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BIOCHEMICAL ENGINEERING APPROACHES TO MIC

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ABSTRACT

Microbiologically influenced corrosion (MIC) is a major problem in the oil and gas industry. A group of bacteria known as sulfate reducing bacteria (SRB) are most frequently the culprits. Although some new mitigation methods have started to emerge, such as adding nitrate to promote the growth of nitrate reducers that outcompete with SRB for nutrients, current mitigation methods still rely on biocides and biostats that either kill planktonic bacteria or inhibit their growth at low concentrations. A much higher concentration is needed to control established biofilms. Microorganisms are capable of developing resistances to biocides after prolonged use. Environmental and safety concerns on biocide uses are becoming more pressing. Non-biocide mitigation approaches are being sought along with enhanced biocide delivery. Biochemical engineers can bring a fresh perspective to the MIC research. By studying SRB growth conditions, important parameters that can be controlled to prevent or slow down SRB growth and biofilm buildup can be identified. The ATCC 7757 strain of *Desulfovibrio desulfuricans* was used in this work. Laboratory experiments were carried out in 100 ml anaerobic vials and 2-L glass cells fitted with a rotating mild steel coupon and instrumentation for electrochemical analysis. Various effects on the growth and corrosion behavior of SRB were investigated including effects of medium composition, flow condition and effect of microcarriers for cell immobilization. A new solid medium that offers fast growth and requires no special hydrogen atmosphere was developed for SRB plating. It is especially suitable for quantification and analysis of sessile SRB cells.

Keywords: Sulfate Reducing Bacteria, Biocorrosion, Microbiologically Influenced Corrosion, MIC, Biocide, *Desulfovibrio Desulfuricans*, Hydrogen Sulfide

INTRODUCTION

Corrosion of metal by microorganisms is a major problem in the oil and gas industry. Microorganisms are involved in the corrosion of pipelines and equipment, plugging of injection or disposal wells and souring of fluids and reservoir[1]. Costerton and Boivin[2] estimated that the microbiologically influenced corrosion (MIC) cost in production, transport, and storage of oil could be some hundred million US dollars in the US every year due to Sulfate reducing bacteria (SRB) alone, not including the costs for lost oil and environmental clean-up. MIC has been defined as an electrochemical process in which the participation of microorganisms is able to initiate, facilitate, or accelerate the corrosion reaction without changing its electrochemical nature[3]. SRB are among the most frequently implicated microorganisms in MIC of iron, copper and ferrous alloys[4]. SRB include all unicellular bacteria capable of reducing sulfate to sulfide[5]. The most common of the genera is *Desulfovibrio*.

SRB are obligate anaerobes that obtain energy for growth from the oxidation of organic substances using sulfate as the external electron acceptor[6, 7]. In the absence of sulfate, some strains can function as fermenters and use organic compounds such as pyruvate to produce acetate, hydrogen, and carbon dioxide[3]. SRB form a physiologically distinctive group of anaerobic bacteria. Their oxidative metabolism is based, not on fermentation, but on the reduction of sulfate or certain other inorganic sulfur compounds. Their physiology has broad analogies to that of nitrate reducing bacteria (denitrifying bacteria), but they are all strict anaerobes and no examples of facultative aerobes are known. The effects of SRB growth and activity on the ferrous metal corrosion process depend on dissolved iron concentrations, nutrient limitations and fluid dynamics. Hence, the influence of dissolved iron concentration on MIC of steel was investigated. MIC due to SRB could cause a localized attack on metal. But this has been in much debate in recent years. Some authors even claimed that “MIC causes pitting corrosion” is a myth[8]. Experiments were carried out to cover a wide range of supersaturation of iron sulfide in this work.

As mentioned earlier sulfate reduction provides the energy for growth and metabolism of SRB. It is found that the initiation of biocorrosion due to SRB only occurs in the presence of sulfate species[9]. However, it is also proposed that high sulfate concentration in the medium could inhibit the sulfate reduction rate of SRB[5]. In some cases, the concentration of sulfate in the system has a direct influence on the growth and activity of SRB and the amount of sulfide produced[10]. This work investigated the growth and corrosion rate of a SRB species at different concentrations of dissolved sulfates in the medium. The effect of flow is important in bacterial corrosion process because it not only affects the transfer of species to the metal surface but also influences the overall bacterial adhesion process and the transfer of nutrients to the metal surface[11]. The SRB activity within biofilm may not be represented by the sample in the broth. It is desirable to develop an efficient solid medium to culture SRB cells dislodged from biofilms for analysis and quantification. In the laboratory identification, agar is generally used as the solid medium to grow microorganisms. Many agar-based media have been attempted by other people to grow these organisms, but usually poor and slow growth (typically 7 days at room temperature) was obtained[12].

Biochemical engineering involves the utilization of microorganisms to produce biomolecules. It relies on the optimizations of cell growth and bioreactor configuration and operation to increase productivity. The same biochemical engineering approaches can be adapted for the investigation of MIC and its mitigation. In order to understand the intrinsic mechanisms of MIC due to SRB, it is necessary to use pure cultures of SRB and to study various conditions affecting SRB growth and its corrosion of metals. This kind of knowledge leads to potential mitigation methods and is essential to a future mechanistic MIC model. This paper shows the results of some of our initial attempts.

EXPERIMENTAL PROCEDURE

The mild steel used for this work was AISI C1018 mild steel machined in the form of small metal coupons. Prior to each use, metal coupons were polished with 200, 400 and 600 grit silicon-carbide papers, rinsed with ethyl alcohol and coated with Teflon leaving only one flat disk surface exposed for anaerobic bottle experiments. The coupons were subjected to three ultrasonic bursts of 15 seconds each in acetone bath to remove dirt and grease on the coupon surface. The coupons used in glass cell experiments were cleaned in a similar fashion. The composition of the metal is given in Table 1.

Experiments were performed in sealed 100-ml anaerobic bottles and special glass cells fitted with a rotating shaft. Disc shaped coupons with a diameter of 11.5 mm and thickness of 3.1 mm were used for experiments in anaerobic bottles. The rotating electrode coupons for glass cells were cylindrical in shape. Necessary care was taken at all times to avoid microbial contamination. All experiments were carried out under anaerobic conditions. All devices involved in the experimentation were sterilized in an autoclave at 121^{o}C. The sterilized medium (Table 2) was cooled down to a temperature of approximately 45^{o}C after autoclaving and transferred to a de-oxygenation bottle that was purged with filtered nitrogen to remove oxygen. De-oxygenation was carried out for approximately 45 minutes. The medium was then transferred to the bottles along with the metal coupons. The liquid medium volume in anaerobic vials was 100 ml and in glass cell 2000 ml. The bottles were inoculated with a 48 hour old culture of the strain *Desulfovibrio desulfurincans* (ATCC 7757). The amount of inoculum was kept fixed at 1 ml inoculum per 100 ml of fresh medium for all experiments in the vials.

Broth samples were taken out at regular time intervals using sterilized hypodermal needles to perform cell count and to measure iron concentrations. Cell counts were done using an improved hemocytometer (Neubauer chamber from Hausser Scientific, Horsham, Pa., USA). At the end of each experiment coupons were cleaned and re-weighed to determine the loss in weight.

For glass cell experiments all corrosion potential and rate measurements were made using a Gamry (www.gamry.com) PC4 monitoring system and analyzed using the accompanying Gamry software. The linear polarization resistance (LPR) method was used to monitor corrosion rates. The sample was polarized ±5 mV around the corrosion potential during the LPR measurement.

RESULTS AND DISCUSSION

Experiments were designed to cover a wide range of initial dissolved Fe^{2+} concentrations. The concentrations tested included 0, 5, 10, 25 and 50 ppm. Iron was added to the system in the form of iron sulfate. The experimental duration was kept at seven days to study the dissolved iron effect in anaerobic vials. The coupons were dried overnight and tested for elements present on the surface using an Energy Dispersive Spectroscopy (EDS). EDS scan showed that iron and sulfur were amongst the most dominant elements on metal surface (Figure 1) before the biofilm removal.

As seen in Figure 2 high corrosion rates were observed for intermediate initial Fe^{2+} concentrations. The corrosion rate was highest at 25 ppm. The metal coupon exposed to 25 ppm initial Fe^{2+} concentration had undergone severe localized attack as can be seen in Figure 3. Concentrations higher than 50 ppm and lower than 5 ppm did not show much localized attack. The localized corrosion at intermediate Fe^{2+} concentrations can be attributed to simultaneous biofilm growth along with iron

sulfide precipitation and film formation. Starosvetsky observed that precipitation of ferrous sulfide products suppresses the growth of pits[13].

Figure 4 indicates that the bacterial growth rate and, hence, the sulfate reduction is decreased as the initial sulfate concentration increases within the range of 1.93g/l to 6.5g/l. It may be due to the increasing toxicity of sulfates towards SRB metabolism or sulfate reduction[5]. Figure 5 shows that lower corrosion rate is observed when the bacterial growth is hindered.

The flow experiments were performed at 0, 1000 and 2000 rpm rotational speeds. Figure 6 shows a schematic of the device. A cylindrical coupon was mounted near the tip of the shaft. The exposed height of the cylindrical coupon was 21 mm and radius 11 mm. Glass cell experiments were used to simulate the flow conditions on the coupon surface in straight pipes. High linear fluid velocities on the coupon surface can readily be achieved with ease. The relationship between the shaft's rotational speed in the glass cell and the linear velocity on the coupon surface that would be experienced in straight pipe flow under similar turbulent conditions is shown in Figure 6 based on a correlation proposed by Silverman[14].

This work extended the flow regime to higher velocities not easily achieved using straight pipes in a laboratory setting. The increase in velocity restricted the cell growth process. A sudden drop in corrosion rate was observed when the solution was super-saturated with FeS. After reaching super-saturation the corrosion rate remained almost stable. Experiments were continued for longer time periods to ensure that a steady corrosion rate was achieved.

Figures 7 and 8 show the results of electrochemical measurements in glass cells. There was a significant increase in the corrosion rate at 1000 rpm as compared to stagnant condition (0 rpm). This could be attributed to an increase in the mass transfer rate of corroding species (HS^- and H_2S) from the bulk of the solution to the metal surface and the increased availability of nutrient species to the sessile SRB cells in the biofilm on the coupon surface. The corrosion rate did not increase significantly as the rotation speed increased from 1000 to 2000 rpm. It was also noted that when the rotational speed was sufficiently large, SRB growth and corrosion rate were inhibited. It indicates that fluid shear can be a potential MIC mitigation method. Further experiments are currently underway.

Figure 9 shows that SRB would grow rapidly on a newly developed agar-based solid medium at room temperature under pure N_2 atmosphere without any other added hydrogen donors such as sodium lactate, or hydrogen gas. At 37^{o}C, excellent SRB growth was achieved overnight. At room temperature, it took one extra day to achieve similar results. To confirm that these colonies are *D. desulfuricans*, one big round colony on the agar surface was used to inoculate the same liquid medium used for experiments in vials and glass cells as described above at 37^{o}C. It took relatively longer time to grow in liquid medium compared to inoculation from liquid broth. Microscopic examinations showed that the viable cells have the same shape and motility as the cells from cultures inoculated using normal planktonic SRB cells. It was found that the medium also performed well when the covered Petri dish was placed in air instead of a nitrogen environment under room temperature. This new solid medium facilitates the testing of SRB cell counts in biofilm. It can also be used to preserve and select SRB cells.

Experiments were also carried out using Celite beads as microcarriers for SRB immobilization. Celite is derived from fossilized shells of diatoms. They are frequently used as porous supports for the immobilization of cells in the fermentation industry. By adding Celite beads, a 3-fold reduction in the planktonic SRB cell count was achieved as shown in Figure 11. However, the effect on the corrosion rate reduction was quite limited. Further experiments are needed.

CONCLUSIONS

The increase of SO_4^{2-} concentration within the range of 1.93 g/l to 6.5 g/l decreases the SRB growth and the corrosion rate of mild steel.

Iron sulfide films can affect the nature of corrosion due to SRB. At intermediate initial iron concentrations the corrosion becomes localized. SRB growth rate increases with the increase of ferrous ion concentration in the medium.

Flow affects the SRB growth and the corrosion process. The overall corrosion rate increases with increase in rotational speed in the glass cell.

A new solid medium was developed for the quantification of SRB cells in biofilms. This medium requires no special hydrogen atmosphere and is faster for growth than those reported in the literature.

Microcarriers reduced the planktonic cell count. However, further experiments are needed to improve the method in order to reduce the corrosion rate.

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TABLE 1
COMPOSITION OF STEEL (C1018)

Element	Wt %	Element	Wt %
Al	0.066	Ni	0.03
As	0.01	P	0.016
B	0.0009	Pb	0.036
C	0.2	S	0.009
Ca	0.0004	Sb	0.009
Co	0.007	Si	0.036
Cr	0.052	Sn	0.005
Cu	0.02	Ta	0.005
Mn	0.84	Ti	0.002
Mo	0.028	V	0.002
Nb	0.012	Zr	0.006

Balance is Fe

TABLE 2
COMPOSITION OF MODIFIED BAAR'S MEDIA USED FOR GROWTH OF SRB

Component I	$MgSO_4$	2.0 g
	Sodium Citrate	5.0 g
	$CaSO_4$	1.0 g
	NH_4Cl	1.0 g
	Distilled water	400.0 ml
Component II	K_2HPO_4	0.5 g
	Distilled water	200.0 ml
Component III	Sodium Lactate	3.5 g
	Yeast Extract	1.0 g
	Distilled water	400.0 ml
Component IV	$FeSO_4$	2.1g
	$(NH_4)_2SO_4$	1.0g
	Distilled water	30ml

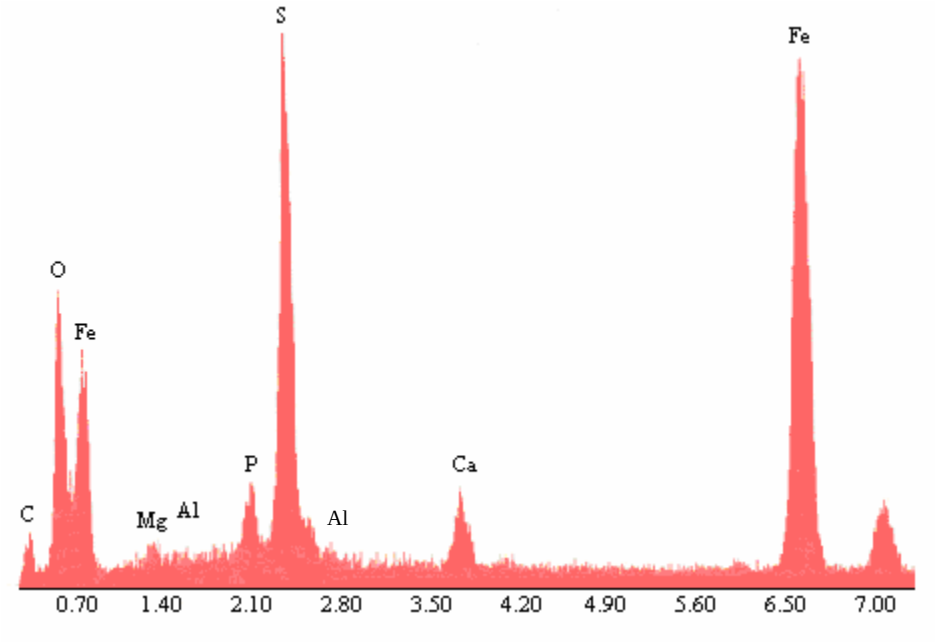

FIGURE 1 - EDS scan of metal coupon surface showing the presence of iron and sulfur

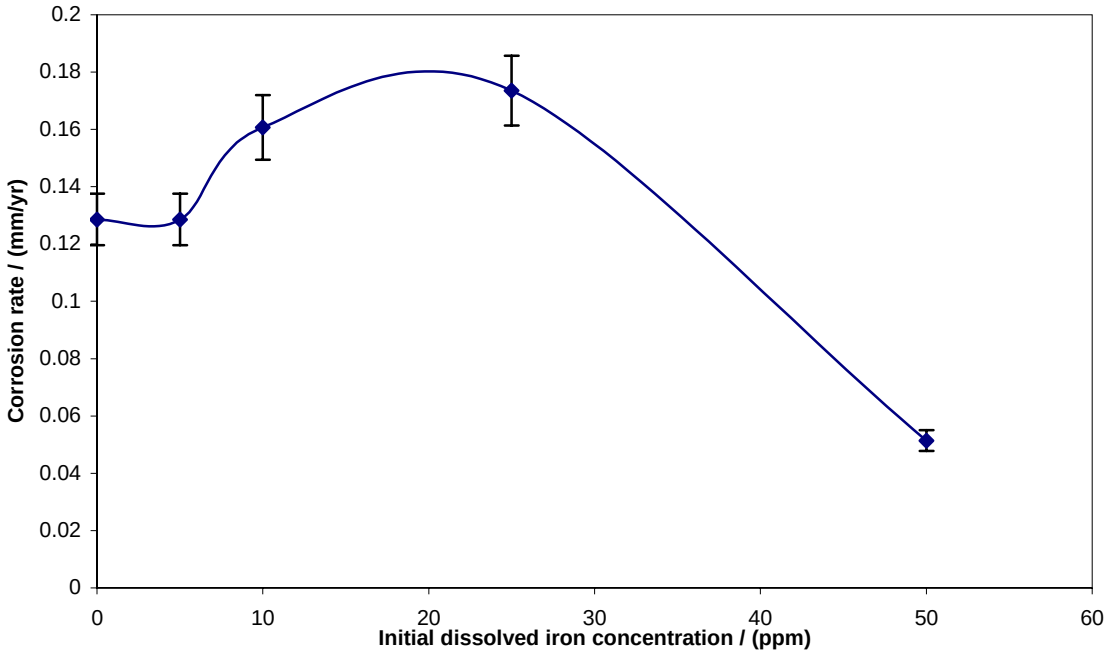

FIGURE 2 - Corrosion rate variation with the change in dissolved iron concentration.

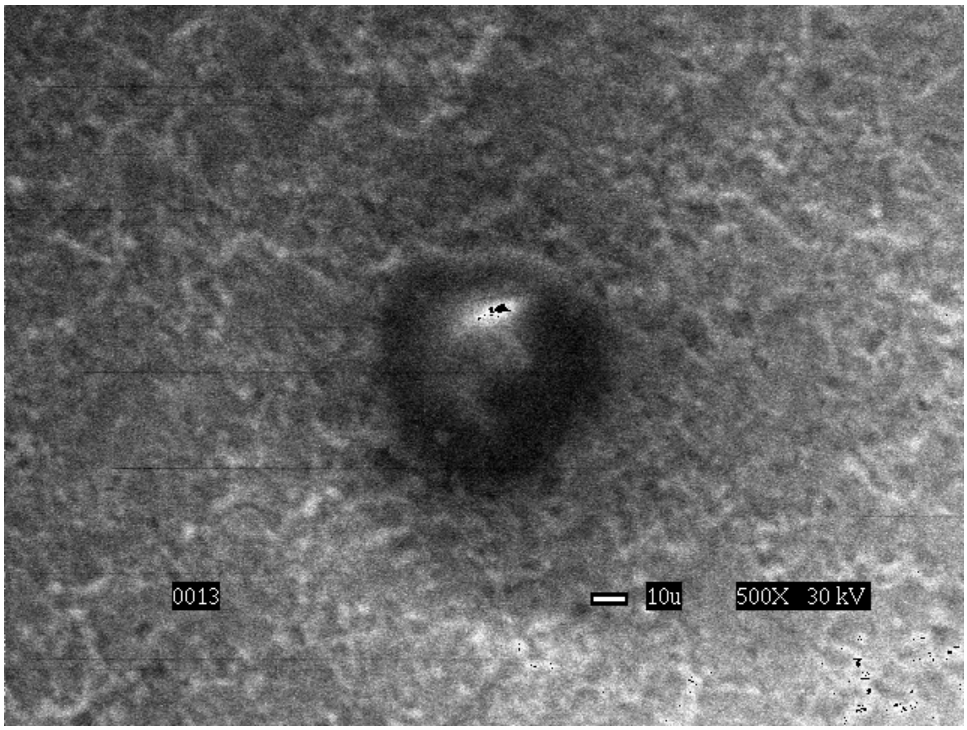

FIGURE 3 - SEM picture of a pit on the coupon surface before film removal.

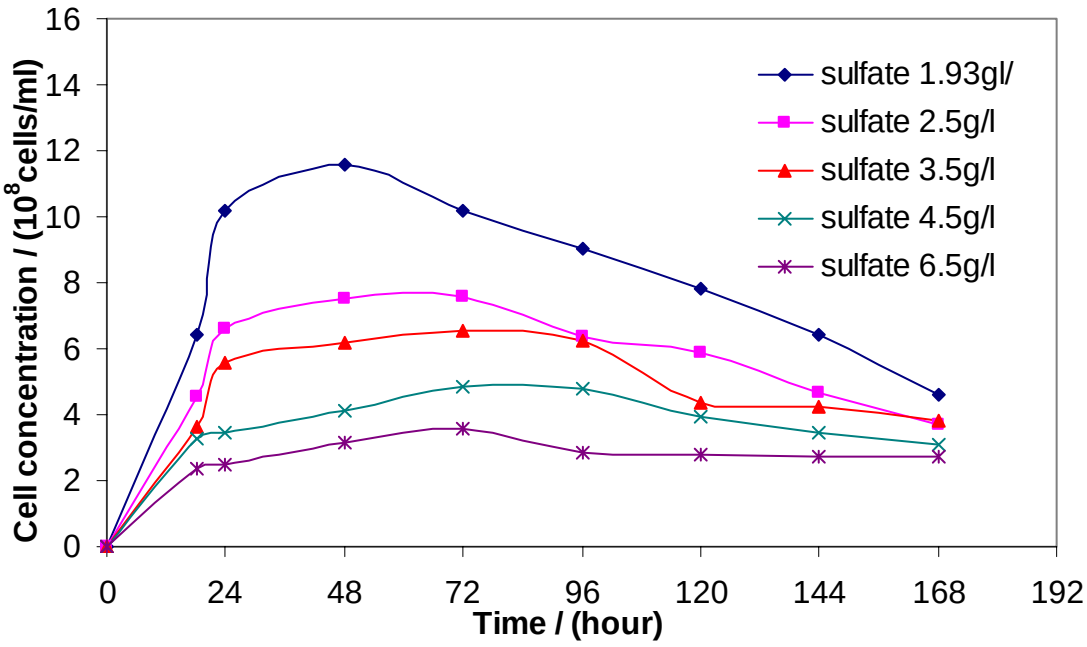

FIGURE 4 - SRB growth with time at different initial sulfate concentrations in anaerobic vials

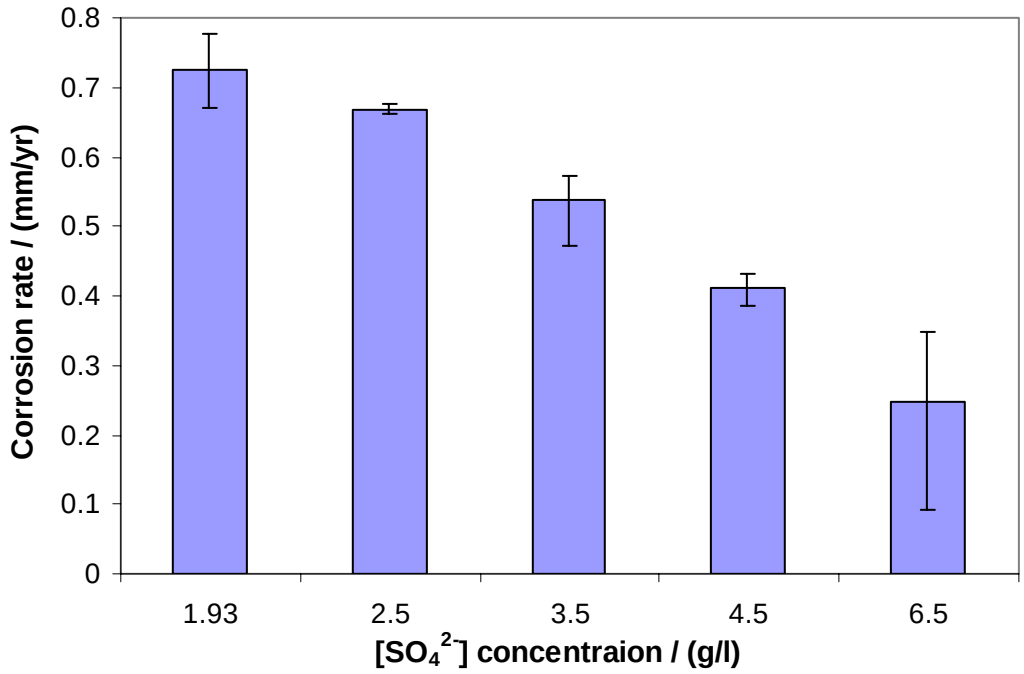

FIGURE 5 - Corrosion rate (weight loss) at different initial sulfate concentrations in the medium after inoculation at 37 ºC. Weight loss was measured at the end of 7 days.

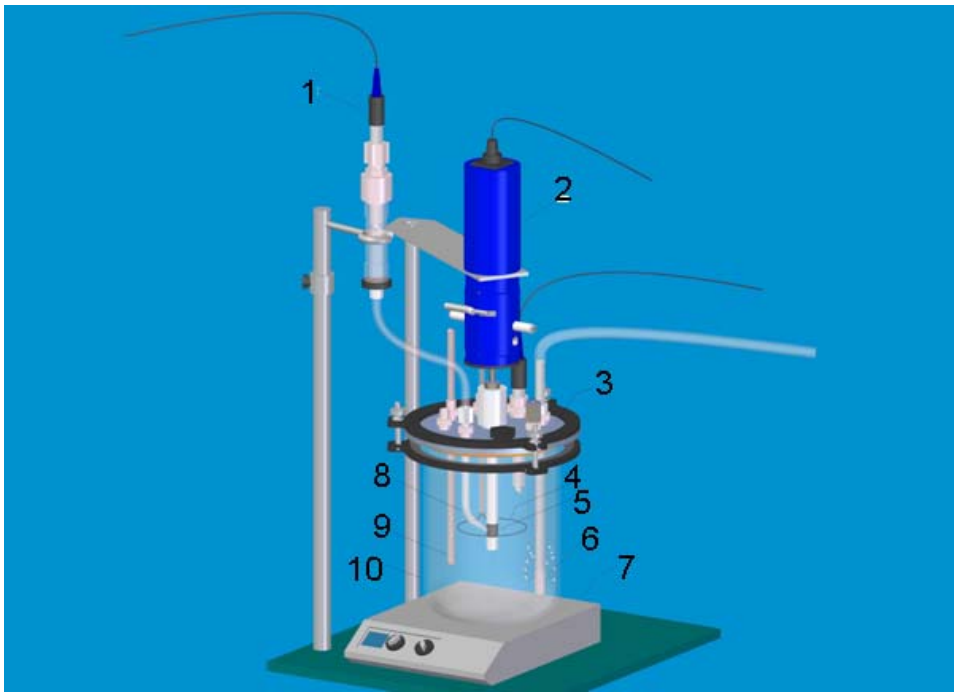

FIGURE 6 - Glass cell setup 1.) Reference electrode, 2.) Rotor, 3.) Lid, 4.) Counter electrode, 5.) Working electrode, 6.) Bubbler, 7.) Hot plate, 8.) Luggin capillary, 9.) Temperature probe, 10.) glass cell. (Courtesy of Daniel Mosser at Ohio University.)

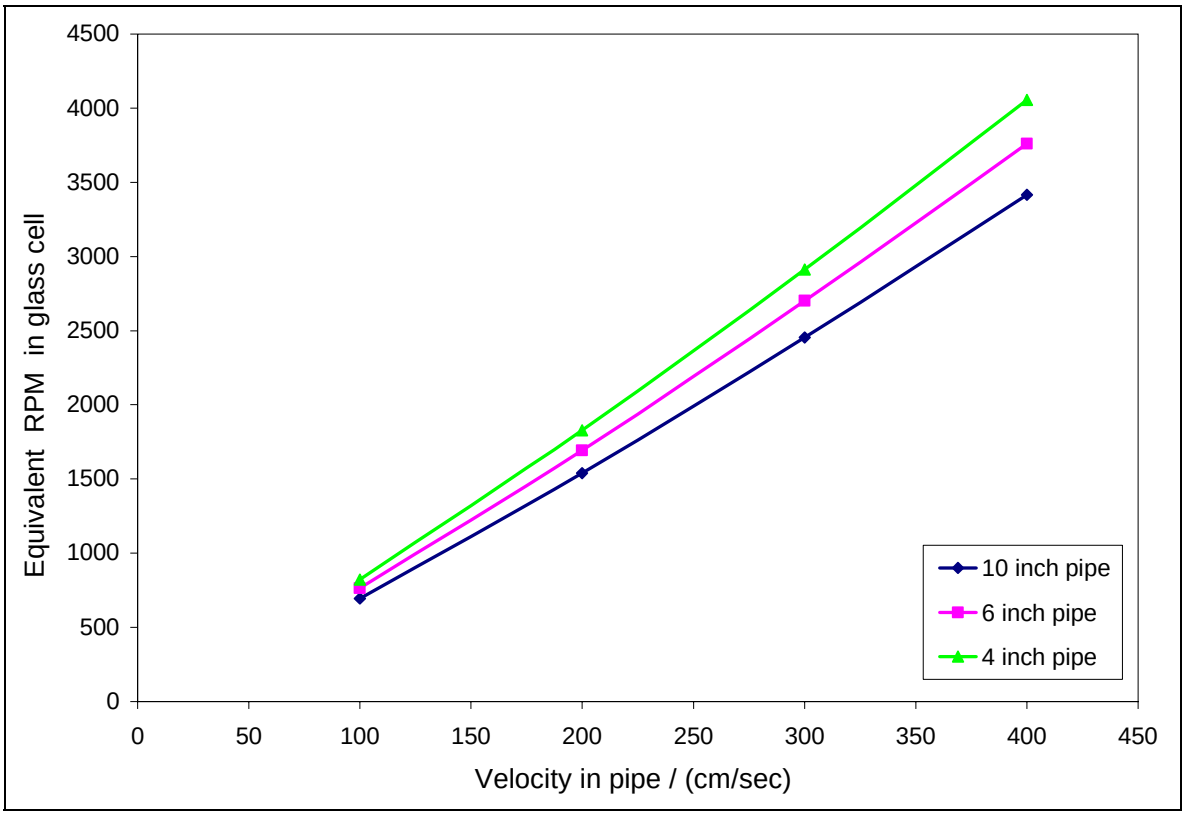

FIGURE 7 - Relation between rotational speed in glass cells and surface velocity for coupons in pipe flow.

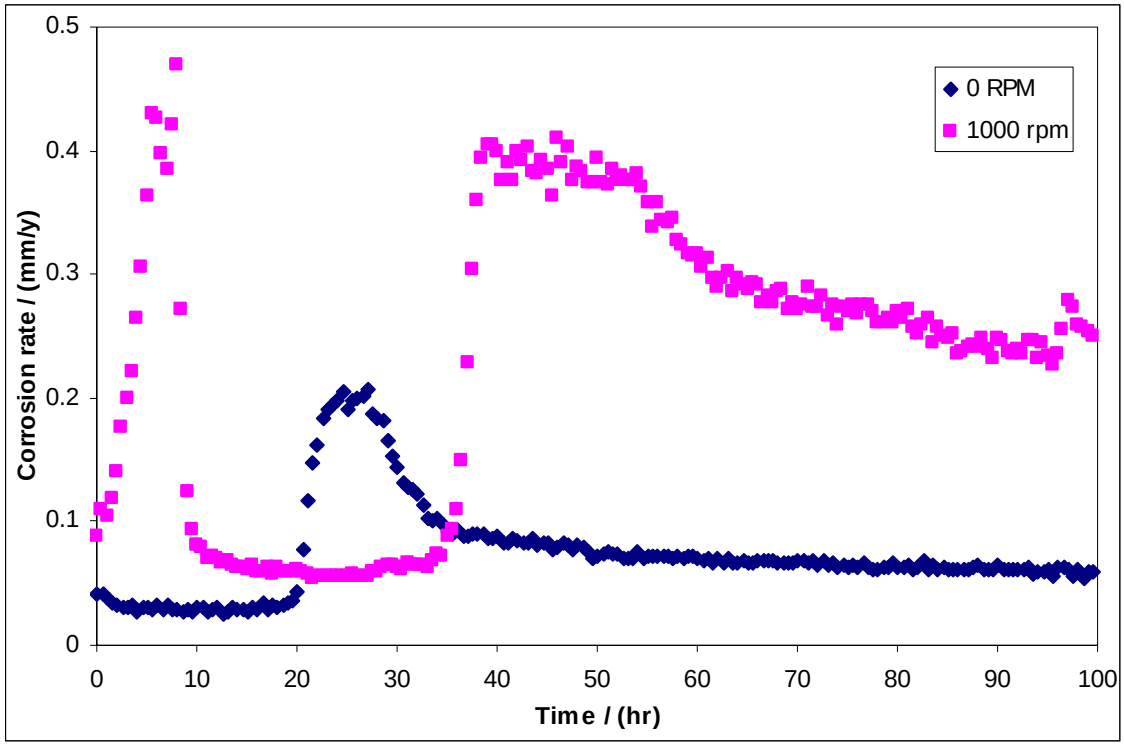

FIGURE 8 - Effect of coupon rotational speed on corrosion at 0 and 1000 rpm.

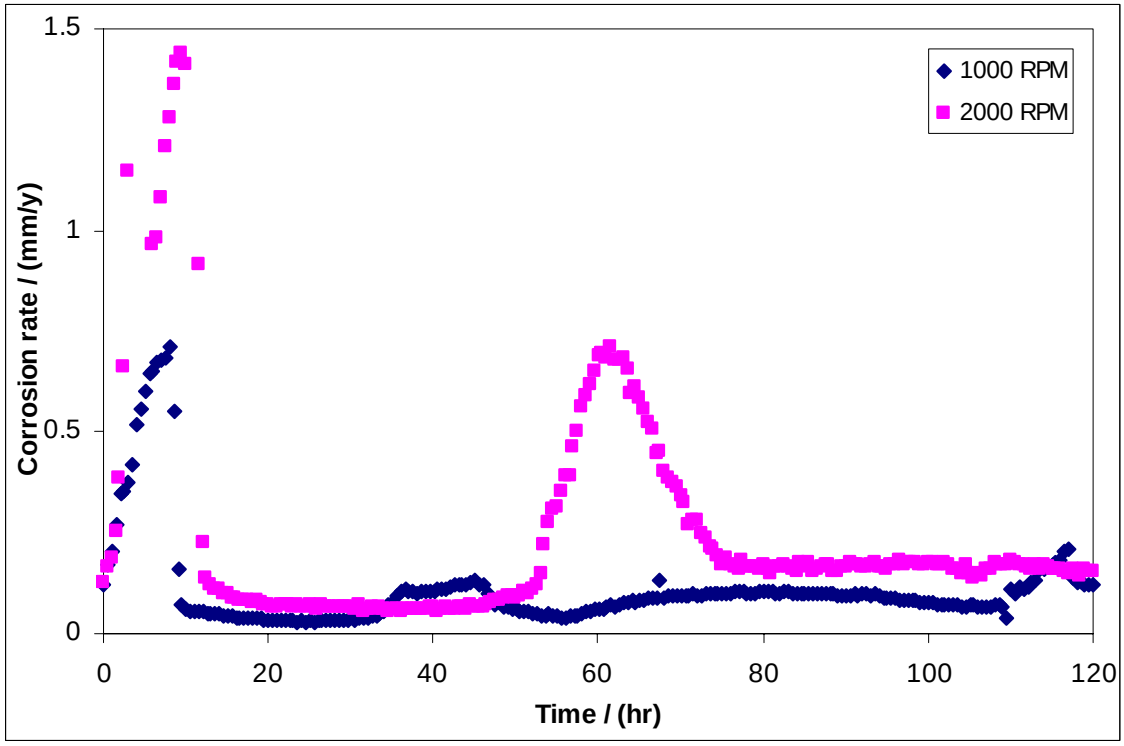

FIGURE 9 - Effect of coupon rotational speed on corrosion at 1000 and 2000 rpm.

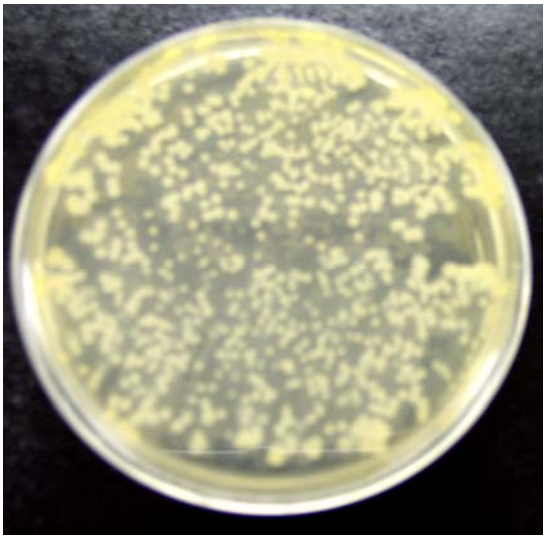

FIGURE 10 - *D. desulfuricans* (ATCC 7757 strain) on agar surface in pure N_2 atmosphere at 37ºC after 24 hours.

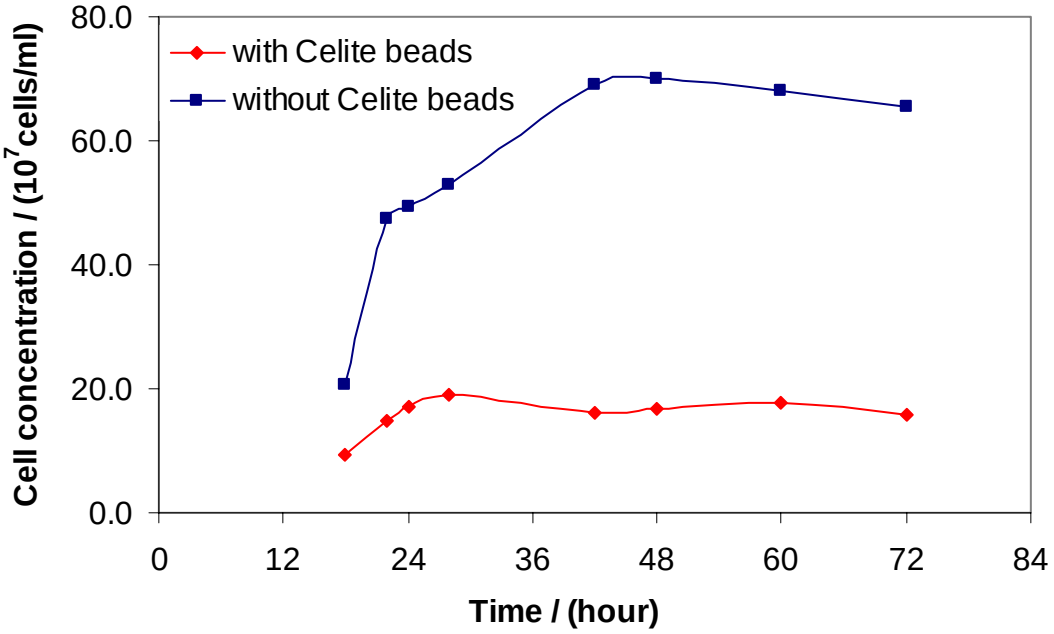

FIGURE 11 - SRB planktonic cell counts with and without Celite beads in a medium with a 25 ppm initial Fe^{2+} concentration in a 2-L glass cell.