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THE IMPEDING OF ACIDITHIOBACILLUS THIOOXIDANS MICROBIAL INDUCED CORROSION (MIC) USING BACTERIAL BIOFILMS MEDIATED INTERACTIONS

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Abstract

Acidithiobacillus thiooxidans has been confirmed to promote for a fast microbial induced corrosion (MIC) on a surface of a metal coupon in 7 days of incubation with oxygen reliable in the peptone medium. This research is conducted to clarify the mutual interactions between biofilm and the metal substratum and to further the study on the direction of biofilm influence on the metal as into a corrosion inhibition. The use of symbiont biofilm between Actinomycete and Pseudomonas aeruginosa has been proved to inhibit the MIC process as early as the third day through growth performance of the biofilm formation. We have studied the impeding process using the mediated interactions and have reported that Acidithiobacillus thiooxidans cells are smaller and thicker apart from its slower growth due to the formation of the biofilm of the microbial cell consortia. All the 3 species have significantly shared the environment, using the peptone in the medium and did not cause any pit formation or started any corrosion sign on the metal surfaces, suggesting that the great tolerance between the species has achieved the aim to reduce bio-corrosion using biofilm consortia. This evidence proved that MIC can be eradicated using other microbes or microbial interaction at the beginning of colonization.

Keywords*: Biogenic sulfide corrosion; Microbial induced corrosion (MIC); Acidithiobacillus thiooxidans; P. aeruginosa; Biofilm; Metal corrosion.*

Introduction

Every year, several cases on pipeline failures due to corrosion were reported which includes lost in billions dollars of money (The U.S federal Highway Administration; FHWA, estimated a direct cost of corrosion $276 billion which equivalent to 3.1% of the nation's Gross Domestic Product [1]. Microbial inhibition of corrosion is another world of MIC; the microbial induced corrosion. As the MIC could start with one single species such as sulfate reducing bacteria (SRB) or even aerobic bacteria, the inhibition has been shown to be an agenda of few or at least two back to back supporting each other species. It is unlikely to see one single mechanism of a single species of microorganisms to inhibit one typical spot of a corrosion starting point in any metal or pipelines. According to *D. Videla and J. Herrera* [2], the development of biofilm by the complex of microorganisms will give some corrosive or an inhibitory effect on the MIC process on certain metal surfaces. The main mechanisms of bacterial corrosion inhibition are always linked to a marked modification of the environmental conditions at the metal–solution interface due to biological activity [2]. The accomplishment of

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the thorough inhibition set up through the complex biofilm was studied by quite a number of researchers and has been reported to be mostly by affecting the growth of the species that starts the MIC. The major feature observed on the biofilm formation is; it starts from free-floating cells growing into 'slime' due to cell-cell attachment supported by the surroundings such as the cell itself, some matrix, polymers, bioreactor wall even living and non-living thing including the metal surfaces they are colonizing on. Biofilm arrangement is well promoted by a lot of biochemical processes and its regulation [3]. In this paper, we are projecting symbiont biofilms of *Actinomycete* sp. and *Pseudomonas aeruginosa* in promoting for the slow growth of *Acidithiobacillus thiooxidans* that has been responsible for microbial induced corrosion and therefore diminish the potential of the MIC to start off. Progress in corrosion control using bacteria biofilms is seen to be one way to a better control strategy.

Materials and Methods

Reagent, chemicals and pH

All reagents and chemical used were from technical grade.

Microorganisms

A Gram-negative *Acidithiobacillus thiooxidans* was maintained at 4°C in peptone water. Both *Actinomycete* and *Pseudomonas aeruginosa* [4] were maintained on nutrient agar (NA) and Pseudomonas agar (PA), respectively, at 4°C.

Source of metal for biogenic sulfide corrosion test

New metal coupon cut into $1.0\times1.0\times0.5cm^3$ with no sign of corrosion were used. All metals used in this experiment were sterile prior to use and handled using aseptic techniques. Prior to each experiment, the weight loss coupons (X65 carbon steel) was polished using isopropyl alcohol as coolant, with silicon carbide abrasive papers of up to 600 grit. All metal were weight into accuracy of 0.515mg.

Biogenic sulfide corrosion simulation test

The corrosion test was simulated in one complete cycle of 10 days incubation at 28°C and 50 rpm [5]. Two metal coupons were kept in 30 ml of sterile peptone water, with only one bottle contains: 5.0×10^5cells/mL 24 hours growth of *Acidithiobacillus thiooxidans* (Metal Coupon 1). The one without the species was used as control (Metal Coupon 2). Experiment was repeated thrice using aseptic techniques. This test was done to confirm that *Acidithiobacillus thiooxidans* can cause biogenic sulfide corrosion on the metal coupons.

Effect of biofilm growth as anti-corrosion test

The anti-corrosion test was simulated at 28°C and 50 rpm for 10 days of incubation. One metal coupon was kept in 30mL of sterile peptone water, containing: 5.0×10^5cells/mL of 24 hours growth of *Acidithiobacillus thiooxidans* and 5.0×10^5cells/mL of 24 hours growth of *Actinomycete* and *Pseudomonas aeruginosa.* One metal coupon was kept in 30mL of sterile peptone water, with only 5.0×10^5cells/mL 24 hours growth of *Acidithiobacillus thiooxidans* and the metal coupon in the sterile peptone bottle without any species was used as control. Experiment was done thrice using aseptic techniques.

Analyses

The H_2S concentration in the bottles was determined by H_2S detector tubes (Agilent). The pH in each of the suspension was determined using Metrohm double electrode (Berchem, Belgium). Bacterial growth, biofilm and pitting on the metal coupon surfaces were examined by Scanning Electron Microscope (SEM) analysis [6, 7]. The sulfate concentration in the suspension was determined according to Standard Methods. Samples were taken on day 10. The weight loss of metal was determined by using Clarke solution according to ASTM G1. The corrosion rate measurement by weight loss method is calculated by Equation (1).

$$CR = \frac{m_{loss} \times 87.6}{\rho_{Fe} \times A \times t} \quad (1)$$

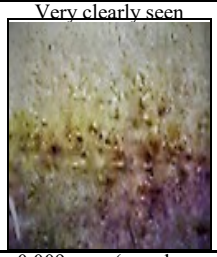
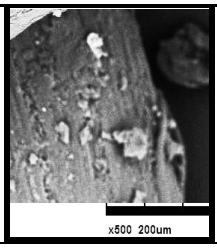
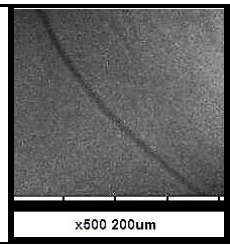
where: CR - calculated corrosion (mm/yr); M_{loss} - mass loss of steel sample (measured in grams); ρ_{Fe} - density of iron (equal to 7.85 g/cm^3); A - surface area (cm^2), t - exposure time (h)

The glucose oxidase-peroxidase enzyme system with syringaldazine as chromagen is suitable for assaying oxygen solubility in complex media. The final quinone concentration, as indicated by A_{max} is a linear function of oxygen added to the assay. Assay sensitivity (A_{max}) is optimum at pH = 7, but depends on the type and volume of medium added. The reaction time (t_{max}) can be shortened to 2 min or less by raising the enzyme and/or glucose concentration used in the assay. Based on these findings an assay composition and procedure has been suggested (Table I). To control for media effects, it is recommended that the medium sample size be standardized and that both reference and sample assays be performed.

Results and discussion

Table 1 confirmed that *Acidithiobacillus thiooxidans* has promoted for the microbial corrosion on Metal Coupon 1 in at least 7 days of incubation with oxygen reliable in the medium.

Table 1. *Acidithiobacillus thiooxidans* in the promotion of Induced Microbial Corrosion (MIC)

	Metal Coupon 1 with *Acidithiobacillus thiooxidans*	**Metal Coupon 2 without *Acidithiobacillus thiooxidans***
Metal's initial/final weight without biofilm (g)	0.515/0.417	0.515/0.514
Initial/Final pH	7.0/3.2	7.0/7.0
Corrosion rate (mpy)	2.62	0.001
Initial color/final color of peptone water	Yellowish/ Light to darker brown	Yellowish/ Yellowish
Positive sign of corrosion on the metal chip	Very clearly seen 	Negative 
H_2S detection	0.009 ppm (very low, confirmed that corrosion was not contributed by the level of H_2S)	0.002 ppm
Oxygen concentration		
Initial/final sulfate concentration	0.00/9.50 mg/ml	0.00/2.00 mg/ml
Formation of biofilm on the metal coupon	Negative	Negative
SEM result of the metal coupon		

This is a very rare result because often, bio-corrosion was reported to take months or year to happen on most metal surfaces. There was no significant value of weight loss or sign of corrosion was recorded from sterile Metal Coupon 2 even it was incubated in the same sterile

peptone medium with the same condition as Metal Coupon 1. The biogenic sulfuric acid corrosion is often a slow corrosion process for example; a corrosion rate of 0.5mm a year was described by *T.K. Mori et al.* [8]. This was not shown in our research. Rapid growth of *Acidithiobacillus thiooxidans* supported by enough oxygen content, has promoted for pits to appear as early as the exponential growth cycle.

A simple colorimetric assay was developed and applied to the analysis of oxygen solubility during alcoholic fermentation. The method was based on the consumption of oxygen by glucose oxidase activity and the production of the pink quinone of syringaldazine by coupled peroxidase activity. Color formation at 526nm progressed through an optimum that was a linear function of the oxygen added to the assay.

Figure 1 shows a general pattern of increment in average percentage weight loss (APWL) and a corresponding increament in corrosion rate (CR) in the Metal Coupon 1 exposed to *Acidithiobacillus thiooxidans* for 7 days, with respect to time.

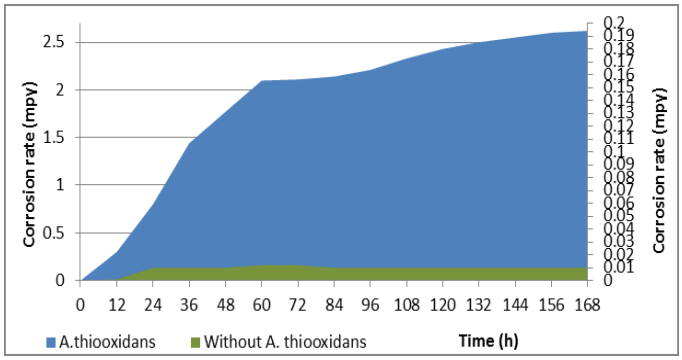


Fig. 1. Corrosion rates in metal coupon1 exposed to *Acidithiobacillus thiooxidans* and without exposure to the cells in 7 days (168h) of incubation.

In order to confirm the possible potential to inhibit the microbial induced corrosion (MIC) by the *Acidithiobacillus thiooxidans* using biofilm corsortia, experimental procedures have been set-up using Metal Coupon 3 incubated in a sterile peptone medium contains the 3 species; *Acidithiobacillus thiooxidans, Actinomycete* and *Pseudomonas aeruginosa.* This was done with regards of the biofilm formation that was targeted to be the inhibition factor for the appearance of bio-corrosion pits. *Acidithiobacillus thiooxidans* growth was recorded as positive with lesser cell numbers indicating slower growth. It was recorded biofilm formation of *Actinomycete* and *Pseudomonas aeruginosa,* which cells have not totally reduced the growth of *Acidithiobacillus thiooxidans* but has helped to reduce the thickness of the biofilm formation with no significant corrosion rate or appearance of the corrosion signs was reported. Biofilm is mostly representing a 'community' of microbial world that surviving the colonization using their exopolysaccharides (EPS).

Basically, the biofilm in their attempt to 'live' shall affect metal surfaces in two ways. It 'uses' the metal compounds for living that gives the result of corrosion behavior on the metal surfaces using sets of their biochemistry and enzymatic potentials. On the other hand, biofilm can also behave as a protective layer on the metal by protecting surfaces from any starting point of the pitting. According to *P. Zuo* [9], "formation of bacterial biofilms on metal surface may accelerate or impede corrosion". The statement by *P. Zuo* [9] has strongly supported our findings.

Figure 2 explains what took place in our findings. Corrosion inhibition using beneficial bacterial biofilms by the mutual arrangement of two aerobic species; the *Actinomycete* and *Pseudomonas aeruginosa* was suggested by manipulating the aerobic respiration of these biofilms which has helped to decrease oxygen concentration on the surface of Metal Coupon

3. Lower concentration of surface oxygen is not the best physical parameter for *Acidithiobacillus thiooxidans* growth, which becomes less eager to colonize the surfaces, instead, it appeared to grow on the surfaces of the *Actinomycete* and *Pseudomonas aeruginosa* biofilm by showing much lesser number of cells since it has to accommodate the oxygen sharing system.

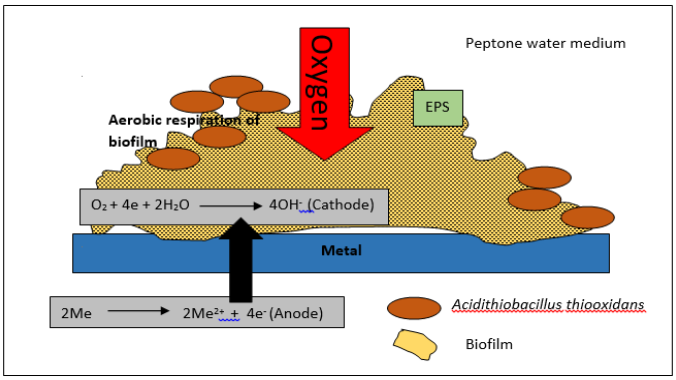


Fig. 2. Mechanism of MIC inhibition using *Acidithiobacillus thiooxidans*, *Actinomycete* and *Pseudomonas aeruginosa*

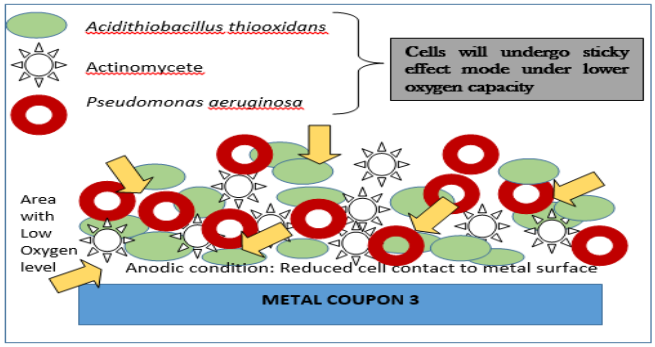


Fig. 3. Mechanism of MIC inhibition using *Acidithiobacillus thiooxidans* respiratory interruption in the biofilm consortia using Metal Coupon 3

The *Acidithiobacillus thiooxidans* cells isolated from the biofilm has appeared smaller and thicker especially on the main spot of the colonization. Oxygen depletion under the layer of the biofilm has promoted for the *Acidithiobacillus thiooxidans* cell shrinking. Figure 3 shows the mechanism of MIC inhibition using *Acidithiobacillus thiooxidans* respiratory interruption in the biofilm consortia. Thicker colonies are formed under high respiration in the low oxygen concentration. This anodic condition will decrease cell contact with the metal surface, thus reducing corrosion**.** The bacteria colonization on the metal coupon has formed a non-uniform patch which, in the presence of aerobic respiration will result in the formation of differential aeration cell. Cells in biofilms experience another growth mode different from planktonic growth. Compared to their planktonic counterparts, bacteria in biofilms show distinct physiological characteristics such as significantly enhanced resistance to antibiotics, increased production of exopolysaccharide, changes in cell morphology or different responses to environmental stimuli [10-13]. The possible mechanisms of microbial inhibition of the corrosion inhibition we are proposing by using the oxygen respiration under aerobic conditions. *T.R. Garrett et al.* [14] have described biofilm attachment as 'sticky effect' of one cell to a respective matrix, polymer or surface; termed as 'adhesion' and attachment of one cell to another cell called 'cohesion'.

Conclusions

We have accomplished the aim of microbial corrosion inhibition through a decrease in the cathodic rate using lower oxygen consumption by the *Acidithiobacillus thiooxidans* respiratory in the biofilm consortia. We have also achieved a proper understanding of the identity and role of microbial consortia in the specific environment on the metal surface which can be exploited to induce corrosion inhibition using bacteria as a useful tool to prevent from frequent MIC effects.

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References

[1] H.A. Videla, L.K. Herrera, *Microbiologically influenced corrosion: looking to the future,* **International Microbiology, 8**, 2005, pp.169-180.
[2] D. Enning, J. Garrelfs, *Corrosion of Iron by Sulfate-Reducing Bacteria: New Views of an Old Problem,* **Applied and Environmental Microbiology, 80**(4), 2014, pp.1226-1236.
[3] K. Shimizu, *Metabolic Regulation of a Bacterial Cell System with Emphasis on Escherichia coli Metabolism,* **ISRN Biochemistry, 2013,** 2013, pp. 1-47.
[4] N.H. Nik Raikhan, A.R. Khairul Izwan. *Novel Treatment of Heavily Oiled Wastewater using Pseudomonas Aeruginosa Nr.22 Producing Usable Free Fatty Acids (FFA).* **International Journal of Conservation Science, 8**(3), 2017, pp. 537-544.
[5] E.V. Steven, V. Joke, M. Willy. *A new test procedure for biogenic sulfuric acid corrosion of concrete,* **Biodegradation, 10**(6), 1999**,** pp. 421-428.
[6] A.V. Sandu, A. Ciomaga, G. Nemtoi, C. Bejinariu, I. Sandu, *SEM-EDX and microftir studies on evaluation of protection capacity of some thin phosphate layers,* **Microscopy Research and Technique, 75**(12), 2012, pp. 1711-1716. DOI: 10.1002/jemt.22120
[7] I. Sandu, O. Mircea, A.V. Sandu, I. Sarghie, I.G. Sandu, V. Vasilache, *Non-invasive Techniques in the Analysis of Corrosion Crusts Formed on Archaeological Metal Objects,* **Revista de Chimie, 61**(11), 2010, pp. 1054-1058.
[8] T.K. Mori T.M. Koga, Y. Hikosaka, T. Nonaka, F. Mishina, Y. Sakai, J. Koizumi J, *Microbial corrosion of concrete sewer pipes, H_2S production form sediments and determination of corrosion rate*, **Water Science and Technology**, **23**, 1991, p.1275–1282.
[9] R. Zuo, *Biofilms: Strategies for metal corrosion inhibition employing microorganisms*, **Applied Microbiology and Biotechnology, 76**(6), 2007, pp.1245-1253.
[10] G. O'Toole, H.B. Kaplan, R. Kolter, *Biofilm formation as microbial development*, **Annual Review of Microbiology**, **54,** 2000, pp. 49-79.
[11] A.V. Sandu, C. Bejinariu, G. Nemtoi, I.G. Sandu, P. Vizureanu, I. Ionita, C. Baciu, *New Anticorrosion Layers Obtained by Chemical Phosphatation,* **Revista de Chimie, 64**(8), 2013, pp. 825-827.
[12] D. Dana, I.G. Sandu, P. Vizureanu, I. Sandu, *Study on the Corrosion Resistance of Welded-Brazed Joints,* **Revista de Chimie, 64**(12), 2013, pp. 1465-1467.
[13] A.V. Sandu, A. Ciomaga, G. Nemtoi, M.M.A. Abdullah, I. Sandu, *Corrosion of mild steel by urban river water,* **Instrumentation Science and Technology, 43**(5), 2015, pp. 545-557. DOI: 10.1080/10739149.2015.1020432.
[14] T.R. Garrett, M. Bhakoo, Z. Zhang, *Bacterial adhesion and biofilms on surfaces,* **Progress in Natural Science**, **18**(9), 2008, pp. 1049-1056.

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