SYNERGISTIC CORROSION OF PIPE-LINE STEEL BY OBLIGATELY ANAEROBIC BACTERIA.

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Abstract-The performance of carbon steels in anaerobic environments is greatly influenced by bacteria which are capable of growth in the absence of oxygen. In oxygen-free gas-transmissions pipelines fermenting bacteria are present which can utilize trace quantities of hydrogen and carbon dioxide to form acetate. This acetate is then available to microorganisms (such as sulphate-reducing bacteria, SRB) which are known to be important in corrosion. In a series of model experiments the effects of Eubacterium limosum, an acetate-producing bacterium, Desulfovibrio Florida strain and Desulfobacter strain 3ac10 were examined on the corrosion of AISI C1020 carbon steel. No organic carbon source was provided. Neither the Desulfovibrio nor the Desulfobacter are capable of growth exclusively upon hydrogen and carbon dioxide. Both require acetate. These studies showed that while no significant corrosion occurred due to Eubacterium limosum alone, the addition of the sulphate-reducing bacteria greatly accelerated the corrosion rate. These results have important implications for industry.

INTRODUCTION

Industry has in place several cubic miles of carbon steel sheet, pipe, rod and wire[1] which in aqueous systems is subject to active corrosion. Generally this is limited by the availability of oxygen for the cathodic process. In early exposure of these steels the rapid formation of a rust film tends to impede further corrosion and permits a useful service lifetime in most environments. Problems occur, however, when this rust film either is prevented from forming or is rapidly removed. Under anaerobic conditions and neutral pH the performance of carbon steels is usually excellent. The major cathodic reactant, oxygen, is not present and thus dissolution is inhibited.

Anaerobic microorganisms can, however, take advantage of such anaerobic systems and promote corrosion. Generally, it has been assumed that corrosion occurs by virtue of the production of acids by fermentative bacteria (which produce excess organic acid). A group of organisms collectively known as the sulphate-

reducing bacteria can reduce sulphate (SO_4^{-2}) to sulphide (HS^{-1}), which is a reactive and corrosive anion by utilizing the products of acid-producing bacteria. Some members of this group of organisms oxidize hydrogen. As a result of these two processes the sulphate-reducing bacteria have been implicated in serious economic losses due to anaerobic microbiologically influenced corrosion[2,3].

Phelps et al.[4] have suggested that significant corrosion may result in gas transmission pipe-lines as a result of not only cathodically-produced hydrogen, but also the trace quantities that occur in natural gas deposits. These authors showed that acetate production from several substrates may be available to the sulphate-reducing bacteria in such pipe-lines. The corrosion rates from such an association were not examined however. In this paper we investigate the interactions of those bacteria with the capability of producing acetate from hydrogen and carbon dioxide (also present in natural gas) and the availability of that acetate to sulphate-reducing bacteria involved in corrosion processes.

EXPERIMENTAL METHOD

Cultures and Growth Conditions

Desulfovibrio Florida strain was obtained by chemostat enrichment from Appalachicola Bay on the northern Gulf coast of Florida. This strain could utilize hydrogen and lactate as electron sources and CO_2 and acetate for carbon.

Desulfobacter 3ac10 was obtained from the Deutsche Sammlung von mikroorganismen # 2035. This is a marine strain, which oxidizes acetate and ethanol only, at the expense of sulphate.

Eubacterium limosum was obtained from the American Type Culture Collection # 8486. This bacterium may grow on H_2/CO_2 to produce acetate or engage in fermentation of other organic substrates such as glucose, alchohols and formate.

The growth medium consisted of basal salts containing (g/L): Na_2SO_4 3, KH_2PO_4 0.2, NH_4CL 0.25, NaCl 20, $MgCl_2.6H_2O$ 4, KCl 0.5, $CaCl_2.2H_2O$ 0.15. Resazurin was added as redox indicator as well as complex vitamins, sodium sulphide[5] and trace elements[6]. The final pH was between 7.0 and 7.5. Operating temperatures were at ambient (approximately 23°C). The gas mix was 5% H_2 with the balance of CO_2. Some carbon was supplied in small amount of yeast extract (0.01%) which was required by the Eubacterium limosum for growth.

Materials Selection

A carbon steel AISI C1020 was selected. This contained (%) C 0.2, Mn 0.47, P 0.012, S 0.013 and the balance Fe. C1020 was selected since much prior data is available and its performance is well-known.

Electrochemical Methods

Electrochemical analysis was performed using a classical three-electrode system: the working (corroding) electrode, a

titanium counter-electrode and saturated calomel reference electrode.

Open circuit potentials were taken with a Princeton Applied Research EG&G model 273 potentiostat. Electrochemical impedance spectroscopy (EIS) was carried out with the same potentiostat in conjunction with the Solartron-Schlumberger 1255 frequency response analyzer. Impedance analysis in this case required a 5 mV rms signal applied to the working electrode while monitoring the resultant current between the frequencies 10 KHz and 5 mHz. Phase angle and impedance were calculated. The instruments were controlled and the data manipulated using software by Scribner Associates, (Charlottesville, Virginia).

Chemical Assays

Sulphate analysis was carried out using the method of Tabatabai[7]. Volatile fatty acids were examined by gas chromatography using a Schimadzu model GC8A with flame ionization detection. The packing utilized was SP-1200 (Supelco, Bellefonte, PA) at 120°C column temperature and injector/detector at 170°C. Carrier gas flow rate was 30 ml/min.

Microbiological Analyses.

Cells in the bulk phase were enumerated directly using a Helber counting chamber (Weber Scientific Ltd., Teddington, England).

Scanning Electron Microscopy (SEM).

Post-exposure examination of the surfaces of the carbon steel coupons was by scanning electron microscopy. Immediately following recovery from the medium/electrolyte the coupons were fixed in anaerobic 2.5% glutaraldehyde (with 5% cacodylate) at Ph 7.0. Subsequently a desiccation series was set up in 50%, 75% and 100% acetone prior to critical point drying. Thereafter the coupons were sputter carbon coated prior to SEM.

EXPERIMENTAL RESULTS

Two experiments were accomplished. Experiment I consisted of a comparison of the corrosion rates exhibited in 1) sterile conditions, 2) the presence of Eubacterium limosum and 3) Eubacterium limosum in coculture with the Desulfovibrio strain. The EIS data permitted evaluation of the charge transfer resistance (R_{ct}) that enables assessment of the corrosion rate. This required calculation of the corrosion rate in mils/yr using a modification of Faraday's law. The corrosion rate was considered to be:

$$\text{Corrosion rate (mils/yr)} = 3350.M \,/\, m.g.A.\, R_{ct} \qquad (1)$$

where "M" is the atomic weight (55.8), "m" the two-electron transfer (2), "g" is the density in g/cm^3 (7.87), "A" is the area (1.3 cm^2) and "R_{ct}" (Ω.cm^2) is the charge transfer resistance. For

the purposes of this experiment the principle assumption has been that the low frequency impedance extrapolated to an infinitely low frequency will give good estimates for the sum of R_{ct} and the uncompensated resistance (R_Ω). However in marine systems, since R_Ω is small by comparison with R_{ct}, it may be neglected. A second assumption was that the anodic and cathodic Tafel parameters remained constant. Previous data have shown very little variation[8] under these conditions.

Figure 1 provides an impedance diagram, in the Bode format, after 12 days exposure of the steel in contact with the three conditions. To the left of the diagram the low frequency data shows a relatively low impedance intercept (R_{ct} = approx. 2 kOhms.cm^2) for the E.limosum/ Desulfovibrio corrosion rate with respect to either the sterile state or the E.limosum alone. Figure 2 applies equation (1) to the R_{ct} values and thus provides the best estimates for average corrosion rates observed during the course of the experiment. Concomitantly, the total number of organisms increased in each vessel (Figure 3) and the sulphate concentration dropped (Figure 4) as was expected for both assimilatory and dissimilatory sulphate-reduction.

Experiment II compared the corrosive effects of E.limosum with Desulfobacter, with that of the fermenter with the Desulfovibrio and of a triculture of E.limosum, Desulfovibrio and Desulfobacter. The results are presented in figure 5. This experiment studies the possibility of synergistic action with respect to the corrosion rate between both sulphate-reducing bacteria. The results show an initial decrease in corrosion rate for all carbon coupons. After day 5 the E.limosum / Desulfovibrio coculture increased the corrosion rate rapidly to about 2.5 mils/year while the triculture achieved a maximum on day 9 of about 5.2 mils/year. At the same time the corrosion rate associated with the E.limosum / Desulfobacter coculture decreased to lower than 1 mil/year.

The coupons exposed to the triculture were examined by SEM which revealed some close association of the Desulfobacter and the Desulfovibrio. Classical morphologies of both types permitted such a distinction. The Desulfovibrio selected is clearly the curved comma-vibrio in figure 6 while the "figure-eight" shaped oval bacteria are common in Desulfobacter.

DISCUSSION

In experiment I it is clear that the bacteria in all cases grew quite rapidly with some uptake of sulphate by assimilatory or dissimilatory processes (figures 3 and 4). Interestingly, sulphate removal by the SRB was not much greater than the assimilatory processes exhibited by Eubacterium limosum alone. Sulphate was monitored as a measure of sulphate-reduction for both assimilatory (sulphur required for amino acids etc.) as well as dissimilatory (generating energy) processes. The concentrations of sulphate in the vessels with the Desulfovibrio decreased slightly more (to about 1.7 mg/L) than those vessels with E.limosum (2.0 mg/L) or the sterile controls (2.3 mg/L). The

limiting nutrient is neither hydrogen (electron donor) nor sulphate (electron acceptor) since they were in excess. It was the production of acetate by the E.limosum which was necessary for the Desulfovibrio for cell carbon. This acetate concentration was kept very low (0-1 mM acetate) during the course of the experiment.

The impedance data consistently showed that the total impedance of the system in contact with the biculture was lower than that of the sterile system or with E.limosum alone (figure 1). We interpret this to indicate a faster corrosion rate due to the presence of the sulphate-reducing bacterium. The data in figure 2 shows that over time the corrosion rate in the sterile system rapidly decreases over time and appears to achieve some stability after five days. This is undoubtedly due to a sulphide film of some kind which formed and prevented further dissolution. Visual observation of the coupons during the experiment showed only blackened deposits which formed rapidly after initial immersion. The data showed that while the presence of the E.limosum did not significantly accelerate the corrosion rate, the Desulfovibrio apparently increased the corrosion rate. Final average corrosion rates for those cases were 1.3 and 0.4 mils/year respectively. This may be due to prevention of the formation of a totally intact sulphide film or a change in the nature of that film. McNeil and Little[9] have pointed out that sulphide films established in the presence of SRB are somewhat different from those established abiotically. Since the Desulfovibrio could not grow alone without the acetate generated by the E.limosum there is clearly a synergistic action which is having a deleterious effect on the carbon steel.

In Experiment II the effect of two sulphate-reducing bacteria coupled with the E.limosum was investigated. This allowed acetogenesis by the fermenter to be funneled to both Desulfobacter and the Desulfovibrio. The physiology of this strain of Desulfobacter permits electrons to be donated from either ethanol or acetate only. The Desulfovibrio on the other hand may utilize several substrates including lactate and hydrogen. In this experiment hydrogen was in excess.

Corrosion rates monitored using EIS during Experiment II are depicted in figure 5. These show that the corrosion rates associated with the coculture of E.limosum and Desulfobacter dropped from approximately 2.0 mils/year to slightly less than 1.0 mil/year over a 12 day period. During the same period the combination of E.limosum and Desulfovibrio increased the corrosion rate from 2.0 to approximately 2.5 mils/year after an initial lag phase. The coculture of all three organisms apparently increased the corrosion rate rapidly to a maximum of 5.2 mils/year after day 5. Interestingly the corrosion rates in all cases decreased initially during the early lag phase of the microorganisms ie. when the bacteria were expected to be less active. At about the time of the rapid exponential increase in cell numbers a concomitant increase in corrosion rate is observed. After Day 9 there was a substantial decrease in the corrosion rate possibly due to some limiting nutrient.

Unfortunately no information is available on the mechanisms involved in these increased corrosion rates. Several mechanisms have been proposed including accelerated corroison via the HS^- anion and the cathodic depolarization hypothesis[10] among many others. In our sterile system we observed that for AISI C1020, the chemical formation of a sulphide film was very rapid, initially in the order of minutes, which further developed over several days. The sulphate-reducing bacteria in coculture were evidently able to penetrate this film and accelerate the overall corrosion rate by synergistic growth with the E.limosum. The sudden acceleration of the corrosion rate with the presence of Desulfobacter in the triculture (figure 5, after day 5) could not be explained except in terms of some interspecies carbon or electron transfer that is currently undescribed. While this strain of Desulfobacter in pure culture is limited to acetate or ethanol as an electron source[11] co-metabolism may permit other supplementary substrates to be utilized[12]. The difference in corrosion rates between the inclusion of Desulfobacter and Desulfovibrio may be explained by the utilization of cathodically generated hydrogen by the Desulfovibrio but would not explain the discrepancy between the E.limosum / Desulfovibrio coculture and the triculture.

Finally, although analysis of the bulk phase enables some conception of the processes occurring at metal surfaces, biofilms are generally very different in nature from those bulk phases. Hence surface examination is required to note the distribution of organisms and corrosion debris on the surface. During Experiment II we observed the numbers of cells in the bulk phase with vibrio morphology were apparently higher than those with the oval morphology associated with Desulfobacter. An SEM survey revealed oval-shaped cells (Desulfobacter-like) on the surface of the carbon steel coupons which were substantially different from the morphologies of those associated with either Desulfovibrio (vibrios) or Eubacterium limosum (straight rods). Figure 6 is a micrograph of a carbon steel surface which depicts cells of the Desulfobacter (fat oval, figure-eight) and Desulfovibrio (vibrio) morphology.

CONCLUSION

The model system investigated in this article demonstrated a 20 fold increase in corrosion rate with the three bacteria in an anaerobic, sulphate-reducing consortium (triculture maximum approx. 5.2 mils/year) above that of the sterile system (final rate approx. 0.25 mils/year). Given the usual caveats associated with comparing laboratory systems with the environment we conclude that certain associations of anaerobic bacteria have the potential to cause deterioration of steel structures in anaerobic environments.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Fig.1. Electrochemical impedance spectra of AISI C1020 carbon steel exposed to sterile marine conditions, the fermenting bacterium E.limosum and a coculture of E.limosum with Desulfovibrio.

Fig.2. Evolution of the corrosion rate of AISI C1020 carbon steel in contact with sterile anaerobic marine medium / electrolyte, E.limosum alone, and a coculture of E.limosum and Desulfovibrio.

Fig.3. Increase in total numbers of cells during carbon steel exposure.

Fig.4. Decrease in sulphate concentration due to assimilatory (E.limosum) and dissimilatory (Desulfovibrio) processes over the sterile controls.

Fig.5. Corrosion rate of AISI C1020 carbon steel exposed to cocultures of E.limosum / Desulfovibrio, E.limosum / Desulfobacter and the triculture in anaerobic, marine medium/electrolyte.

Fig.6. Scanning electron micrograph of oval-shaped (Desulfobacter) and vibrio-shaped bacterial cells on a carbon steel, surface.

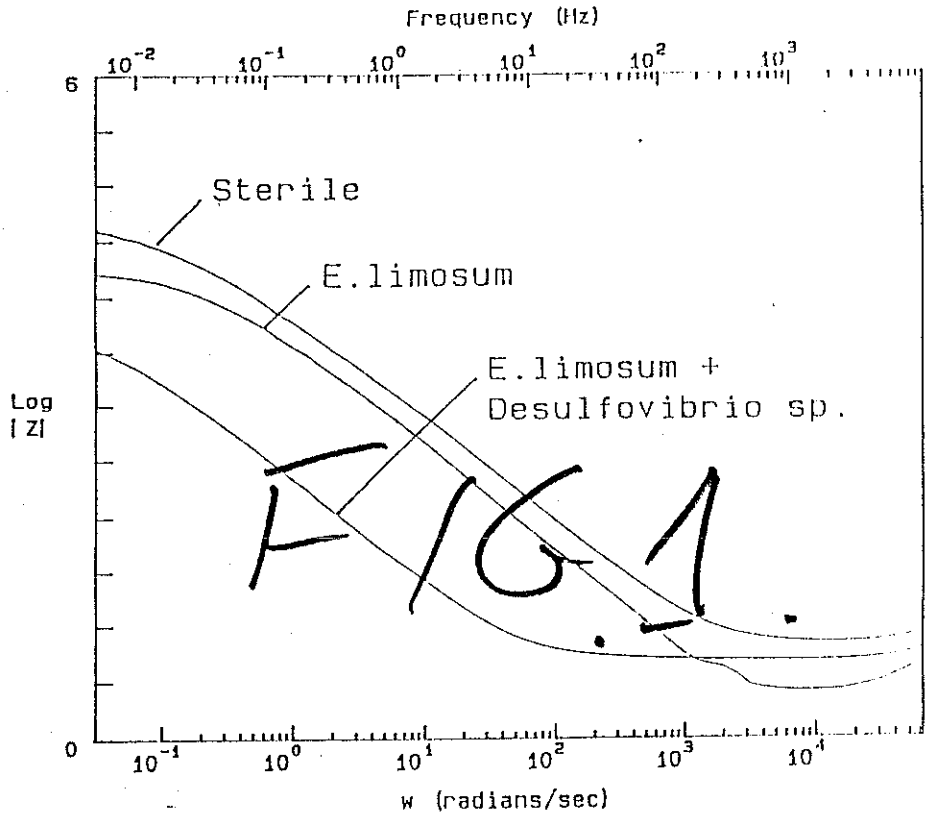


FIG. 1.

CORROSION RATE IN MILS/YR
due to marine bacteria of various types.

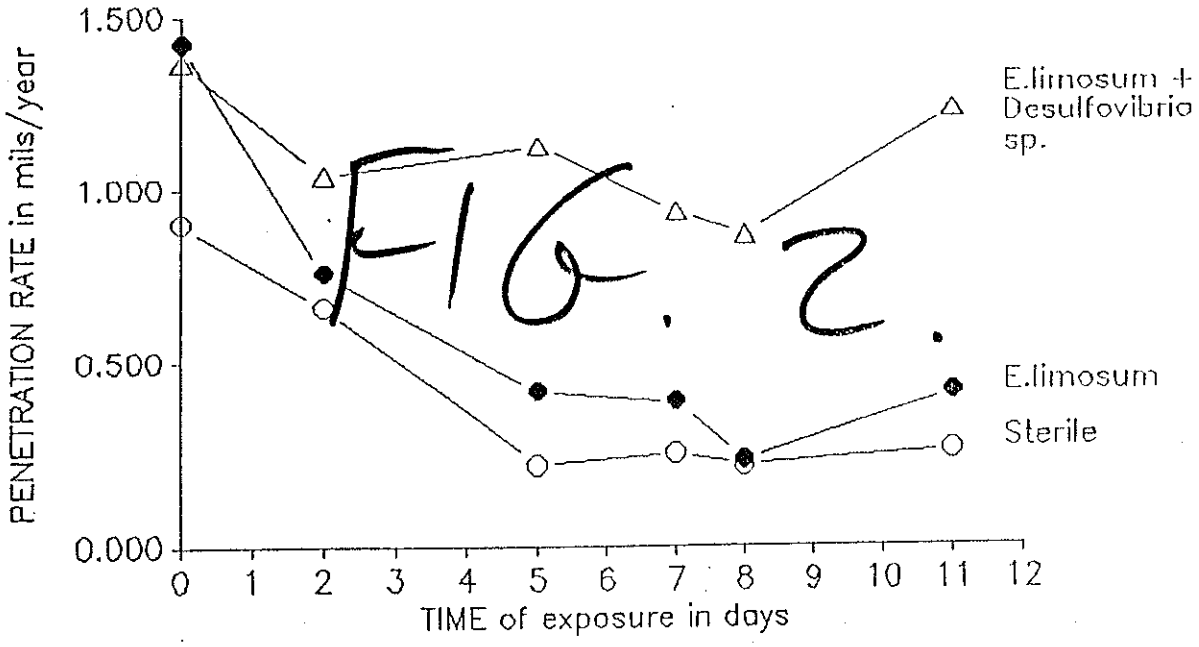


FIG. 2.

INCREASE IN TOTAL CELL NUMBERS DURING EXPOSURE OF PIPE-LINE STEEL TO OBLIGATE ANAEROBIC BACTERIA

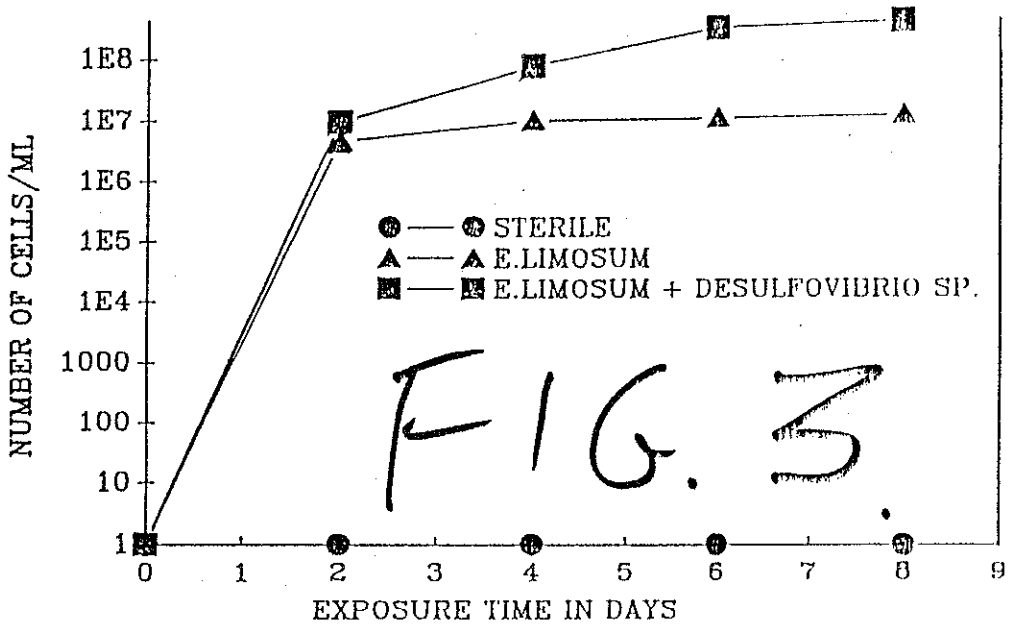


FIG. 3.

SULFATE CONCENTRATIONS AS A FUNCTION OF TIME OF EXPOSURE

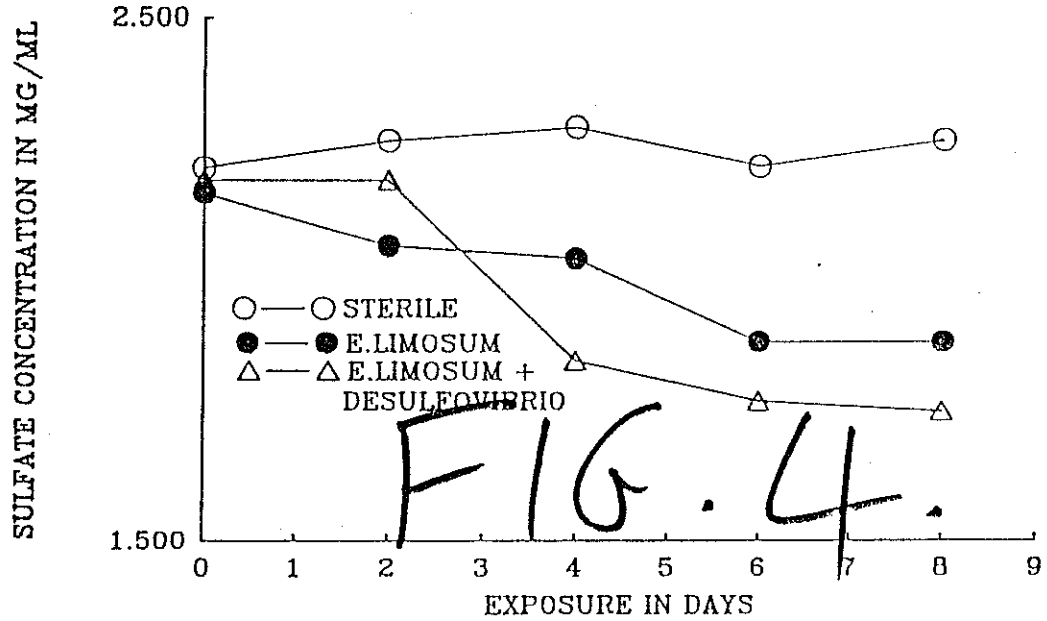


FIG. 4.

CORROSION RATE OF CARBON STEEL EXPOSED TO OBLIGATE ANAEROBIC BACTERIA IN CONSORTIAL ARRANGEMENTS

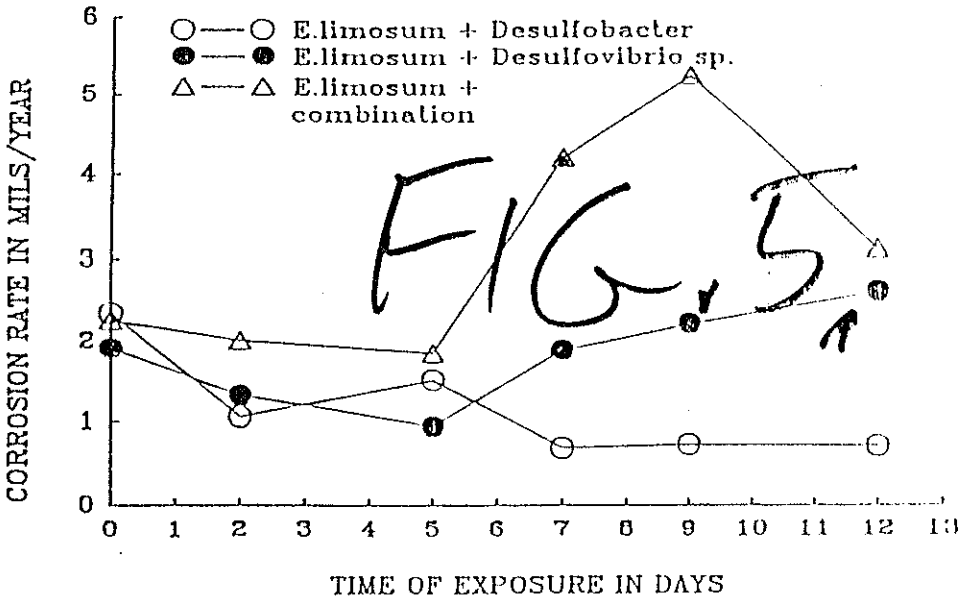


FIG. 5

FIGURE 5

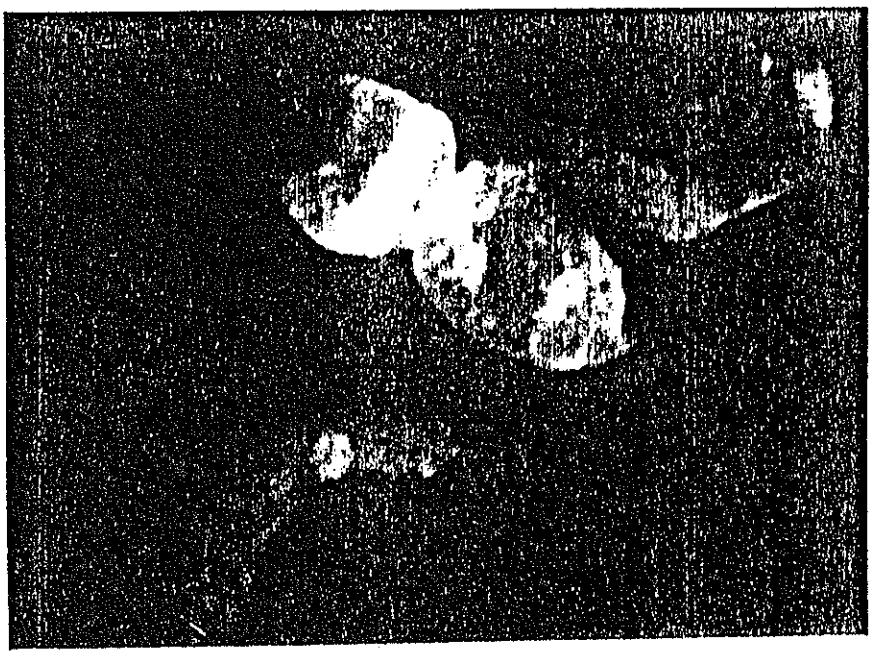
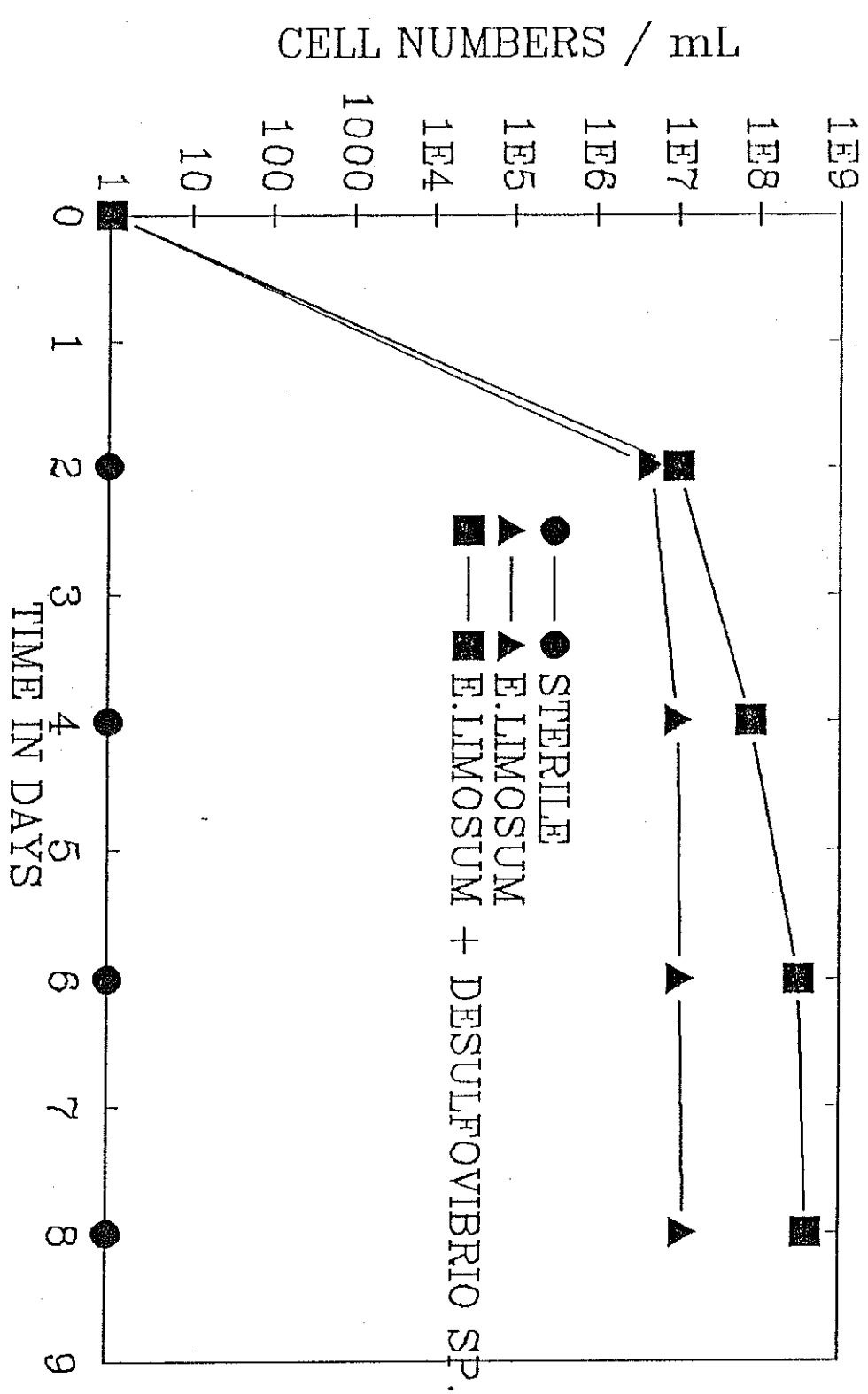
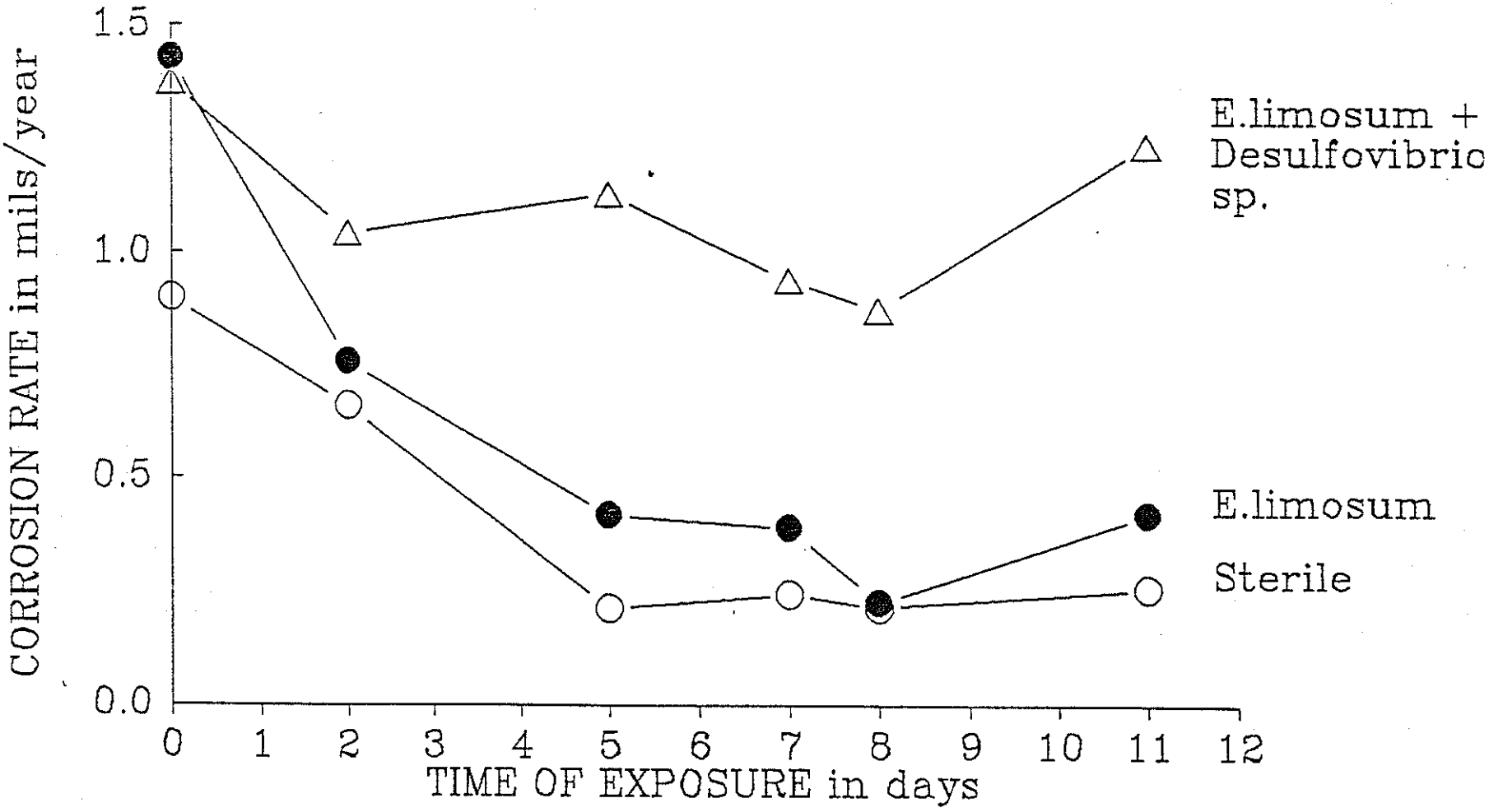
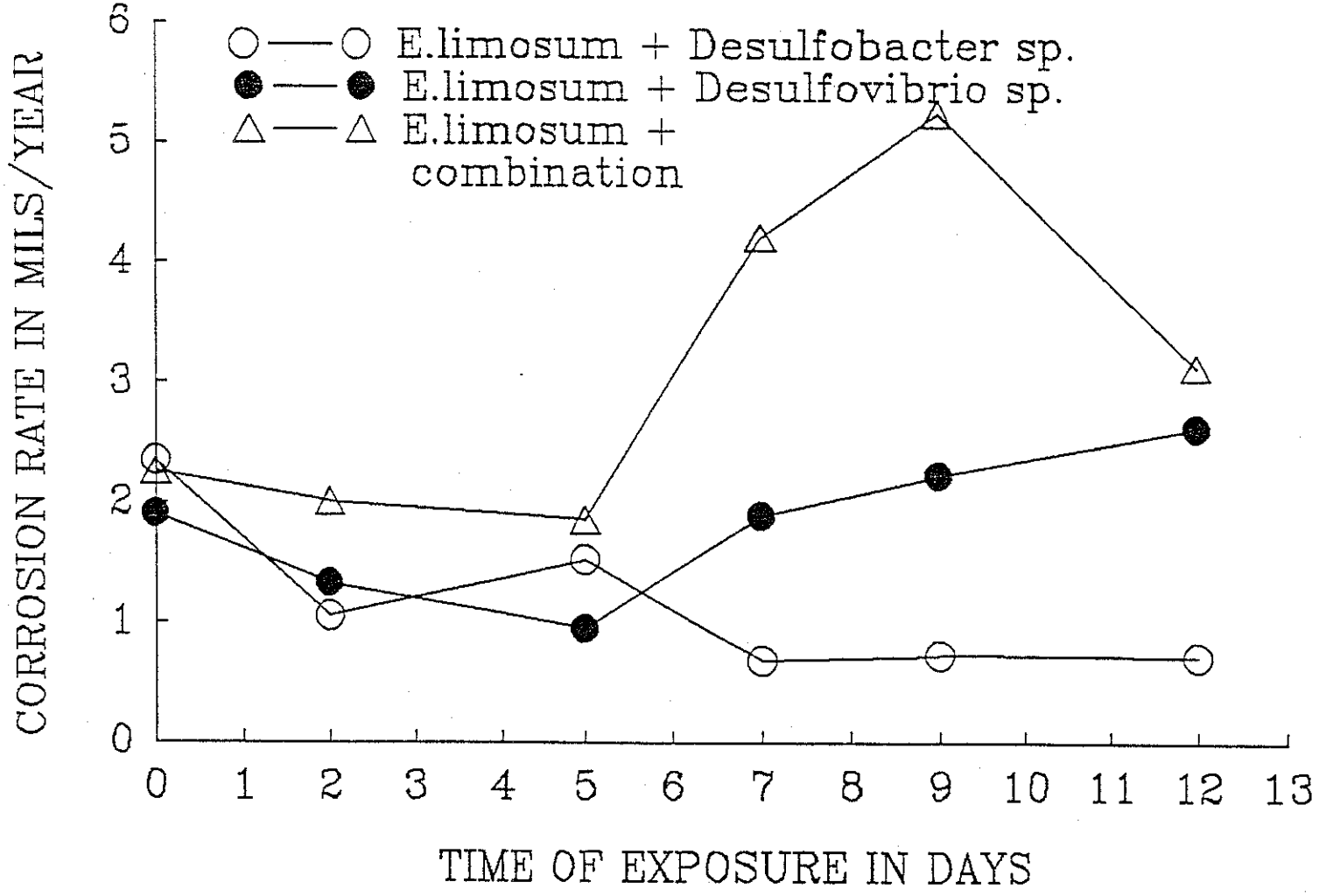


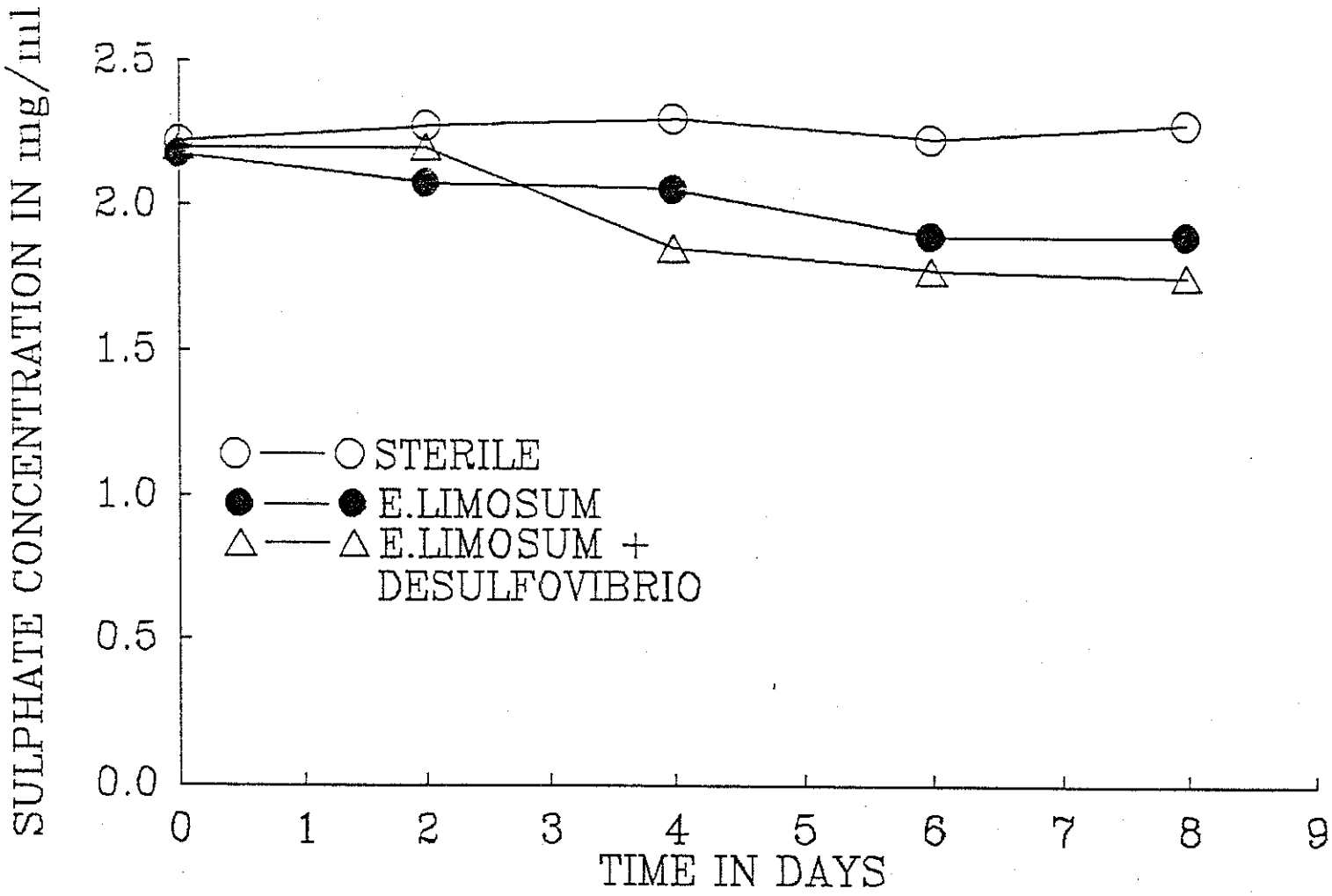
FIGURE 6: Desulfobacter and Desulfovibrio on the surface of a carbon steel coupon.

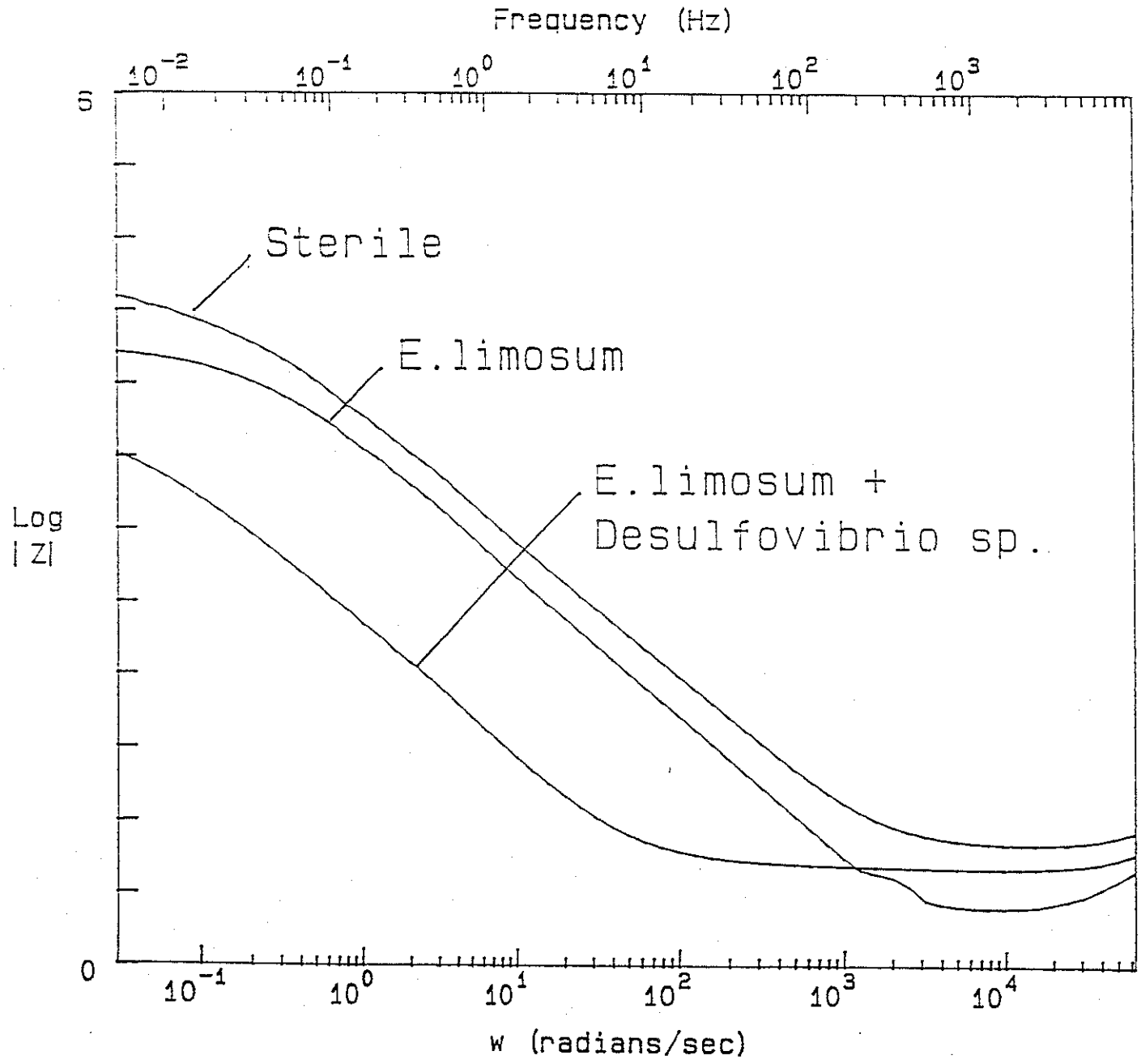
FIG. 6.


CELL NUMBERS / mL
1E9
1E8
1E7
1E6
1E5
1E4
1000
100
10
1
0
1
2
3
4
5
6
7
8
9
TIME IN DAYS
STERILE
E.LIMOSUM
E.LIMOSUM + DESULFOVIBRIO SP.


CORROSION RATE in mils/year
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME OF EXPOSURE in days
E.limosum + Desulfovibrio sp.
E.limosum
Sterile


E.limosum + Desulfobacter sp.
E.limosum + Desulfovibrio sp.
E.limosum + combination
CORROSION RATE IN MILS/YEAR
0
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13
TIME OF EXPOSURE IN DAYS


SULPHATE CONCENTRATION IN mg/ml
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
9
TIME IN DAYS
STERILE
E.LIMOSUM
E.LIMOSUM +
DESULFOVIBRIO


Frequency (Hz)
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
6
Sterile
E.limosum
E.limosum +
Desulfovibrio sp.
Log
|Z|
0
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
w (radians/sec)