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Amaricf_Basra office@yahoo.com
abdulalwan@yahoo.com
.marshbulletin@yahoo.com

Activity of sulphate reducing bacteria isolated from Shatt Al Arab water on corrosion mild steel specimens *in vitro*

W. H. A. S. Al Tamimi
Science College - University of Basrah

Abstract

The research included study of the corrosion effect of mix cultures of sulphte reducing bacteria isolated from non artificial environmental include waters of Shatt Al Arab river. The corrosion effect study on mild steel coupons supplied from Nalco Company during three exposure time include (30, 60 , 90) days , the rate of microbial corrosion was measured by lost weight, the result showed that the rate of corrosion increase with period time , the high corrosion rate was (0.8468) mg / cm^2/ day through (90) days period , and the lowest corrosion rate was (0.4014) mg / cm^2/ day through (30) days period.

1- Introduction

Sulfate- reducing bacteria (SRB) are the main group of taxonomically drivers microorganisms which are classified as a strictly anaerobic and which are distributed within two domains : archaea and bacteria. SRB use lactate, acetate or hydrogen as electron donors and sulfate, sulfide, thiosulfate and sulfur itself as the final electrone acceptor in their anaerobic respiration leading to produce a large amounts of hydrogen sulfide (Hao *et al.* , 1996 ; Rzeczycka *et al.*, 2004 and Sahrani *et al.*, 2009).

SRB causes biocorrosion of cast iron, carbon and low alloys , stainless steel high nickel alloys and copper alloys (Zuo *et al.*, 2004). These corrosion has been mainly attributed to the production of corrosive hydrogen sulfide or cathode depolarization by biological consumption of hydrogen at the cathode by the hydrogenase enzyme and production sticky exopolymers (Hamilton, 1985 and Yu *et al.*, 2002). The

main corrosion products formed on the iron surface are ferrous sulfide which can be productive of aggressive to the under lying metal (Beech, 2002). These corrosion by SRB primary realized as a pitting, localized attack , crevice , under deposit corrosion and stress corrosion cracking which occurs as a result of activity of physiologically dives SRB species present within biofilm on the metals surface (Zuo *et al.*, 2004 and Sahrani *et al.*, 2009). Biofilm consist of microbial cells their extra cellular polymeric substance (EPS) and adsorbed organic matter in addition inorganic precipitate may originate from the bulk aqueous phase or present as corrosion products.(Beech and Coutinho, 2003).

The corrosion by SRB occurs in aquatic and terrestrial inhabitants varying nutrient content , temperature, pressure and pH value under anoxic and oxygenated condition (Beech, 2002).many factors effects on the SRB growth in environments such as amounts of sulfate, SRB only flourish and cause damage if they can obtain sufficient sulfate to act as acceptors in their metabolism also carbon source, minor nutrients and trace metals necessary for their growth, in economically environments the present of biocide and toxic agents that use as a inhibitors and extreme condition high temperature, pressure and extreme pH all these not preclude the growth of SRB (Sequera and Tiller, 1988). These factors controlling the numbers and distribution of SRB and then sulfate reduction which caused the corrosion and damage of metals , The aim of this study is to investigate the ability of SRB communities from non artificial environments to in causing mild steel allays corrosion.

2- Material and methods

Samples collection

Water samples were collected from Shatt- Al- Arab river in Basrah , the samples collected from subsurface water about (50) cm by clean bottles sealed and transport immediately to the laboratory.

Isolation and cultivation of sulfate-reducing bacteria:

SRB were isolated from water samples by using liquid API medium (API , 1975) as selective growth medium which has the following composition : yeast extract (1 g) ; $MgSO_4$. $7H_2O$ (0.2 g) ; Fe $(NH_4)_2$ $(SO_4)_2$. $6H_2O$ (0.2 g) ; NaCl (1 g); $K2HPO_4$ (0.01 g) ; Ascorbic acid (0.1 g) and Sodium lactate (2.24 g). All of the dry chemical were measured out first and prepared by adding to each a liter of distal water , the pH was adjusted to (7.2) using (1 M) NaOH solution , then sterilized by autoclave under (121) °C and pressure

(15) bound / inch² for (15) minute , left to cool at room temperature. This medium treated with added oxygen reducing agents as the following: Sodium dithionate (0.3 g / l) and L–systein (0.28 g / l) these compounds were sterilized by heat , and saturated under gas phase (90% N2) with (10% CO2) before being inoculated with the samples (Rabus *et al.*, 1996 and Teske *et al*, 1996). The soiled medium was prepared by added (1.5 % wt. / vol.) agar.

To isolated of SRB from samples (1) ml of sample was added to screw tubes approximately full of liquid API medium , tubes were sealed by screw cover and coated with paraffin tape (parafilm) to prevent diffusion of O2 into medium, and incubated at (37) °C until blackening of the medium was recorded as positive for SRB presence. (Hirnes *et al. ,* 1999 and Carignan *et al.*, 1994).

Community of sulfate reducing bacteria (Mix cultures):

The Community of sulfate reducing bacteria was obtained by mix pure cultures of SRB which isolated by using roll tubes technique (Hungate , 1969) with API solid medium at (45) °C tubes was stopped well , coated with parafilm and incubated at (37) °C until well formed colonies become visible , the colonies picked by means of final drawn sterile Pasteur pipettes and immediately transferred into tubes of fresh liquid API medium , community of SRB obtain by mixing of many pure colonies of bacterial communities originating from samples (Rabus *et al.* , 1996)

Corrosion test (Bell and Lim , 1981)

The metal samples was provided from Nalco Europe B.V. Company figure (1) the corrosion of mild steel samples (coupons) occurred in cultures was determined in a duplicated corrosion test, the coupon rods were abraded with No.1 emery papers degreased in acetone and then acid etched in (2N) HCl for (30) minutes. This pretreatment process leaves the specimens clean , bright and highly susceptible to corrosion experiment because of the removed of the any protective surface film pretreated specimens were weighted (mg) and measured surface area (cm^2), sterilized by passing through a bunsen flame and then placed into sterile (250) ml conical flask contains liquid API medium. each test flask was inoculated with (3) ml of active mix culture before the flask were tightly Stoppard , sterile API medium was added until the flask were completely filled. The test flask were incubated at (37)°C, control samples containing API medium without inoculums were also prepare at intervals of (7) days, (100) ml of fresh sterile API medium was introduced aseptically into the lower portion of each flask with a sterile

(100) ml pipette resulting in the upward displacement of an equal volume of exhausted medium , at the end of the test period (30,60,90) days , the test specimens were removed from both test and control flask , loose corrosion products , black FeS was removed by washing in a stream of hot water, the specimens were place into glacial acetic acid for (30) minute to remove adherent corrosion products , after washing in distilled water the specimens were dried on filter papers and reweighted. The corrosion rate was measurement by formula at the following:

Corrosion rate (mg/cm^2 /day) = Weight lost / Surface area / Exposure time.

3- Result

The bacterial cultures showed good growth anaerobically in API medium and on agar medium that supplied with oxygen reducing agents and presence of sodium lactate as carbon source under (90% N2) with (10% CO2). Rapid growth was observed on this medium as it only took about three days for the liquid medium to turn blacking due to sulfide production , the observation of black color might imply the presence of SRB.

The results of corrosion test showed forming biofilm on the surface of mild steel metal (figure 2) corrosion rate measurement was appear that the highest corrosion rate was (0.8464) mg/cm^2 /day during (90) days, which equal to (304.849) mg/ cm^2/ year, while the lowest corrosion rate was (0.4014) mg / cm^2 / day during (30) days, which equal to (144.504) mg/ cm^2 /year. Table (1) and figures (3and 4).

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Table (1) Measuring of Corrosion rate by lost weight method

Samples	Lost weight mg	Surface area cm^2	Corrosion rate $mg/cm^2/day$	Net microbial corrosion $mg/cm^2/day$	Net microbial corrosion $mg/cm^2/year$
		30 days			
Control	13.1	22.189	0.0211	0.0000	0.0000
Mild steel	262.5	22.189	0.4225	0.4014	144.504
		60 days			
Control	28.5	22.189	0.0459	0.0000	0.0000
Mild steel	459.8	22.189	0.7401	0.6942	249.912
		90 days			
Control	69.4	22.189	0.1117	0.0000	0.0000
Mild steel	595.5	22.189	0.9585	0.8468	304.849

Figure (1): Once coupon of mild steel supply from Nalco Company

(Figure (2): Coupon of mild steel : **A**_ Exposure to mix culture of SRB , **B**_ Control coupon in API medium only, **C**_ Coupon non treated.

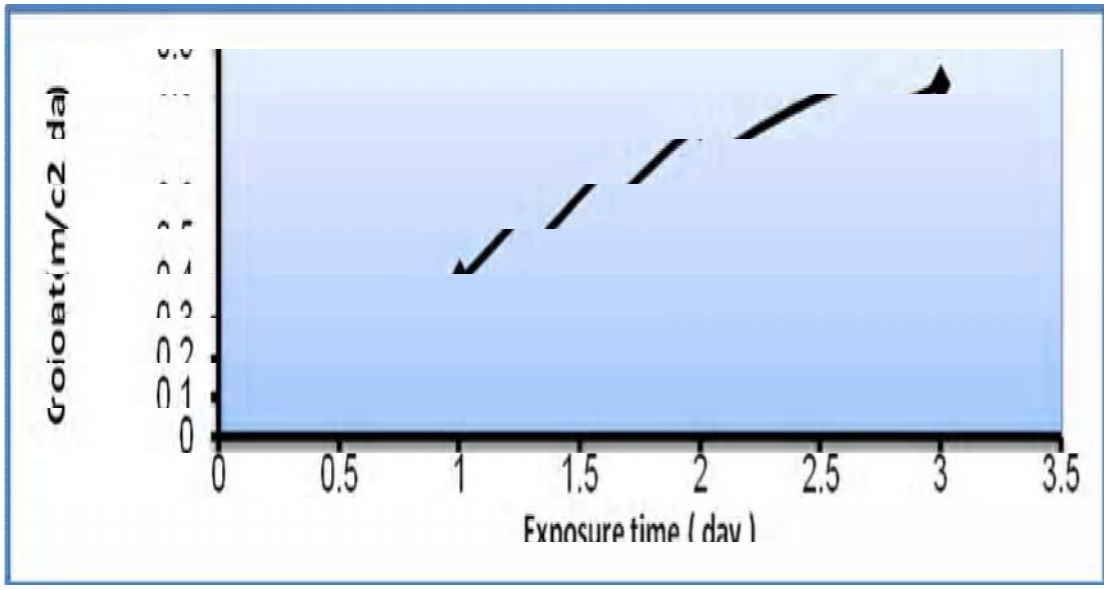

Figure (3) Relation between the Corrosion rate of mild steel and exposure time per day

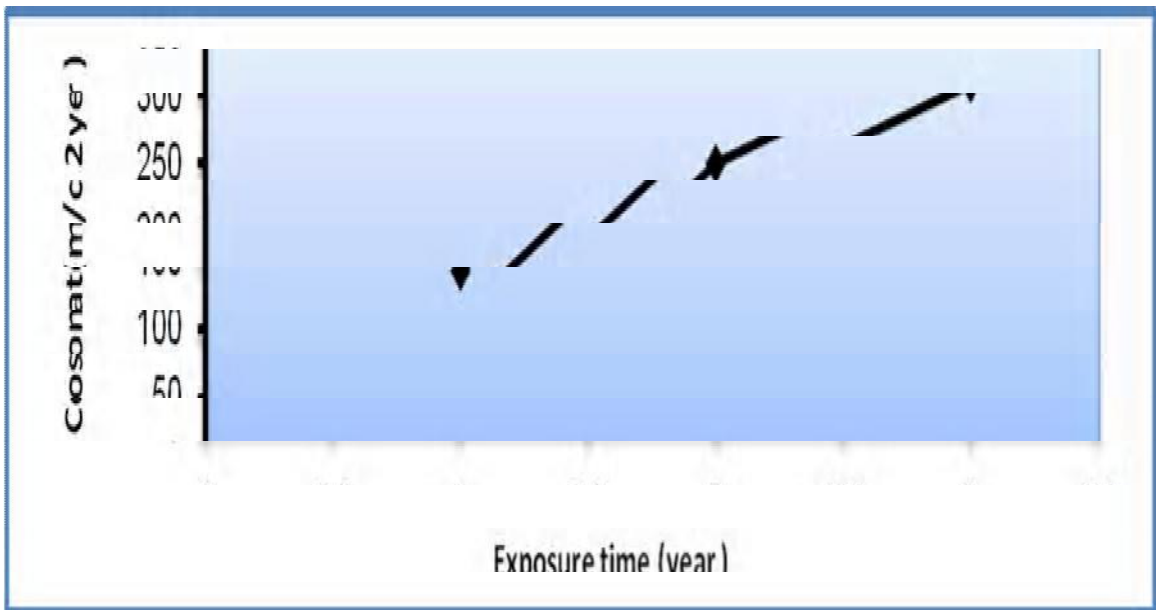

Figure (4): Relation between the Corrosion rate of mild steel and exposure time per year

4-**Discussion**

The SRB cultures grown in API medium with sodium lactate through three days , the growth was estimated on the basic of the amount of sulfate reduced in the medium to turn blacking (Hanselmann *et al.*, 1995 and Rzeczycka *et al.*, 2004).

The result of corrosion test showed increase corrosion rate with period time of exposure, the corrosion occurs by accumulation of microbial metabolic products aggressive to the protective layers and to metal itself , harbouring enzymes which are able to effect reduction reaction at cathode site , providing matrix for binding sorption of diverse metal cations and demons tating the presence of an a corroded metal surface (Beech , 2002).In this study we test the SRB ability to corroded metals isolated from non artificial environment and the result show lower corrosion rate (0.8468) mg / cm^2 / day through (90) days compared with study of AL-Tamimi (2001) which to refer higher corrosion rate (1.6266) mg / cm^2 / day through (84) days by mix culture of SRB isolated from cooling water system of fertilizer company.

The condition that SRB can grow at very high temperature or pressures in environments of extreme pH or in the presence of toxic agents such condition are encountered in the artificial environment ,

these extreme condition do not preclude the effects of either direct or indirect microbial corrosion by SRB and may accentuate problem (Blson *et al.*, 1981). The concentration of sulfate may different between the artificial and non artificial environments and these effect on activity of SRB to corrosion of metal.

The different in rate of corrosion potential due to the SRB itself these bacteria vary in their ability to influence deterioration of metallic material to date no clear consensus has been reached in elucidating the importance of species specificity in corrosion processes (Beech and Coutinho , 2003).Also may be the deference in hydrogenase enzyme activity account for the variation in corrosion potential

(Booth and Warmwell , 1961) and this agreement with study of (Bell and Lim , 1981) which showed that the higher corrosion activity was associated with marine and brackish water isolated compared with isolated from fresh water environment.

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فعالية الجراثيم المختزلة للكبريت المعزولة من مياه شط العرب على تآكل عينات الحديد المطاوع مختبرياً

وجدان حسين عبد الصاحب التميمي
جامعة البصرة / كلية العلوم / قسم علوم الحياة

الخلاصة

تضمن البحث الحالي دراسة التأثير التآكلي لمزارع خليطة من الجراثيم المختزلة للكبريت معزولة من بيئة غير صناعية متمثلة بمياه شط العرب وتم دراسة قابليتها على إحداث التآكل في عينات الحديد المطاوع المجهزة من قبل شركة نالكو وقد درست قابلية الجراثيم المعزولة على تآكل النماذج الحديدية مختبرياً في وسط API السائل خلال ثلاث فترات زمنية هي (30 و 60 و 90) يوماً وتم حساب معدلات التآكل بالاعتماد على الطريقة الوزنية وقد أظهرت النتائج زياد واضحة في معدلات التآكل مع زيادة الفترة الزمنية للتعرض إذ بلغ أعلى معدل للتآكل (0.8468) ملغم/ م^{٢}/ يوم خلال فترة (90) يوماً و أقل معدل للتآكل بلغ (0.4014) ملغم / سم^{٢} / يوم خلال (30) يوماً من التعرض.