on brass in a neutral medium using weight loss measurements conducted for a period of 28 days. The morphology of the samples after corrosion was studied using scanning electron microscopy. The changes in the elemental compositions of the coupons before and after corrosion were studied using energy dispersive X-ray analysis. The work also tests the ability of two plant extracts in controlling the bacterial activity namely ginger and neem extract.

2 EXPERIMENTAL

2.1 Preparation of sample brass coupons

Brass coupons with a dimension of 10 mm x 10 mm x 0.5 mm were used for the studies. Prior to each experiment, the coupons were polished with various grades of sand paper and washed with acetone and distilled water. Every coupon is punched with a hole, such that a nylon thread can be fastened into that.

2.2 Nutrient preparation and bacterial culturing

Pure strains of *Arthrobacter sulfureus* were purchased from NCIM, Pune. The strains were subcultured on nutrient agar plates. Nutrient agar solution was prepared by adding 2.8 g of nutrient agar in distilled water and was allowed to boil till it dissolved completely. To it, 1.5 g/l of yeast extract, 15 g/l agar, 5 g/l peptic digest if animal tissue, 5 g/l of NaCl and 1.5

g/l of beef extract were added. The final pH was adjusted to 7.4±0.2. This solution was sterilized at 1.5 kg/cm^2 gauge pressure for 20 min. Finally, the nutrient medium was poured into sterile petri plates under aseptic conditions, and medium was allowed to solidify. Upon solidification, a loop full of microorganism from the stock was streaked on the solidified media under sterile conditions. The sub cultured petri plates were then incubated at a temperature of 30±1^{o}C in the incubator.

2.3 Optical density (O.D) measurement

Prior to the corrosion studies, to know the growth characteristics of the *Arthrobacter sulfureus* species, nutrient medium was prepared using distilled water with 1.5 g/l of yeast extract, 5 g/l peptic digest of animal tissue, 5 g/l of NaCl and 1.5 g/l of beef extract. The final pH was adjusted to 7.4±0.2. This solution was sterilized at 1.5 kg/cm^2 gauge pressure for 20 min. To the medium taken in test tube, one loop full of culture was transferred and incubated at 30±1^{o}C in the incubator. The optical density at 600 nm was measured at regular intervals.

2.4 Sample preparation for corrosion studies

The corrosion studies were conducted by immersion of the metal coupons in media with and without bacterial culture. The nutrient medium was prepared in the similar manner reported in Section 2.2. The sterile nutrient medium was distributed evenly in multiple test tubes. Out of those tubes, twelve were termed as control and bacterial culture was not added to them to check the corrosive nature of the nutrient medium. To the rest of the tubes, loop full of bacterial culture from agar plates was added under sterile conditions. To all the test tubes, sterile metal coupons were immersed and sealed using sterile cotton. All the test tubes were incubated at a temperature of 30±1^{o}C in the incubator. Three metal coupons were taken out at regular intervals and their weight loss was measured. The average of the same has been reported here.

2.5 Inhibitor preparation and antimicrobial activity

The ginger and neem leaves were dried individually and powdered. The obtained powder was individually soaked in ethanol for 24 h and filtered. The obtained filtrate of ginger and neem leaves was individually refluxed in reflux setup at 55 ^{o}C for 5 h. The obtained extract was then concentrated using rotary evaporator for concentration.

The concentrated extract obtained from ginger and neem leaves were tested for antimicrobial activity. The nutrient broth containing one loop full of bacterial culture were taken in conical flasks. To the conical flasks the ginger and neem leaf extract were added individually. The volume % of the inhibitor extracts varied from 1 vol% to 3 vol%. After the addition of the inhibitor extract the flasks were sealed and incubated. The optical density was measured for the samples periodically.

3 RESULTS AND DISCUSSION

3.1 Growth of bacteria

Table 1 shows the values of optical density measured at 600 nm for the nutrient medium with bacterial culture alone at different time durations. It is clearly observed that the OD values increases continuously till 16 h and was found to saturate at 2.63 at 20 h which is an indication of stationary phase attainment. This shows the attainment of stationary phase. It was ensured that the bacterial strains used for corrosion studies were in stationary phase at the time of adding the strains into the nutrient broth containing metal coupons.

Table 1 Optical density measured at regular intervals of time to measure *Arthrobacter sulfureus* growth

Duration h	Optical density
4	1.67
8	1.75
12	1.96
16	2.63
20	2.63
24	2.63
28	1.67

3.2 Weight loss studies

Table 2 shows the weight loss of the brass coupons after immersion for various periods in control and bacterial cell containing neutral medium. It is clearly seen that the weight loss of the coupons significantly increases from 1.48 mg for 7-week immersion to 11. 7 mg for an immersion period of 14 days. With further increase in immersion period the weight loss increases, however the magnitude of the weight loss observed is less than that observed after 2-week immersion. The corrosion rate *CR*, can be calculated using the equation 1[5].

$$CR = \frac{87.6 \times w}{\rho \times a \times t} \quad (1)$$

where ρ is the density of brass in g/cm^3, *A* is the area of coupon in cm^2 and *t* is the immersion period in hours. It is seen from the Table 2 that the corrosion rate is the maximum for two weeks and decreases slightly for third week and increases again for fourth week. *Arthrobacter sulfureus* is known to form biofilm. The biofilm thickness will be at smaller at initial immersion period. With progress of bacterial activity, the biofilm thickness increases which poses diffusion limitations for oxygen and nutrients [5,6]. With increased thickness of the biofilm, its mechanical strength weakens causing detachment of the biofilm at some places which results in enhancing corrosion again like that observed for fourth week [5,6].

From Table 2 it can also be seen that the sample immersed for 28 days in medium without bacterial cell (control) presented a weight loss of 13.69 mg after 28 days which indicates that the medium is mildly corrosive to the metal.

Table 2 Results of weight loss studies for brass coupons in media with and without bacteria.

Immersion period h	Weight loss mg	Corrosion rate mm/y
Bacterial Medium		
7	1.78	0.11
14	11.7	0.35
21	14.4	0.29
28	22.4	0.33
Control		
28	13.69	0.2

3.3 Scanning electron microscopy (SEM)

Fig. 1 shows the scanning electron microscopy results of the brass coupons before immersion into the bacterial medium and the image of the brass coupon immersed in the bacterial medium for three weeks. It is clearly seen that the brass coupons after immersion for three weeks in bacterial medium has developed pits which is an indication of pitting corrosion. The biofilm is known to consist of 97% water into which the solutes such as extra cellular polymeric substances secreted by the bacteria, organic and inorganic acids due to the metabolic activity of the microbes are in dissolved condition [4]. The biofilm in the case of *Arthrobacter sulfureus* is reported to capture dissolved copper ions due to the opposite charge. Thus the zones covered with biofilm are slightly positive than the other areas resulting in localized cathodic and anodic regions resulting in pitting corrosion [6].

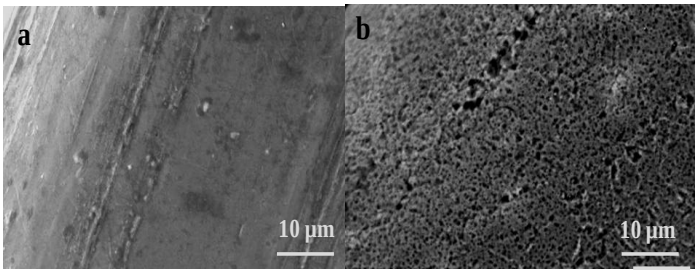

Fig. 1 Scanning electron microscopy images of brass coupon a) before immersion into the bacterial medium b) after immersion for three weeks in bacterial medium.

Table 3 shows the elemental composition of the coupons before and after immersion (for 3 weeks) into the bacterial medium. It is clearly seen that for the sample before immersion into the bacterial medium, the copper content was about 39.52% which has significantly dropped to 25.64% after immersion into the bacterial medium for three weeks. Similarly there is an increase in oxygen content from 4.87 % to 23.92% after immersion into the bacterial medium. This could be due to the formation of copper oxides due to the bacterial activity. Similarly phosphorus which is absent in sample before immersion shows its presence to significant extent which could be due to the extracellular polymeric substances consisting the biofilm [5].

Table 3 Elemental analysis of the brass samples before and after corrosion in the presence of bacteria for an immersion period of 3 weeks

Element	Before corrosion Wt%	After 3 weeks corrosion Wt%
Copper	38.52	35.79
Zinc	37.42	32.73
Carbon	19.19	13.31
Oxygen	4.87	14.00
Phosphorus	-	1.85
Magnesium	-	0.72
Silicon	-	1.26
Calcium	-	0.33

3.4 Antimicrobial activity of the inhibitors

Table 4 shows the optical density values taken after 28 h of incubation with ginger extract and neem leaf extract. It is clearly seen that the 3 vol% ginger extract is able to decrease the bacterial population only slightly. The neem leaf extract decreases the growth of the bacteria when the concentration is 1 vol% and 2 vol% but stops the growth when its concentration was increased to 3 vol% which is reflected by the very low optical density value. The optical density value of 0.04 may be due to the initial culture that has been added to the system.

Table 4 Optical density measurements at 600 nm for the medium containing only *Arthrobacter sulfureus* strains after 28 hours of incubation in the presence of inhibitor extract

Extract concentration vol%	Optical density
Ginger	
1	2.2
2	2.2
3	1.9
Neem	
1	2
2	1.2
3	0.04

3.5 Mechanism

Studies on bacterial corrosion have shown two types of mechanisms occurring individually or together resulting in microbial corrosion [8], i) metal oxidation coupled with intracellular reduction called as type I MIC ii)material degradation due to corrosive products formed from bacterial activity [5,10]. Three possible mechanisms have been proposed for copper reactions in microbial system [11] which is anodic half reactions resulting in Cu^{+} and Cu^{2+}. The first is simultaneous mechanism shown by reactions 2 and 3.

$$2Cu + H_2O \rightarrow Cu_2O + 2e^- + 2H^+ \quad (2)$$

$$Cu \rightarrow Cu^{2+} + 2e^- \quad (3)$$

The second mechanism proposed is sequential mechanism given by equations 4 and 5 [11]

$$2Cu + H_2O \rightarrow Cu_2O + 2e^- + 2H^+ \quad (4)$$

$$Cu_2O + 2H_2O \rightarrow Cu^{2+} + H_2 + 2e^- + 2OH^- \quad (5)$$

The third mechanism is redeposition mechanism shown by equations 6 and 7.

$$Cu \rightarrow Cu^{2+} + 2e^- \quad (6)$$

$$Cu + Cu^{2+} + H_2O \rightarrow 2Cu_2O + 2H^+ \quad (7)$$

Arthrobacter sulfureus are electrogenic bacteria capable of electron transfer [5]. Thus type I microbially induced corrosion occurs in the present situation. Moreover, the biofilm formed has greater affinity for copper ions. The copper ions that diffuse out to the bulk liquid from the biofilm readily react with dissolved oxygen to form cuprite, Cu_2O. It was observed that the solution upon immersion of the brass coupons turned to bluish green. Blue green deposits that occur during copper and its alloys corrosion could be due to the formation of malachite or some sulfur compounds. Since the energy dispersive X-ray analysis results did not show the presence of any sulfur, the blue green color could be due to malachite [12]. Earlier studies on the analysis of copper and brass samples used for water distribution by X-ray absorption spectroscopy also indicate the formation of malachite on the surface [11]. Thus based on the earlier theories and the obtained results it can be said that the *Arthrobacter sulfureus* acts as electrogenic species and thus type I MIC occurs in the system. The biofilm formed varies in its thickness and causes mass transfer limitation which is verified from the weight loss studies. The formation of blue green deposits on the brass coupons and the energy dispersive X-ray analysis results suggest the formation of Malachite which is due to the reaction of Cu_2O with the ions available in the solution. The formation of corrosion products along with biofilm also suggests the presence of type II microbially induced corrosion caused by corrosive products formed by bacterial metabolism.

4 CONCLUSIONS

An attempt to study the microbial corrosion of brass using aerobic bacteria *Arthrobacter sulfureus* was made. The gravimetric studies have shown that the bacterial population is active enough to cause corrosion for a period of 28 days. The scanning electron microscopy results suggest pitting corrosion which is supported by the theories which state the formation of concentration cells due to biofilm formation. Further analysis using electrochemical techniques such as potentiodynamic polarization and electrochemical impedance spectroscopy will be helpful in understanding the behavior clearly. Extensive studies using 3 vol% neem inhibitor has to be carried out.

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