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Biocorrosion inhibition effect of 2-aminopyrimidine derivatives on SRB

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Abstract

In the study the biocidal activity and the corrosion inhibitor activity of 2-aminopyrimidines on growth of *Desulfotomaculum* sp. B2-1 and B2-2 strains and biocorrosion of X65 steel in 63a medium were determined. For this purpose 4 synthetic compounds (2-amino-4-chloro-6-methylpyrimidine (AMP1), 2-amino-4-chloro-6-morpholinepyrimidine (AMP3), 2-amino-4-chloro-6-(4-methylpiperazine-1-yl) pyrimidine (AMP8), 2-amino-4-morpholine-6-methylpyrimidine (AMP11)) were tested for biocidal activity on *Desulfotomaculum* sp. Corrosion experiment system were prepared with standard corrosion cell were incubated at 30°C in incubator for bacterial growth. Electrochemical Impedance Spectroscopy (EIS) and potentiodynamic polarization methods were used to identify corrosion with CH Instruments 660B. The AMP1 and AMP3 compounds reduced the growth of *Desulfotomaculum* sp. at 25 mg L^{-1} concentration. Moreover, these synthetic compounds reduced the biocorrosion of X65 steel by B2-1 strain 95% and 91% for AMP1 and AMP3 sequentially.

Key words: *steel, microbiological corrosion, potentiostatic curves, 2-aminopyrimidines.*

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1. Introduction

Corrosion which occurs due to biofilm formation on metallic material and microbial activity is termed as Microbial Induced-Influenced Corrosion (MIC) or biocorrosion and a major problem for the oil and gas industry. Biocorrosion is responsible for greater than 20% pipeline system failure and biocorrosion of metals and metal alloys drew considerable attention in recent years [1, 2].

Sulfate-reducing bacteria (SRB) are the most important and largest microorganism group that cause dissimilative sulfate reduction and are the largest bacteria group associated with MIC [3–5].

Corrosion inhibition refers to deceleration of corrosion reaction with various compounds and involves electrochemical reactions like corrosion. Corrosion inhibitor mixtures form a recyclable film layer to prevent the reaction between the solution and corroded surface [6].

Chemical compounds that control microbial activity are called biocides. In addition to inhibiting microbial activity, biocides should be environmentally applicable [2, 7–8]. Cationic type imidazoles, amines, diamines, amino-amines and film forming inhibitors are widely used in oil and gas industries [6]. Cationic type imidazoles, amines, diamines, amino-amines are used as biocidal and corrosion inhibitor in oil systems [6, 9]. The biocidal activity of glutaraldehyde and EDDS determined on SRB and reported that 30 mg L^{-1} glutaraldehyde and 2000 mg L^{-1} EDDS controlled the growth of SRB [10]. In literature different synthetic compounds were used for corrosion inhibition [5, 11, 12–15]. The oxidiazole derivatives such as 2-MPOX, 3-MPOX, 4-MPOX, were used as biocides on brass and low carbon steel that slowed down cathodic and anodic process [16, 17]. The biocidal effect of AMP1 which was used in this work is higher than that of MPOX derivatives.

Pyrimidines, a class of heterocyclic compounds including imidazoles, triazoles *etc.* have been known to show considerable biological activity [18–20]. In addition to their biological activities (biocidal properties), pyrimidines are effective inhibitors for the corrosion because of nitrogen-containing heterocyclic compounds [21]. Therefore, synthesis of new pyrimidine derivatives and investigation of their biocidal and corrosion inhibitor properties are very important.

Desulfotomaculum sp. has extremely high biocorrosion effect. In this study alternative biocidal compounds were investigated that reduce biocorrosion effect of *Desulfotomaculum* sp. on X65 steel which is used in oil pipelines. For this purpose, newly synthesized compounds of 2-aminopyrimidines were determined for the biocidal and corrosion inhibition effect on *Desulfotomaculum* sp. and X65 steel.

2. Experimental

2.1 Synthesis of the organic compounds

In this work, 2-aminopyrimidine derivatives (2-amino-4-chloro-6-methylpyrimidine (AMP1), 2-amino-4-chloro-6-morpholinepyrimidine (AMP3), 2-amino-4-chloro-6-(4-methylpiperazine-1-yl)pyrimidine (AMP8), 2-amino-4-morpholine-6-methylpyrimidine (AMP11)) were synthesized and the structures of these compounds were shown in Figure 1.

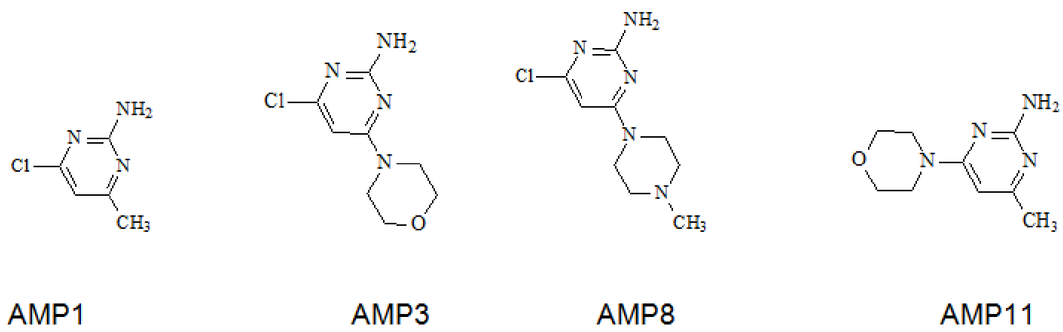

Figure 1. The forms of synthethized 2-aminopyrimidines.

2.2 Growth conditions of Desulfotomaculum sp.:

B2-1 and B2-2 strains of *Desulfotomaculum* sp. which were isolated from Adıyaman-Turkiye oil well were used in the study; also 63a medium was used for growth of *Desulfotomaculum* sp. The composition of the medium was as follows: K_2HPO_4 0.5 g L^{-1} (Merck), NH_4Cl 1.0 g L^{-1} (Merck), Na_2SO_4 1.0 g L^{-1} (Merck), $CaCl_2·2H_2O$ 0.1 g L^{-1} (Merck), $MgSO_4·7H_2O$ 2.0 g L^{-1} (Merck), Na acetate 2.0 g L^{-1} (Merck), Na pyruvate 5.0 g L^{-1} (Fluka), yeast extract 1.0 g L^{-1} (Scharlau), $FeSO_4·7H_2O$ 0.5 g L^{-1} (Riedel-de Haen), Ascorbic Acid 0.1 g L^{-1} (Merck), Mercapto Acetic Acid 0.1 g L^{-1} (Aldrich), Resazurin 1 mg L^{-1} (Aldrich). The pH of the medium was adjusted to 7.2 ± 0.1 with NaOH and H_2SO_4. To achieve anaerobic conditions, medium were subjected to nitrogen gas; sealed with rubber plugs and were autoclaved. Bacteria cultures used in the experiments were growth at 30°C for 24 hours for continuing experiments [22].

2.3 Identification of biocidal activities of synthetic organic compounds

Synthetic organic compounds were added 25, 50 and 100 mg L^{-1} final concentrations into 10 ml 63a medium to identify their biocidal activities. *Desulfotomaculum* sp. B2-1 and B2-2 isolates were inoculated with a proportion of 1/10 v v^{-1} and they were incubated at 30°C for 24 hours. The growth of *Desulfotomaculum* sp. strains was determined with the formation of characteristic iron precipitates and undesired rotten egg odor.

2.4 Corrosion determination studies

Working electrode was produced from API SL X65 (BOTAŞ–Yumurtalık–Turkiye) steel. The steel used in working electrode contained 98.3% Fe, 0.074% C, and 0.243 Si%, 1.18% Mn, 0.013% P, 0.0047% S. Working electrode was cleared with sandpaper and washed with alcohol and distilled water and dried. Working electrode was sterilized with UV light for 15 minutes. Saturated calomel electrode (SCE) was used as reference electrode and platinum electrode was used as counter electrode.

Electrochemical experiments were designed to identify the effects of *Desulfotomaculum* sp. isolates on corrosion of X65 and determine biocidal and corrosion inhibition activities of synthetic organic compounds [22, 23]. Corrosion experiment system were prepared with standard corrosion cell were incubated at 30°C in incubator for bacterial growth.

2.5 Electrochemical analyses

Electrochemical Impedance Spectroscopy (EIS) and potentiodynamic polarization methods were used to identify corrosion with CH Instruments 660B. EIS analyses were performed on determined OCP value at an interval of 0.005–100000 Hz with 5 mV amplitude. Solution resistance (R_s) and polarization resistance (R_p) were calculated with Zsim demo 3.22d version using the circuit in Figure 2.

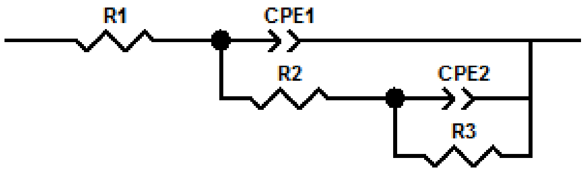

Figure 2. The equivalent circuit for EIS calculations, R_s (solution resistance): *R*1, R_p (Polarization resistance): *R*2 + *R*3.

Potentiodynamic polarization graphs were obtained with 0.001 mV/s scan rate between the range of –1.4 to 0.15 V potential. Corrosion potential (E_{corr}, mV), corrosion current density (I_{corr}, μA cm^{-2}) were calculated based on potentiodynamic polarization graphs.

Corrosion inhibition effectiveness (*IE*%) of synthetic organic compounds used in the study were calculated using the below formula:

$$IE\% = \left[\left(i_{corr} - i_{inh}\right) / i_{corr}\right] \times 100 .$$

3. Results and Discussion

3.1 Biocidal activities of synthetic organic compounds

In the study the compounds which can be used to prevent biocorrosion were investigated, after determining the effects of *Desulfotomaculum* sp. isolates on the corrosion of X65 steel.

2-Aminopyrimidine group synthetic organic compounds were used in the present study. Biocidal effects of four 2-aminopyrimidine compounds (AMP1, AMP3, AMP8 and AMP11) were presented in Table 1 and AMP1 and AMP3 substances decreased the growth of *Desulfotomaculum* sp. at 25 mg L^{-1} concentration and completely ceased growth of the bacterium at 50 mg L^{-1} concentration. Moreover 25 mg L^{-1} 2-aminopyrimidine concentration was chosen for biocorrosion inhibitor activity for AMP1 and AMP3. However, AMP8 and AMP11 substances had no biocidal activity on *Desulfotomaculum* sp.

Table 1. The biocidal effect of 2-aminopyrimidines on *Desulfotomaculum* sp. B2-1 and B2-2 strains in anoxic conditions.

		25 mg L^{-1}	**50 mg L^{-1}**	**100 mg L^{-1}**
B2-1	AMP1	±	–	–
	AMP3	±	–	–
	AMP8	+	+	+
	AMP11	+	+	+
B2-2	AMP1	±	–	–
	AMP3	±	–	–
	AMP8	+	+	+
	AMP11	+	+	+

(+): positive growth; (–): negative growth; (±): weak growth.

3.2 The effect of Desulfotomaculum sp. isolates on biocorrosion of X65 steel

Nyquist curves of *Desulfotomaculum* sp. strains were presented in Figure 3. Variances of solution resistance (R_s) and polarization resistance (R_p) values calculated from the curves were presented in Table 2 and the situation is coherent with the literature [24]. The R_s value of X65 steel was decreased with the growth of *Desulfotomaculum* sp. B2-1 and B2-2 strains to 10 $\Omega \cdot cm^2$ and 9.5 $\Omega \cdot cm^2$ from 13.9 $\Omega \cdot cm^2$ respectively. In addition to this B2-1 and B2-2 decreased the R_p value to 3667 $\Omega \cdot cm^2$ and 1071 $\Omega \cdot cm^2$ from 11061 $\Omega \cdot cm^2$ consequently.

Table 2. The biocorrosion of x65 steel under anoxic conditions by *Desulfotomaculum* sp. B2-1 and B2-2 strains and the inhibitor activity of AMP1 and AMP3.

		E_{corr} **(mV)**	I_{corr} **(μA·cm^{-2})**	R_s **(Ω·cm^2)**	R_p **(Ω·cm^2)**	***IE*%**
63a		–937	1.5	13.9	11061	
B2-1		–741	791.0	10.0	3667	
B2-2		–888	60.0	9.5	1071	
B2-1	AMP1	–780	39.5	13.8	4679	95
	AMP3	–797	71.4	15.2	194701	91
B2-2	AMP1	–778	233.6	17.3	1296	
	AMP3	–921	262.7	15.0	195464	

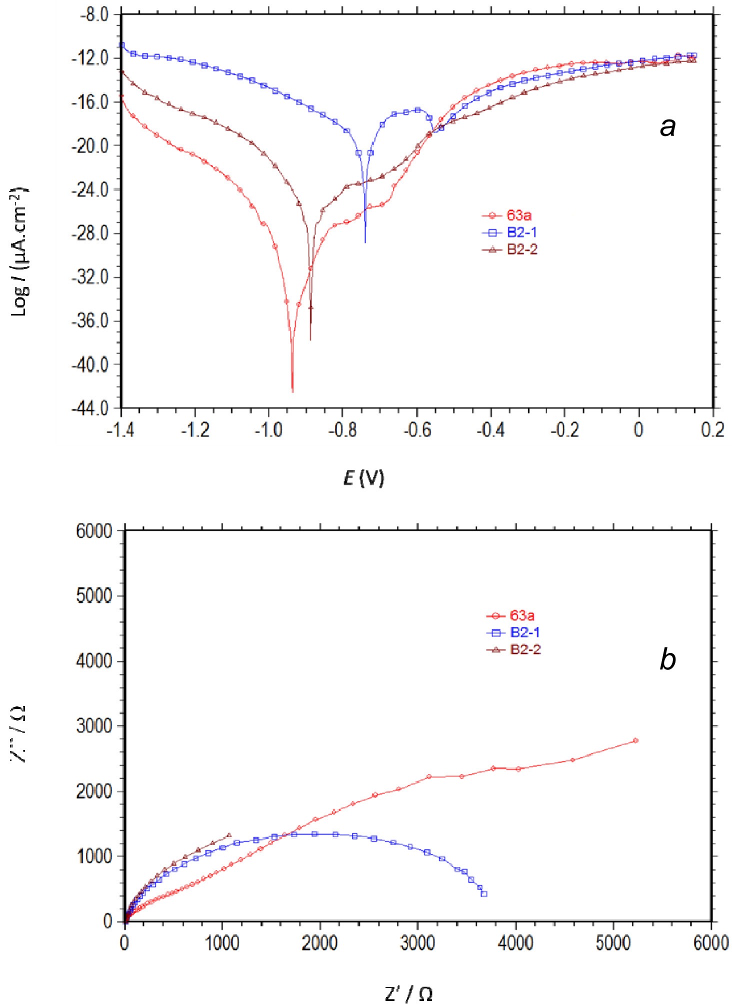

Figure 3. The potentiodynamic and EIS graphs of 63a medium and *Desulfotomaculum* sp. B2-1 and B2-2 strains. (*a*) Potentiodynamic graphs; (*b*) Nyquist plots.

The potentiodynamic polarization curves of *Desulfotomaculum* sp. B2-1 and B2-2 strains showed that at the end of 168-hour incubation period the I_{corr} value of X65 steel increased to 791.0 μA cm^{-2} and 60.0 μA cm^{-2} from 1.5 μA cm^{-2} (Figure 3, Table 2). *Desulfotomaculum* sp. strains formed biofilm and increased corrosion of X65 steel at the end of 168-hour incubation that used to determine corrosion inhibition activities of synthetic organic compounds, consistent with previous studies in the literature [11, 23].

3.3 The inhibitor effects of synthetic organic compounds

The potentiodynamic polarization curves of X65 steel in the presence of *Desulfotomaculum* sp. provide information about corrosion rate of steel (Figure 4, Table 2). The AMP1 reduced I_{corr} to 39.5 μA cm^{-2} from 791.0 μA cm^{-2} for *Desulfotomaculum* sp. B2-1 and increased the R_s to 13.8 Ω·cm^2 from 10 Ω·cm^2 and R_p to 4679 Ω·cm^2 from 3667 Ω·cm^2. On account of this AMP1 reduced the biocorrosion of X65 steel by *Desulfotomaculum* sp. B2-1 strain.

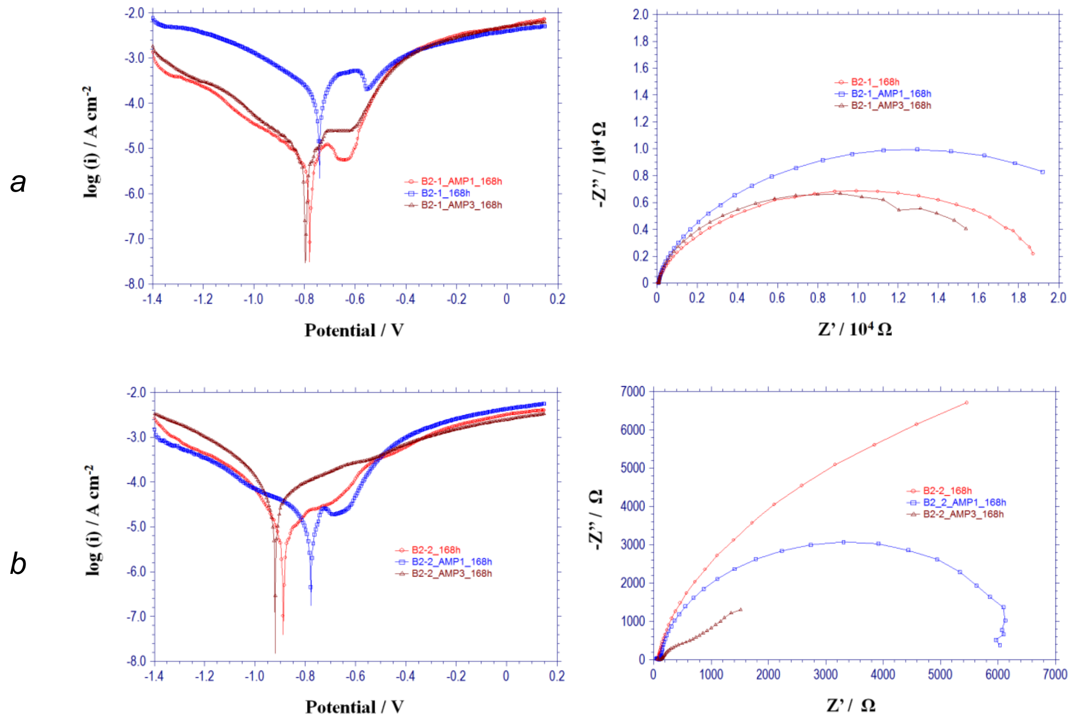

Figure 4. The effect of 2-aminopyrimidines on biocorrosion of x65 steel by *Desulfotomaculum* sp. B2-1 and B2-2. (*a*) *Desulfotomaculum* sp. B2-1; (*b*) *Desulfotomaculum* sp. B2-2.

The AMP3 decreased I_{corr} to 71.4 μA cm^{-2} from 791.0 μA cm^{-2} and increased R_s and R_p to 17.3 Ω·cm^2 and 194701 Ω·cm^2 respectively for *Desulfotomaculum* sp. B2-1 strain. AMP1 and AMP3 substances prevented corrosion caused by *Desulfotomaculum* sp. B2-1 isolate with 95% and 91% inhibition ratios respectively. As it is seen in the results the AMP1 was more effective than AMP3. The solution resistance has not changed too much but the polarization resistance was very different. The methyl group was used instead of morpholine group in AMP1. This difference caused the increasing of R_p values with AMP3.

At first, the increase in both I_{corr} and R_p, due to the effect of AMP1 and AMP3 on the B2-2 strain, may seem like an anomaly. However, when this effect is taken into account along with the Tafel graphic patterns, the change in AMP1 and AMP3 Tafel patterns, with respect to steel, can easily be attributable to individual processes. This shows the presence of a mixed control process. Indeed, while AMP1 shows decrease in the cathodic direction and an increase in the anodic direction, the opposite is true for AMP3. Consequently the I_{corr} values increased to 233.6 μA cm^{-2} and 262.7 μA cm^{-2} and the R_p values increased to 1296 Ω·cm^2 and 195464 Ω·cm^2 with the presence of *Desulfotomaculum* sp. B2-2 strain for AMP1 and AMP3. These results are indicated that the 2-aminopyrimidine group synthetic organic compounds have biocidal effect on *Desulfotomaculum* sp. but have not any effect on biocorrosion of steel.

The AMP1 and AMP3 had a small difference (methyl group *vs*. morpholine group) in their structure but this situation caused to big difference in the corrosion inhibition effect of

AMP1 and AMP3. As seen in Table 3, the presence of morpholine has effected the molecules quantitative structure-activity relationship (QSAR) properties. When the data in Table 2, where the polarization of AMP3 is higher than AMP1 in the presence of both strains, and Table 3 are both taken into account the biocidal effect of AMP3 has been evaluated to be higher than that of AMP1.

Table 3. Calculated QSAR properties for AMP1 and AMP3 using HyperChem 8.0.10 [25].

	Polarizability (Å^3)	**Molecular Volume (Å^3)**	**Molecular Surface (Å^2)**	**Partition Coefficient, Log *P***
AMP1	14.13	441.58	300.94	1.76
AMP3	20.85	607.10	387.20	0.95

In literature different synthetic compounds were used for corrosion inhibition [5, 11, 12–15]. 20 ppm MPOX concentration reduced the biocorrosion of brass and low carbon steel, as 91%, 90%, 50% for 3-MPOX, 4-MPOX, and 2-MPOX sequentially [16, 17]. The biocidal effect of AMP1 which were used in this work is higher than MPOX derivatives.

Based on these data, this study AMP1 and AMP3 compounds in 2-aminopyrimidine class were used and it was found that these compounds were significantly effective in preventing the corrosion of X65 steel by *Desulfotomaculum* sp. B2-1 strain. In addition, 25 mg L^{-1} concentration is considered as suitable for a biocidal compound.

4. Conclusion

The study found that *Desulfotomaculum* sp. increased the corrosion of X65 steel dramatically in 168-hour incubation period. AMP1 and AMP3 substances among synthetic organic compounds belonging to 2-aminopyrimidine class were found to have biocidal activity on both strains; while AMP8 and AMP11 substances had no biocidal effect. As for the corrosion inhibition activities of the compounds which show biocidal activity, it was found that AMP1 and AMP3 substances prevented biocorrosion by *Desulfotomaculum* sp. B2-1 strain with a ratio of 95% and 91% respectively. Based on these results, it was thought that AMP1 and AMP3 compounds among 2-aminopyrimidine can be used to prevent biocorrosion of X65 steel by *Desulfotomaculum* sp. B2-1 strain.

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