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The Effects of Culture Conditions for Microbially Influenced Corrosion

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The experimental methods to rapidly and stably reproduce Microbially Influenced Corrosion (MIC) of stainless steel by sulfate-reducing bacteria such as *Desulfovibrio vulgaris* were developed. In this study, using two types of stainless steel, 304 and 444, obtained from Pohang Steel & Iron Co., Ltd. (POSCO)., three major factors were tested; overall medium composition, dilution ratio, and chloride concentration. In the overall medium tests, three different media were prepared according to $FeSO_4$ concentration; PM (original Postgate's medium No. 2), MPM 1 (modified PM, no $FeSO_4$), MPM 2 (modified PM, 1/10 $FeSO_4$). The effects of various dilution ratios (3, 1, 1/3, 1/10, 1/30, and 1/100 times) and chloride concentrations (0.0067M, 0.01M, 0.05M, and 0.1M) were examined during 2 months cultivation. Through SEM (Scanning Electron Microscopy) observation, the diluted and modified media, particularly the 1/3 x MPM 1 medium, showed more micro-pitting points on surfaces compared to the original PM medium. High concentrations of chloride ions (above 0.05 M) were not adequate for observation of MIC since those brought about non-microbiologically induced corrosion. From this study, the optimization of medium composition was very effective to routinely observe MIC in a laboratory system.

Keywords : *MIC, modified Postgate's medium, SRB, stainless steel, chloride*

1. Introduction

Microbially influenced corrosion (MIC) in stainless steels has long been known to occur in cooling water system, water and wastewater facilities, power generating industry, and so on., however, it was not generally recognized until recently. MIC activities are the results of several metabolic functions associated with bacteria. These include the production of organic acids, and the development of differential metabolite cells as a result of bacteria biofilm formation.

These days, new stainless steel products containing different metal compositions are consistently developed from steel companies under more competitive market environment. It is important to estimate how much the product is persistent to corrosion. However, it takes too long time to observe MIC phenomena in the same as field conditions. Furthermore, it is difficult to exclude non-biological effects completely. Therefore, it is needed to develop the methodology to be able to consistently reproduce and accelerate MIC in a laboratory.

In this study, *Desulfovibrio vulgaris*, one of sulphate reducing bacteria (SRB) was used to investigate corrosion phenomena in anaerobic environments. The objectives of this study are: 1) to investigate the effects of different culture conditions and chemical compositions; 2) to find test conditions to consistently induce MIC on stainless steels in a laboratory system; and 3) to accelerate MIC by the variation of chloride concentration with chemical effects excluded.

2. Experimental

2.1 Stainless steel coupons

Two types of stainless steels coupons (10 mm x 10 mm) with 3mm thickness, 304 and 444, were obtained from Pohang Steel & Iron Co., Ltd. (POSCO). The coupons were degreased and cleaned with ultrasonic cleaning equipment and polished with 120 grits of special silicon carbide grinding paper (Buehler, USA).

2.2 Culture of microorganism

The microorganism, *Desulfovibrio vulgaris*, was purchased from Korean Collection for Type Cultures. It was cultivated in an incubator at 30℃ with nitrogen sparging for 30 minutes every day. Postgate's medium was used for cell growth, colony counting, and corrosion tests. Cell concentration was measured as three methods: dry weight,

protein analysis, and optical density. Total organic carbon (TOC) was measured for the consumption of carbon source along with cell growth.

2.3 Corrosion tests

The stainless steel coupons were submerged in the media in 250-mL Erlenmeyer flasks with various test conditions as shown in Table 1. In order to avoid the interference of precipitate from $FeSO_4$ in Postgate's medium, various concentrations of $FeSO_4$ (original, 1/10 of original, no $FeSO_4$) were tested. Also, 6 different dilution ratios of the whole medium (3, 1, 1/3, 1/10, 1/30, and 1/100 times) and 4 different chloride concentrations (0.0067M, 0.01M, 0.05M, and 0.1M) were tested.

Table 1. The compositions of Postgate's Medium #2 and Modified Postgate's Media.

	Postegate's Medium #2	Modified Postegate's Medium #	Modified Postegate's Medium #2
K_2HPO_4	0.5 g	10.5 g	0.5 g
Na_2SO_4	1.0 g	1.5 g	1.45 g
$CaCl_2 \cdot 2H_2O$	0.1 g	0.1 g	0.1 g
$MgSO_4 \cdot 7H_2O$	2.0 g	2.0 g	2.0 g
Sodium lactate (70%)	5.0 ml	5.0 ml	5.0 ml
Yeast extract	1.0 g	1.0 g	1.0 g
Sodium thioglycollate	0.1 g	0.1 g	0.1 g
Sodium ascobate	0.1 g	0.1 g	0.1 g
$FeSO_4$	0.5 g	-	0.05 g
Distilled water	1.0 L	1.0 L	1.0 L
	Adjust pH to 7.3		

2.4 Electron and optical microscopy analyses

The coupons were taken out of the flasks after exposure for 30 days and examined with scanning electron microscopy (SEM) and optical microscopy. Rinse the coupons with sodium phosphate buffer and fixed with 3% glutaraldehyde buffer with phosphate buffer. After 8 hrs, the coupons were washed, post-fixed with 1% osmium tetroxide for 4 hrs and dehydrate the coupons in an ethanol series. The coupons were dried with a critical point dryer using liquid carbon dioxide as transitional fluid and kept in a desiccator.

3. Results and discussion

3.1 SRB growth

The effects of nitrogen sparging on cell growth were shown in Fig. 1. Without nitrogen sparging, carbon sources

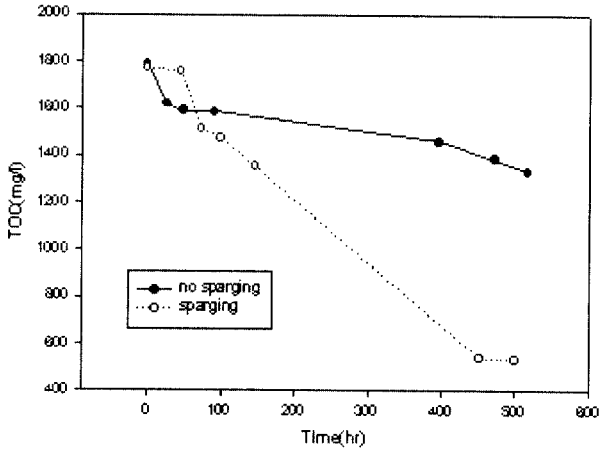
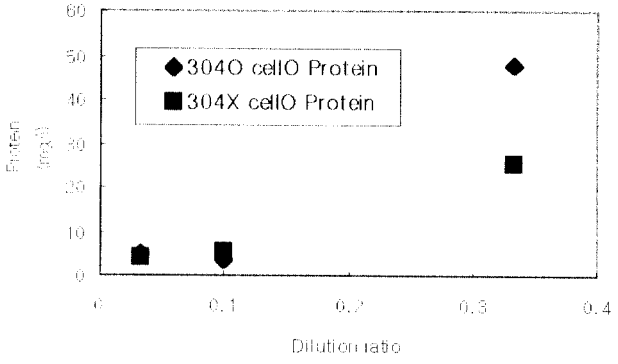
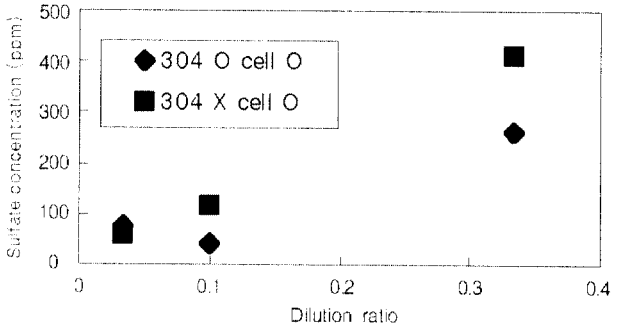


Fig. 1. The carbon source consumption according to cell growth under different nitrogen-sparging scheme.



(a)



(b)

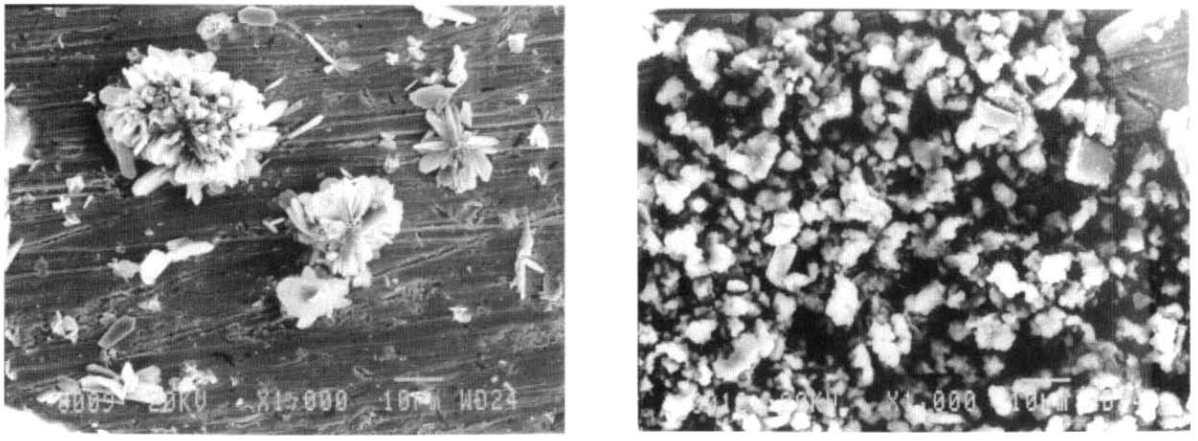
Fig. 2. Effects of the addition of a stainless-steel coupon with various dilution ratios. (a) Cell growth as a protein amount. (b) Sulfate concentration.

were not consumed any more after 50 hrs, i.e., the cell growth was seriously inhibited. It was considered that the nitrogen sparging was critically needed in the two points: to provide strictly anaerobic environment and to remove toxic metabolic gases, mainly hydrogen sulfide.

The effects of coupon addition on cell growth were shown in Fig. 2. The sulfate consumption and cell growth with coupon addition were greatly higher than without coupon addition, particularly at x 1/3 and x 1/10 dilution ratios compared to x 1/100 dilution ratio. It was suggested that the stainless steel coupon provides the surfaces that bacteria can survive effectively on. However, these effects were not observed in the cases of high nutrient concentration above original PM, since the bacterial cell growth in the bulk liquid was very high and the effects of the coupon surface did not become significant.

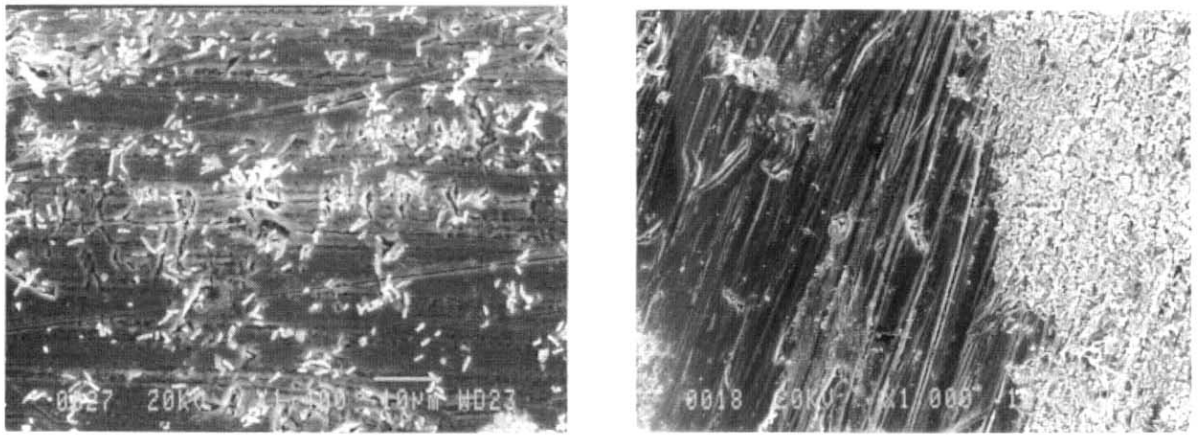
3.2 Modification of mediumure

The composition of Postgate's medium was shown in Table 1. This composition was not for the purpose of the observation of MIC but for the cell growth. Original Postgate's medium has some limitations to properly study MIC. Those are the formation of precipitates in the step of media preparation and the formation of black particles along with cell growth, which interfere the measurement



(a) (b)

Fig. 3. Scanning electron microscope photographs of SS 304 in the different PM concentration without bacterial inoculum. (a) PM x 3. (b) PM x 1.



(a) (b)

Fig. 4. The formation of biolfilm on the surface of SS 304 coupon using a tenth diluted MPM1. (a) Scattered or colonized bacteria. (b) Layer formation of bacteria on the right-hand side.

of cell mass. Thus, PM needed to be modified in terms of $FeSO_4$ concentration and dilution ratio. Without $FeSO_4$ addition, the precipitation of black particles could be avoided. The effects of $FeSO_4$ on cell growth was not significant and the patterns of cell growth were very similar in both PM and MPM1 media.

3.3 Effects of dilution ratio

The effects of the dilution ratio were investigated in two points of view. First, the precipitations on the coupon were shown in Fig. 3. The precipitation and the partial biofilm formation at decay phase were observed above the original concentration of PM medium. These precipitations induced chemical effects and obstructed the formation of biofilm. Second, the formation of biofilm on the coupon was shown in Fig. 4. After about 10 days, biofilm developed on the surface of the coupons. MIC occurred by several steps, such as inoculation, cell growth, biofilm formation, cell growth on the surface, and development of MIC. Biofilm formation is the important step of MIC, due to the role of biofilm on the localization of micro-environment around biofilm that accelerates MIC.

3.4 Effects of chloride concentration

The effects of chloride were shown in Fig. 5. The chemical effect tests were based on x 1/3 case and these tests were measured by an optical microscope. The different results were obtained at 0.01M and 0.05M. With low chloride concentration (0.01M), any corrosion phenomena were not observed without SRB inoculation (Fig. 5(a)), while the pitting were observed on the surface with SRB inoculation (Fig. 5(b)). On the other hand, with high

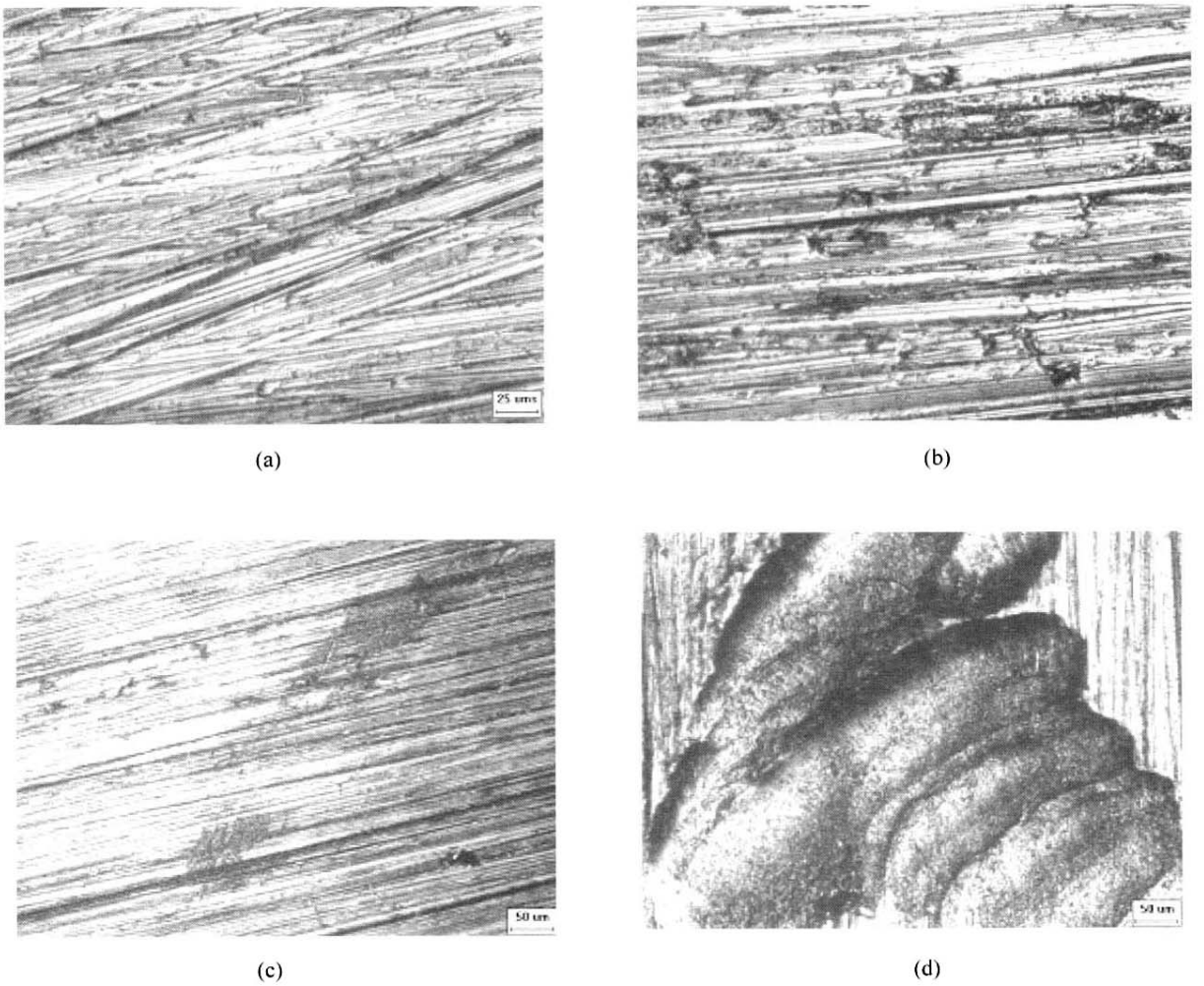


Fig. 5. Optical microscope photographs with different chloride concentration based on MPM1 and x 1/3. (a) SS 444, Cl 0.01M, without SRB. (b) SS 444, Cl 0.01M, with SRB. (c) SS 304, Cl 0.05M, without SRB. (d) SS 304, Cl 0.05M, with SRB.

chloride concentration, the pitting occurred even without SRB inoculation (Fig. 5(c)). With high chloride concentration (0.05M), MIC was significantly accelerated and could be observed within 30 days test.

3.5 Biofilm formation and MIC observation

After 2 months cultivation with the MPM1 x 1/3 medium, a big hole (0.15 mm x 0.5 mm) could be observed on the surface as shown in Fig. 6. In the highly magnified photographs, a number of bacteria were observed on the pit. Also, in another part, long cracks (0.01 mm x 0.0001 mm) and numerous pin points (0.001 mm diameter) were observed. Such phenomena were observed on both stainless steel 304 and 444 coupons. The medium of MPM1 x 1/3 was best for MIC observation, since it was transparent solution to measure microbial growth, could minimize chemical influences without inhibition of microbial growth compared to original PM.

4. Conclusions

The experimental methods were developed to be able

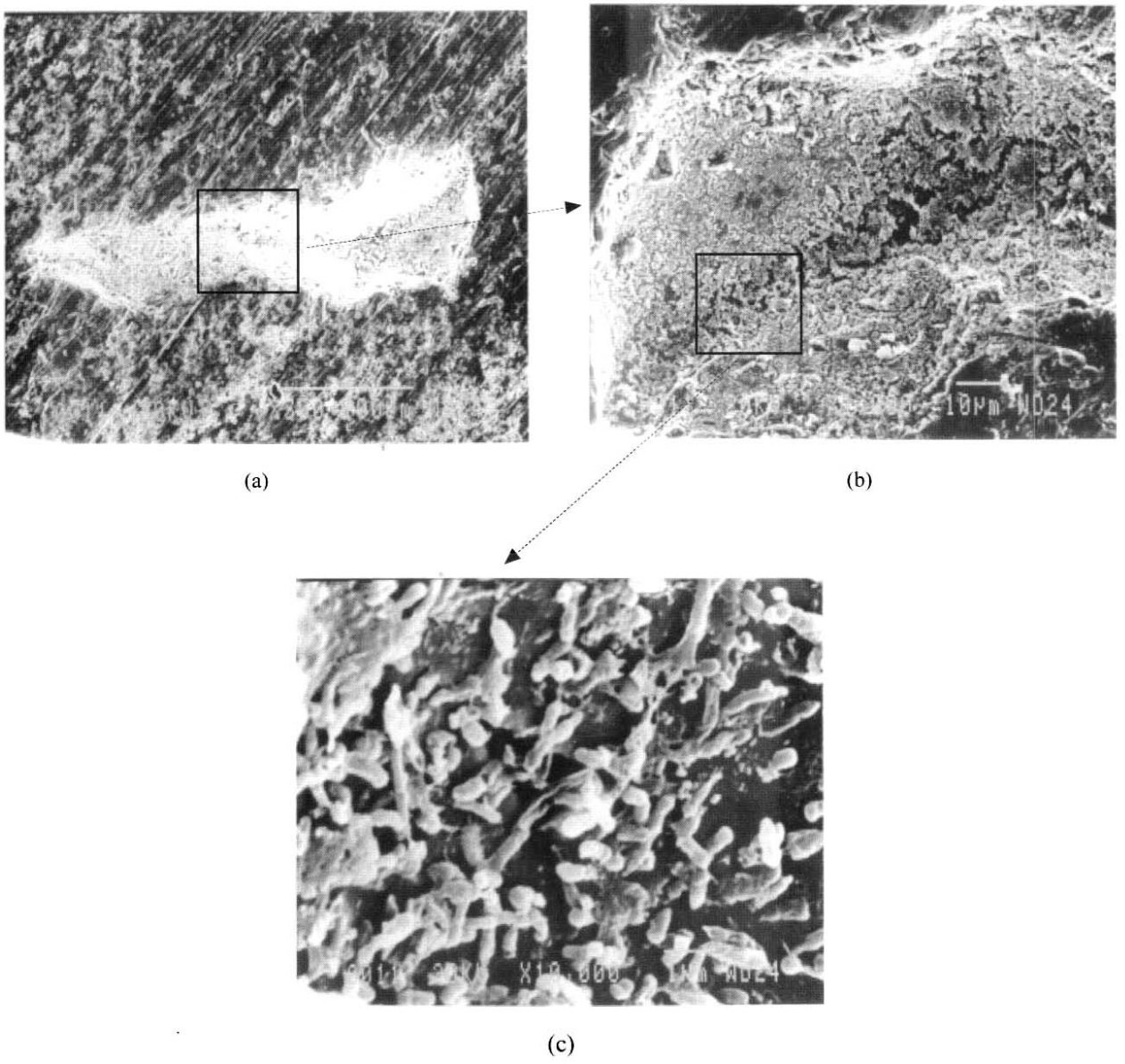


Fig. 6. Scanning electron microscope graphs of the surface of SS 304 with biofilm and MIC using MPM x 1/3 medium. (a X 300 magnified. (b) X1000 magnified. (c) X10000 magnifid.

to consistently reproduce microbially influenced corrosion of stainless steels and accelerate it in a laboratory system. Four major factors were tested using two types of stainless steel (304 and 444); overall medium composition, dilution ratio, chloride concentration, and sulfate concentration. In the scanning electron microscopic observations, the diluted and modified media, particularly the 1/3 times diluted medium without $FeSO_4$ addition, showed more micro-pitting points on the surfaces compared to the original Postgate's medium. High concentration of chloride ion (above 0.05 M) was not adequate to test the MIC, since severe chemical corrosion also occurred and interrupted the observation of MIC. However, sulfate did not interrupt the MIC (as well as biofilm formation). It was suggested that diluted and modified Postgate's medium would be an alternative to observe MIC within short test time in a laboratory system.

Acknowledgements

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