Influence of \( \text{Cu}^{2+} \) on Sulfate-reducing Bacteria Associated with Magnesium Alloy

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(Received on April 19, 2007; accepted on July 13, 2007)

Influence of \( \text{Cu}^{2+} \) on sulfate-reducing bacteria associated with magnesium alloy was investigated. X-ray diffraction results reveal that the reaction products of magnesium alloy from the SRB and sterile medium containing \( \text{Cu}^{2+} \) consist of \( \text{Mg(OH)}_2 \) and Cu phases. \( \text{Cu}^{2+} \) can inhibit the growth of SRB, but it can not die out SRB cells in biofilm and medium. \( \text{Cu}^{2+} \) is a deleterious ion, which can form Cu–Mg micro-galvanic corrosion electrocircuits on substrate surface leading to the severe corrosion of magnesium alloy. Although extracellular polymeric substances (EPS) of SRB have the ability to bind \( \text{Cu}^{2+} \), severe micro-galvanic corrosion was still observed on magnesium alloy surface. This indicates that \( \text{Cu}^{2+} \) can not act as a sterilization reagent or a composition of sterilization reagent for SRB associated with magnesium alloy.

KEY WORDS: corrosion; \( \text{Cu}^{2+} \); sulfate-reducing bacteria; magnesium alloy.

Table 1. Chemical composition of the AZ91 magnesium alloy (wt%).

<table>
<thead>
<tr>
<th>Element</th>
<th>Composition (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>8.67</td>
</tr>
<tr>
<td>Zn</td>
<td>0.77</td>
</tr>
<tr>
<td>Mn</td>
<td>0.28</td>
</tr>
<tr>
<td>Fe</td>
<td>0.0008</td>
</tr>
<tr>
<td>Si</td>
<td>0.0019</td>
</tr>
<tr>
<td>Mg</td>
<td>balance</td>
</tr>
</tbody>
</table>

1. Introduction

In natural or man-made environment, microorganisms tend to attach to the metal surface and subsequently form biofilm. The biofilm consists of water, microorganisms, extracellular polymeric substances (EPS) and forms a protective layer, reducing the exposure of the metal surface to the corrosion environment.\(^1\) The formation of biofilm on metal surface could result in corrosion increase or corrosion inhibition.\(^2\) The main types of microorganisms associated with metals corrosion or corrosion inhibition are sulfate-, iron- and \( \text{CO}_2 \)-reducing bacteria, sulfur-, iron- and manganese-oxidizing bacteria.\(^5\) Among them, sulfate-reducing bacteria (SRB) are recognized as a major group of microorganisms, and have been the most commonly studied group of microorganisms.\(^9\) Historically, the majority of investigations of metals associated with microorganisms had been dealt with iron, copper, aluminum and their alloys for practical industry applications.\(^7\) but the effect of microorganisms associated with magnesium alloy was seldom talked about. At present, magnesium alloys have been used in the fields of aviation, spaceflight, automobile and communication. With increasing use of magnesium alloy in industrial applications, the potential significance of the effect of microorganisms associated with magnesium alloy should be concerned.

As to microorganisms, environmental conditions must be favorable to the growth and metabolic activity of the microbial producers.\(^11\) Among these environment factors, \( \text{Cu}^{2+} \) was known as a toxic heavy metal ion, which was commonly used as a composition of sterilization reagents to inhibit the growth of bacterium. However, by far, the correlative works relating to \( \text{Cu}^{2+} \), SRB and magnesium alloy have not been reported. It is thus of interest to investigate the influence of toxic \( \text{Cu}^{2+} \) on the growth of SRB associated with magnesium alloy.

2. Experimental Details

A commercial AZ91 magnesium alloy was used as experimental material, and its composition is shown in Table 1. Coupons (2.0×1.5×2 cm\(^3\)) were wet polished on progressively finer silicon carbide papers to a final grit size of 800. After polishing they were rinsed in distilled water and then in acetone for degreasing. Later, the coupons were sterilized using ultraviolet radiation for 20 min before exposure to the test medium.

SRB seed was obtained from Key Laboratory of Metal Corrosion of Nation, Institute of Metal Research Chinese Academy of Sciences. The American Petroleum Institute...
(API) standard medium \(^{12}\) was prepared by adding to each liter of distilled water with 0.5 g Na\(_2\)SO\(_4\), 1.0 g NH\(_4\)Cl, 0.1 g CaCl\(_2\), 0.5 g K\(_2\)HPO\(_4\), 3H\(_2\)O, 2.0 g MgSO\(_4\), 7H\(_2\)O, 3.5 g C\(_3\)H\(_5\)NaO\(_3\), 1.0 g yeast.

Experiments were carried out in parallel in 12 enclosed 600 mL glass reactors containing the API medium, and initial Cu\(^{2+}\) concentrations (0, 50, 100, 150, 200 and 250 mg/L Cu\(^{2+}\)) in the medium were adjusted using CuSO\(_4\), 5H\(_2\)O. The pH was maintained at 7.2. These medium were autoclaved at 121°C for 20 min. An appropriate volume (2‰) of a pure SRB culture was inoculated in the test medium resulting in an initial concentration of 6.50 × 10\(^4\) cells/mL. Subsequently, the sterile coupons were immersed in the test systems, which were incubated at 30°C for 8 d. Control systems without SRB containing sterile medium and Cu\(^{2+}\) were run simultaneously with the test systems.

SRB enumeration was performed after 8 d. Live SRB on the coupon surface and in the medium were counted using optical microscope (Model COIC XSZ-HS7) and haemacytometer. Corrosion rate was measured using weight loss method. The values of the corrosion rate were determined by applying the equation proposed by the ASTM standard G 1-72.\(^{13}\)

\[ V = \frac{(K \times W)}{(A \times t \times D)} \] ........................(1)

Where: \( V \) is Corrosion rate (mg/dm\(^2\), \( K = 2.4 \times 10^6 \times D \), \( W \) is weight loss after cleaning (g), \( A \) is coupon area (cm\(^2\)), \( t \) is time of exposure (h) and \( D \) is density of coupon (g/cm\(^3\)).

The coupon surfaces were observed using optical microscope (OM) (Model XTS 30, China). Phase analyses were investigated using X-ray diffraction (XRD) (Model D/Max 2500PC, Rigaku, Japan).

3. Results and Discussion

After 8 d exposure, microbial product of H\(_2\)S was confirmed by its characteristic odour. Corrosion morphologies of six coupons after 8 d immersion are shown in Fig. 1. Figures 1(a) and 1(b) illustrate that there was a kind of transparent crystal salt on the coupons from the sterile and SRB medium not containing Cu\(^{2+}\). The former work of us had confirmed that this salt is NH\(_4\)MgPO\(_4\), 6H\(_2\)O. Figures

![Fig. 1](image_url)
I(c)–I(f) illustrate that the same corrosion products were detected on the coupons from the medium containing Cu\(^{2+}\). The scraped out corrosion products on the coupons from the sterile and SRB medium were finely powdered, respectively, and then the powders were analyzed using XRD. Figure 2 shows the XRD patterns of the typical corrosion products of the coupons from the SRB and sterile medium. XRD analysis reveals that the corrosion products consist of Mg(OH)\(_2\) and Cu phases. However, the intensity of diffraction peaks of the coupon from the SRB medium is weaker than that from the sterile medium. This indicates that SRB biofilm inhibited the formation of Mg(OH)\(_2\) and Cu phases. It may attribute to the generation of acid environment within the biofilm\(^6\) and the binding of Cu\(^{2+}\) by EPS.\(^5\)

Figure 2 illustrates the XRD patterns of the corrosion products of AZ91 coupons from (a) SRB medium containing 250 mg/L Cu\(^{2+}\), and from (b) sterile medium containing 250 mg/L Cu\(^{2+}\).

Metal binding by EPS involves interaction between the metal ions and anionic functional groups that are common on the protein and carbohydrate components of exopolymers.\(^5\) EPS bound Cu\(^{2+}\) and prevented the contact between Mg and Cu\(^{2+}\), so the reaction of the formation of Cu micro-particles at the substrate surface was inhibited by EPS.

Figure 3 illustrates that variation of SRB count vs. Cu\(^{2+}\) concentration in biofilm and medium. It was observed that SRB count in biofilm reaches the minimum at the concentration of 50 mg/L Cu\(^{2+}\). This indicates that the lower Cu\(^{2+}\) concentration is beneficial to inhibit the growth of SRB. One reason should be explained that the higher initial Cu\(^{2+}\) concentration led to the formation of large numbers of Mg\(^{2+}\), which further reacted with OH\(^-\) to form Mg(OH)\(_2\). The thicker and incomplete Mg(OH)\(_2\) protective layer was in favor of the colonization of bacteria and prevented SRB cells from having the toxic environment. The other reason may be explained that the higher Cu\(^{2+}\) concentration caused the death of the majority of SRB cells. However, some drug-fast cells thus attained enough nutrition for their propagation, which resulted in the increase of the sessile bacteria. The cell count of planktonic bacteria reaches a maximum in the SRB medium without Cu\(^{2+}\) and begins to decrease quickly thereafter. Accession of Cu\(^{2+}\) in the test medium resulted in a distinct decrease of the cell count of planktonic bacteria, indicating that Cu\(^{2+}\) inhibits the growth of planktonic bacteria. In addition, Cu\(^{2+}\) can not die out the planktonic bacteria. To avoid exposure to toxicity, microbes may tend to aggregate in order to reduce the total surface area in contact with the environment.\(^1\)

Even a slight Cu\(^{2+}\) concentration (50 mg/L) had induced serious corrosion. In order to clarify this problem, the corrosion morphologies of four kinds of coupons after removal of the corrosion products are shown in Fig. 4. Only few pittings were observed on the two coupons from the sterile and SRB medium not containing Cu\(^{2+}\). The main feature of the two coupons was pitting corrosion. However, evident corrosion pits appeared on the surfaces of the coupons from the sterile and SRB medium containing 50 mg/L Cu\(^{2+}\). The Coupons show localized corrosion. These results indicate that Cu\(^{2+}\) is a deleterious composition in the API medium, which accelerates the corrosion of magnesium alloy.

Figure 5 illustrates average corrosion rate vs. Cu\(^{2+}\) concentrations in sterile and SRB medium. Corrosion rate measurement also proves that Cu\(^{2+}\) is a deleterious composition in the control and test medium, which led to the severe corrosion of magnesium alloy. Corrosion rate at 50 mg/L Cu\(^{2+}\) is about 7.5–8.1 times as large as that at 0 mg/L Cu\(^{2+}\). The corrosion rates of the coupons in the test systems increases to 491 mg/dm\(^2\)-d quickly at the concentration of 50 mg/L Cu\(^{2+}\) and begins to keep a steady state with a mean value of 529 mg/dm\(^2\)-d thereafter, and then increased to 654 mg/dm\(^2\)-d at the concentration of 250 mg/L Cu\(^{2+}\). This indicated that a steady corrosion products layer were formed in the SRB medium containing Cu\(^{2+}\) from 50 to 200 mg/L. The corrosion type in the SRB medium is galvanic corrosion. Cu\(^{2+}\) reacted with Mg substrate to form Cu micro-particles depositing on the surface of magnesium alloy, which resulted in formation of a great deal of micro-galvanic corrosion electrocircuits. However, EPS have the ability to bind Cu\(^{2+}\), and prevent the transmission of Cu\(^{2+}\) from the solution to the metal surface. Hence, the number of micro-galvanic corrosion electrocircuits did not increase with the increase of Cu\(^{2+}\) from the concentration of 50 to...
200 mg/L. When Cu\(^{2+}\) increased to 250 mg/L in the test medium, the transmission ability of Cu\(^{2+}\) enhanced, so the number of micro-galvanic corrosion electrocircuits increased leading to the increase of corrosion rate. In addition, SRB causes a mean decrease in corrosion rate compared to the control systems, indicating that SRB inhibits the corrosion of magnesium in the test systems.

In conclusion, Cu\(^{2+}\) is a deleterious ion, which can form Cu–Mg micro-galvanic corrosion electrocircuits on magnesium alloy surface leading to the severe corrosion of magnesium alloy. Although EPS has the ability to bind Cu\(^{2+}\), severe corrosion is still observed on the coupon surface. This indicates that Cu\(^{2+}\) can not act as sterilization reagent or a composition of sterilization reagent for SRB associated with magnesium alloy.

4. Conclusions

From the present study, the following conclusion can be drawn:

(1) X-ray diffraction result reveals that the reaction products of magnesium alloy immersed in the SRB and sterile medium containing Cu\(^{2+}\) consist of Mg(OH)\(_2\) and Cu phases.

(2) The lower Cu\(^{2+}\) concentration is beneficial to inhibit the growth of sessile SRB on magnesium alloy surface. In addition, Cu\(^{2+}\) can inhibit the growth of SRB, but it can not die out SRB in biofilm and medium.

(3) Cu\(^{2+}\) is a deleterious ion, which can form Cu–Mg micro-galvanic corrosion electrocircuits on magnesium alloy surface leading to the severe corrosion of magnesium alloy.

(4) Although EPS has the ability to bind Cu\(^{2+}\), severe corrosion is still observed on the coupon surface. This indicates that Cu\(^{2+}\) can not act as sterilization reagent or a composition of sterilization reagent for SRB associated with magnesium alloy.

Acknowledgements

The authors acknowledge the financial support of Keystone Project of Science & Technology of Jilin Province, China (20040315). We wish to acknowledge Prof. S. J. Zhao, School of Biological & Agricultural Engineering for the provision of experimental equipments.

REFERENCES