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Effect of Quinizarin Dye on the Aluminum Corrosion by *Pseudomonas Aeruginosa*

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Formation of a passive oxide film on aluminum enhances its corrosion resistance. However, this can be damaged by microbiologically species. *Pseudomonas aeruginosa* and *Cladosporium* sp. have been commonly associated with the microbiologically influenced corrosion (MIC) of aluminum and its alloys. In this study, the effect of an organic dye (quinizarin), on resistance of aluminum plates to MIC was studied. An overnight culture of *Pseudomonas aeruginosa* in a chemically defined medium was inoculated in each test tube containing artificial seawater plus various prepared aluminum plates and investigated by various electrochemically methods consisting: Cyclic and AC voltammetry and scanning electron microscopy. The results obtained showed that the growth of the *P. aeruginosa* in the medium containing quinizarin dye had not significantly difference with that of the control medium. Scanning electron microscopy micrographs and electrochemical impedance measurements showed that the amounts of the bacteria on the surface of coloured plate were more than that of on uncoloured plates, however, the corrosion rate in the later plates was more than coloured plates. It seems that quinizarin dye is useful for inhibiting of aerobic corrosion of aluminum materials by *P. aeruginosa*.

Key Words: Aluminum, Microbiologically influenced corrosion, Quinizarin, Scanning electron microscopy, *Pseudomonas aeruginosa*.

INTRODUCTION

Aluminum is a widely occurring metal, its insolubility assures its low bioavailability. However, acid rain, industrial wastes and anthropogenic activities have led to a sharp rise in the concentration of biologically active forms of this metal. This situation has become a major cause of concern, as aluminum is known to interfere with the normal functioning of most cellular systems[1].

Biofilms can be made on natural terrestrial and aquatic environments and human-made substrates *e.g.*, artificial organs, contact lenses and dental tubing[2]. Biofilms can have negative effects on human activities in

many ways, including energy waste, heat transfer resistance and requirement for excess equipment capacity, decreased life of equipment, quality control problems and safety problems[3].

Development of microbial biofilms or attaching of selfish larvae to hull caused to markedly increased fuel consumption[4-7]. Over the last two decades, study of the microbiologically influenced corrosion (MIC) of metallic materials has received considerable attention.

Numerous techniques are available for studying bacterial biofilms, *e.g.* weight loss and infrared spectroscopy have been employed for detecting and characterizing the objective of the present study is the resistance monitoring of coloured aluminum plates with a organic dye to microbiologically influenced corrosion during the biofilm development of *Pseudomonas aeruginosa* on aluminum plates exposed in an artificial seawater by electrochemically methods (cyclic and AC voltammetry) and scanning electron microscopy.

EXPERIMENTAL

All of the chemical compounds were purchased from Merck chemical company (Darmstadt, Germany). *Pseudomonas aeruginosa* ATCC 15528 was used in this study. The strain was maintained in 30 % phosphate buffered, glycerol (pH 7.0) at -80ºC until used. 3 cm × 2 cm of 99.5 % pure, smooth and homogenous aluminum plates were used for this study so that the biofilm could develop uniformly over the plate surface. Two treatments were done on the plates:

1) Coloured: The plates were polished using successively finer grades of emery paper (240, 400, 800 mesh) mounted on a Grinder-polisher (Buehler, UK) and etched in a 5% solution of NaOH at 50-60ºC, rinsed by 50 % (w/v) solution of HNO_3, then immersed in a brightening solution (H_3PO_4 85%, H_2SO_4 16%, HNO_3 3 % w/v) and rinsed with distilled water. The prepared specimens were anodized with 12 V direct current voltage for 0.5 h in 18 % (w/v) H_2SO_4 solution at 22ºC. Then they were washed with distilled water and placed in a solution of 2 g/L of quinizarin (1,4-dihydroxy anthraquinone, Merck, Germany) for 20 min at 50ºC and sealed by immersing in distilled water at 100ºC for 0.5 h. The structure of the dye was shown in Fig. 1.

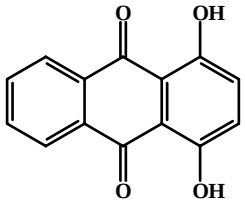


Fig. 1. Structure of Quinizarin

2) Uncoloured: The plates were only polished as the same as above mentioned conditions. The cyclic and impedance voltammetric measurements were performed by using potentiostat[8,9]. The solution for electrochemical measurements was 0.01 M H_2SO_4 solution. The auxiliary electrode was a Pt electrode (1 cm × 0.1 mm diameter) and the reference electrode was a standard calomel (Ag/AgCl) electrode. Scanning electron microscopy (SEM) apparatus (DSM 960 A, Zeiss, Germany) was used for confirmation the results.

A chemically defined medium (CDM) containing (L^{-1} solution), 1.5858 g $(NH_4)_2SO_4$, 0.788 g $MgSO_4$. $7H_2O$, 0.2237 g KCl, 0.1753 g NaCl, 5.6 mg $FeSO_4.7H_2O$ and 0.209 g K_2HPO_4 was used for preparing inoculums in all experiments. It was prepared and sterilized at 121ºC for 15 min, then 50 m mol L^{-1} MOPS (pH 7.0, sterilized by 0.2 µm filtration) and 100 m mol L^{-1} glucose were added. An artificial seawater (ASW)[10] was used for corrosion study. Its compositions was as follows (L^{-1} solution) : 0.7 mg NH_4Cl, 24 g NaCl, 0.6 g KCl, 8.0 g $MgCl_2.6H_2O$, 0.3 g $CaCl_2$, 0.01 g KH_2PO_4 , 1 mg $FeCl_3$, 5.32 g *tris*-HCl and 1.97 g *tris*-base . The pH was adjusted to 8.0 and the medium supplemented with 25 m mol L^{-1} glucose.

An overnight culture of *Pseudomonas aeruginosa* ATCC 15528 in Brain heart infusion agar was used for preparing of bacterial suspension. 100 mL Erlenmeyer flask containing 25 mL of CDM was inoculated by 1 mL of bacterial suspension containing 1.5×10^8 CFU/mL and incubated at 30ºC, 180 rpm for 24 h. In aseptic conditions, 0.5 mL of this culture was added to each 200 × 25 mm test tube containing 30 mL sterilized artificial seawater plus various treatments. The treatments are as follows: artificial seawater (ASW) (control), ASW + quinizarin, ASW + coloured aluminum plates, ASW + uncoloured aluminum plates. The inoculated test tubes were incubated at 25ºC for 60 d. Each experiment was done in triplicates in three independent batches. At the end of incubation, a sample of each tube was cultured in BHI agar plates to ensure that the bacteria were live and culturable. This aluminum plates were used for electrochemically measurements and SEM images.

RESULTS AND DISCUSSION

It is well known that *Pseudomonas aeruginosa* forms biofilms on natural surfaces, medical biomaterials and CF (cystic fibrosis) lung epithelial cells[11-13]. The effects of adhesion and subsequent fouling biofilm formation are similar for both biomaterials and other engineered surfaces.

In this study, we can understand that the quinizarin as an organic dye coated the surface of aluminum plates and cause to protection from biocorrosion by *Pseudomonas aeruginosa*. These results were confirmed by SEM images (Fig. 2), cyclic (Fig. 3) and AC voltammetry (impedance and admittance, Fig. 4).

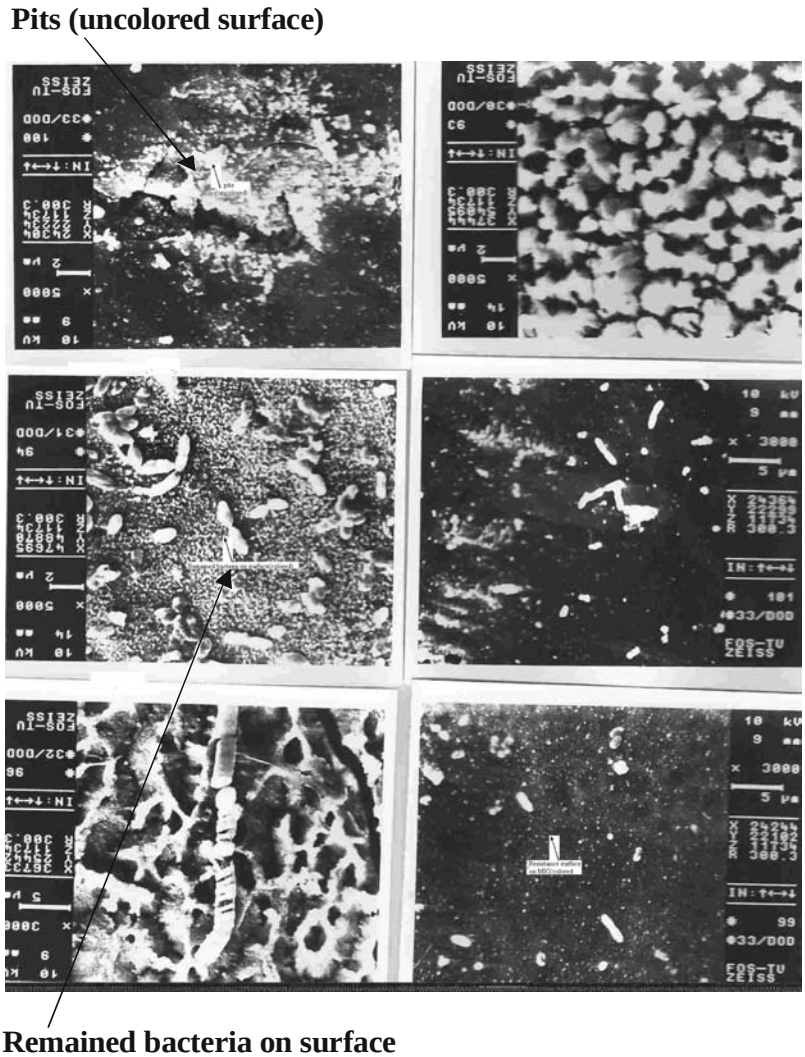
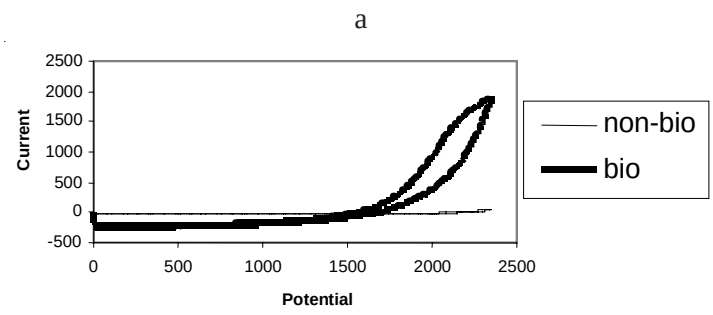


Fig. 2. The SEM images of various colored and uncolored aluminum plates that influenced by microbiologically corrosion



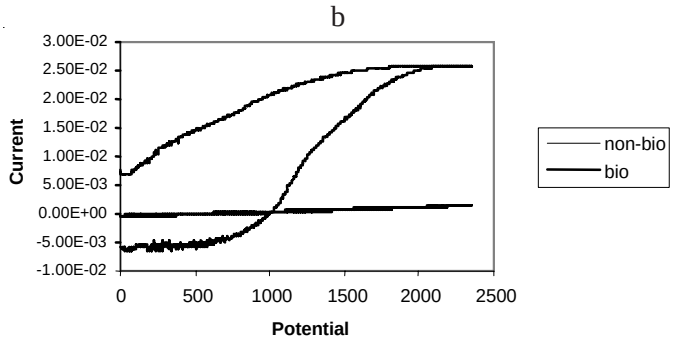


Fig. 3. Cyclic Voltammograms of uncoloured (a) and coloured (b) aluminum plates

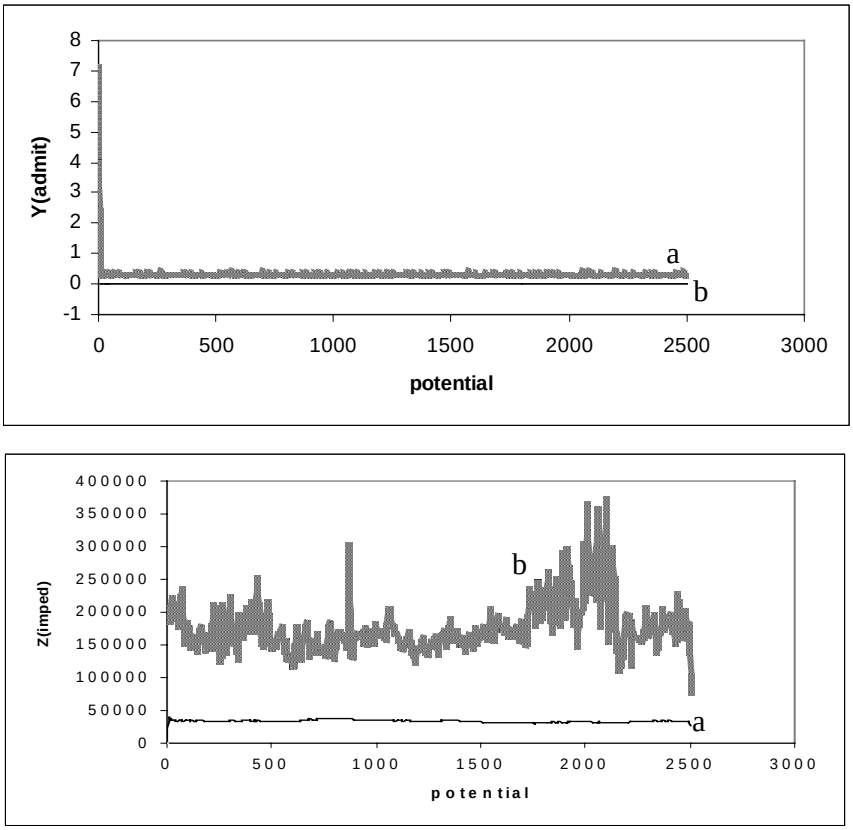


Fig. 4. Impedance and admittance measurements of uncoloured (a) and coloured (b) aluminum plates

The results showed that the mentioned colour was caused to decrease the growth of bacteria, because of colour coated a protected layer on the surface of aluminum.

This characteristic can reduce intensity of biocorrosion on aluminum plates, so the coloured plates have most resistance to MIC and the bacteria

were remained on the colour coated surfaces of them without damaging to the surface (Fig. 2) and the cyclic voltammograms of them have a low corrosion current and admittance and high impedance values.

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