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**A Surface Analytical Investigation of the Influence of
Sulphate Reducing Bacteria on Metallic Corrosion**

Principal Investigator: Clive R. Clayton

Final Report
Contract No. N0001492J1550

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**Prepared for:
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9 ABSTRACT (Continue on reverse if necessary and identify by block number)

It has long been recognized that Sulphate Reducing Bacteria (SRB) found in natural and industrial waste waters promote Microbiologically Influenced Corrosion (MIC) of certain metals and alloys. X-ray Photoelectron Spectroscopy (XPS) is used in conjunction with conventional microbiological and quantitative chemical analytical techniques to better understand the effect of environmental conditions on microbial behavior as well as the ability of SRB to alter local environmental conditions (such as pH) in such a way as to accelerate corrosion. Specifically, the interactions of Fe, Cr, Ni and Mo ions with *Desulfovibrio* sp. under anoxic conditions were studied in order to determine the resulting speciation of the metal ions and sulfur.

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Application of XPS to the Study of MIC

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The biotic and abiotic factors that contribute to microbiologically influenced corrosion (MIC) involve the transformation of chemical species at a metal surface. X-ray photoelectron spectroscopy (XPS) is used in conjunction with conventional microbiological and quantitative chemical analytical techniques to better understand the effect of environmental conditions on microbial behavior as well as the ability of bacteria to alter local environmental conditions. Specifically, the interaction of Fe, Cr, Ni, Mo ions with Desulfovibrio sp. under anoxic conditions were studied. This is the first phase of a systematic study of microbial activity and the effects of alloy elements and thermomechanical treatments on the MIC resistance of stainless steels.

Sulfate-reducing bacteria (SRB) are found extensively in natural and industrial waters and have been frequently implicated as a cause of accelerated corrosion of certain metals.¹ The extent to which the metabolic products of bacteria are involved in the breakdown of passive alloys has yet to be established. The presence in corrosion products of unusually high concentrations of certain chemical elements that may have a microbiological origin has been proposed to be conclusive proof of the role of bacteria in a degradation process. For example, the presence and activity of SRB in water systems is often associated with the pungent odor of hydrogen sulfide and the formation of metal sulfides. Although metal sulfides and hydrogen sulfide (H₂S) may be

present at a corrosion site, these factors are not necessarily the cause of accelerated corrosion. There are both biotic and abiotic sources of sulfur compounds and H₂S in natural environments.

X-ray photoelectron spectroscopy (XPS) is a well-established technique for the study of the surfaces of inert biological materials as well as the surface of living cells. In this study, the application of the technique has been extended to study the effect of environmental conditions on microbial behavior as well as the ability of bacteria to alter local environmental conditions. Specifically, the interaction of iron (Fe), chromium (Cr), nickel (Ni), and molybdenum (Mo) ions with *Desulfovibrio* sp. in a modified Postgate's medium C was examined.

The concentration and type of metal ions used represent those that might be present at a clean, passive (approximately 10⁻⁵ A/cm²) stainless steel surface. The characterization of the interaction of metal ions and bacteria will serve as the basis of a systematic study of microbial activity and the effects of alloying elements and thermomechanical treatments on the MIC resistance of stainless steels.

Experimental

Desulfovibrio sp. was grown in the presence of metal ions in a modified Postgate's medium C.² The resulting microbial transformations were assessed by chemical and spectroscopic means.

Microbiology

Culture

Desulfovibrio sp.; isolated from a leachate sample that had the following characteristics: reduces sulfate, desulfovibridin positive, gram negative, vibrioid rod, produces acetic and propionic acids from lactic acid.

Medium

Modified Postgate's medium C consisting of (g/L): 1 NH₄Cl, 2.25

ENVIRONMENTAL EFFECTS

TABLE 1
Effect of Sulfate-Reducing Bacteria on Metal Ions

Metal Ion Added	Treatment	Metal in Solution (mM)	% Metal Remaining in Solution	Precipitate	Solution Color
Chromic	Control	0.20 ± 0.01	90	None	Yellow ^(a)
	Inoculated	0.18 ± 0.02			
Ferric	Control	0.37 ± 0.01	3	None	Yellow
	Inoculated	0.013 ± 0.007			
Molybdate	Control	0.47 ± 0.01	<1	None	Yellow
	Inoculated	<0.01			
Nickelous	Control	0.51 ± 0.02	4	None	Yellow
	Inoculated	0.02 ± 0.01			

^(a)Yellow color is due to media ingredients peptone and yeast.

lactic acid; 0.06 MgSO₄ · 7 H₂O; 4.5 Na₂SO₄; 1 yeast extract; 0.5 KH₂PO₄; 0.06 CaCl₂ · 2H₂O; 0.004 FeSO₄ · 7H₂O and 1,000 mL deionized water. (The modification consisted of the use of lactic acid instead of sodium lactate.) The medium contained 1,800 µM sulfate and 900 µM lactic acid. The medium was pre-reduced by boiling and purging with N₂, and dispensed in 60 mL serum bottles in an anaerobic glove box filled with 95% N₂ and 5% H₂. The bottles were capped with butyl-rubber stoppers, crimped and autoclaved at 121°C at 20 psi for 20 min. The final pH of the medium after sterilization was 7.4.

Metals

0.2 mM of ferric chloride (FeCl₃ · 6H₂O), sodium molybdate (Na₂MoO₄ · 2H₂O), chromic chloride (CrCl₃ · 6H₂O) and nickelous chloride (NiCl₂ · 6H₂O) were separately added to sterile medium. The solutions containing the metal ions were added to the sterile medium after filtration through a 0.22 µm filter. The final concentrations of metal ions in the medium are given in Table 1. The pH of the medium after the addition of the metal ions was readjusted to 7.4. A 2.5% v/v inoculum of a two-day-old culture was used in the experiments.

Microbiological

and Chemical Analysis

At the end of the incubation period, the following were measured: (1) lactic acid consumption; (2) production of acetic acid and propionic acid; (3) head-space gas pressure; (4)

changes in pH; and (5) sulfate reduction. Turbidity could not be used as a measure of growth because of the formation of metal-sulfide precipitates. The head-space gas pressure was checked with an analog pressure gauge attached to a 22-gauge needle. The culture samples were transferred into 40 mL acid-washed centrifuge tubes and centrifuged at 10,000 rpm for 15 min. The supernate was decanted; the pH was determined; the sample was filtered through a 0.22-µm filter and analyzed for metals, sulfate, and organic acids. The cell-pellets (bacterial cells and sulfide precipitates) were placed in a desiccator and allowed to dry, under anoxic conditions for two days. The uninoculated samples, which served as experimental controls, were also analyzed for pH, metals, sulfate, and organic acids.

Quantitative Chemical Analysis

Metals were analyzed by atomic adsorption spectrophotometry. Fe was analyzed by a colorimetric method. Organic acids were quantified by high-performance liquid chromatography (HPLC) with a UV/VIS detector and refractive index detectors. Sulfate was determined spectrophotometrically by precipitation with barium chloride. The pH was measured with a pH meter and combination electrode.

Sample Preparation for XPS Analysis

The dried cell-pellets were stored under nitrogen and transferred to an argon-purged glove box

attached to the XPS unit. Cell-pellets were placed onto an Indium foil and mounted onto the XPS holder. The samples were then transferred under argon from the glove box to the spectrometer.

XPS Analysis

All XPS measurements were performed with a modified spectrometer controlled by a computer-based data acquisition system. An aluminum K_{α1} x-ray source was operated at 400 W. Special features of the XPS unit include an environmental cell, multiple injection ports and a probe with heating and nitrogen cooling capabilities. A cold stage was specifically added to prevent the degradation of biological samples during analysis and to avoid contaminating the chamber. Ultra-high vacuum conditions (base pressure was 1 to 2 × 10⁻⁹ torr) were maintained in order to optimize the quality of the signal coming from the specimen surface to the detector and to prevent accumulation of contaminants on the surface from the gas phase.

All XPS measurements were conducted at a high take-off angle (50 degrees) measured with respect to the plane of the sample. In each case, 1,000 eV survey scans were run to locate the most intense peaks. These peaks were repeatedly scanned to improve the sensitivity and the signal-to-noise ratio. The metal and sulfur peaks were first identified, and then separate narrow scan peaks were obtained.

The ion bombardment operation that is typically done (with Krypton at 2 keV) to remove surface contaminants was found to reduce sulfur species. Contaminants found in the vacuum system were attributed to the egress of certain chemicals within the biomass when under vacuum. In the future, a freezing and mechanical scraping operation will be used to remove surface contaminants and to avoid ion damage of the sample.

Results and Discussion

Microbiological and Quantitative Chemical Analysis

The consumption of lactic acid and the production of acetic and propionic acids by *Desulfovibrio* sp. in

the presence of the Fe, Cr, Ni, Mo ions are presented in Figure 1. The associated total production of gas and reduction of sulfate are presented in Table 2. Exposure of *Desulfovibrio sp.* to solutions containing 0.2 mM concentrations of Fe, Cr, Ni and Mo metal ions was not found to inhibit microbial growth, as indicated by acid production, sulfate reduction, and total gas production (Figure 1 and Table 2).

XPS Analysis

The XPS technique involved the irradiation of the cell-pellet sample by an x-ray beam which induced the ejection of electrons from the outermost layer of the sample surface. The kinetic energies of the emitted electrons were analyzed to determine their binding energies in the sample. Peaks in the recorded spectra were associated with specific elements (e.g., S, Fe, Cr, Ni, Mo) by comparison to standards. The way in which the atoms were bound on the surface was deduced from the shape and binding energy position of the peaks.

The chemical state of the metal inoculates and sulfur compounds present in the biomass was determined, when possible, from the deconvoluted XPS spectra. Figure 2 shows the presence of metal sulfides, sulfite, and elemental sulfur in the biomass that had been inoculated by both cationic (Fe, Cr, Ni) and anionic (Mo) metal complexes. The highest rate of sulfate reduction by *Desulfovibrio sp.* occurred in the culture inoculated with molybdate. This observation indicates that bacteria may have a significant effect on the efficacy of molybdate corrosion inhibitors. Compounds that are isostructural to sulfate, such as molybdate, are generally regarded as inhibitory to the growth of *Desulfovibrio sp.*² However, the molybdate concentration (0.2 mM) that was used in this study may have been too low to inhibit growth. The molybdate concentration used is representative of that which might be present at the surface of a clean, passive stainless steel (approximately 10^{-5} A/cm² current density).³ Further work will demonstrate the critical concentration at which molybdate will affect the activity of SRB.

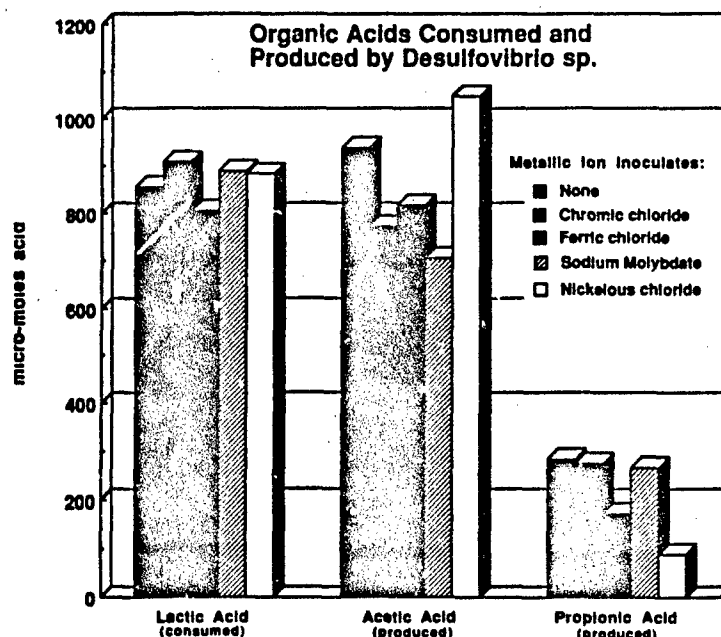


FIGURE 1
Organic acids consumed (lactic) and produced (acetic and propionic) by *Desulfovibrio sp.* as determined by HPLC.

TABLE 2
Effect of Metal Ions on Sulfate-Reducing Bacteria Activity

Treatment	pH	Gas Produced (mL)	Sulfate Reduced (%)
No metal added Uninoculated (control)	7.48 ± 0.06	0	0
No metal added Inoculated	6.96 ± 0.05	6.8	15
Chromium Inoculated	6.95 ± 0.05	6.8	30
Iron Inoculated	7.0 ± 0.01	9.5	23
Molybdenum Inoculated	6.85 ± 0.01	6.8	39
Nickel Inoculated	7.10 ± 0.01	6.8	24

The formation of metal sulfides from cations in neutral pH medium was not surprising, but evidence of molybdenum disulfide was. One possible mechanism is that molybdenum disulfide may form from the reaction with biogenic hydrogen sulfide, but this requires acidic conditions.⁴ In the bulk medium with neutral pH, the formation and stability of a sulfide would require microbial production of hydrogen sulfide gas in a region of low pH, which could possibly be created by the acetic acid and propionic acids that were

observed to be produced by the bacteria.

The S 2p spectra revealed the presence of sulfide in all cases. The smallest amount of sulfide observed was found in the case of no inoculate. In all cases, the S 2p spectra revealed four sulfur species: sulfur, sulfate and sulfite and sulfide. The smallest amount of sulfur species observed was found in the case of no inoculate.

The XPS metal spectra appear in Figure 3. Unlike the sulfur spectra, the signal-to-noise ratios for the

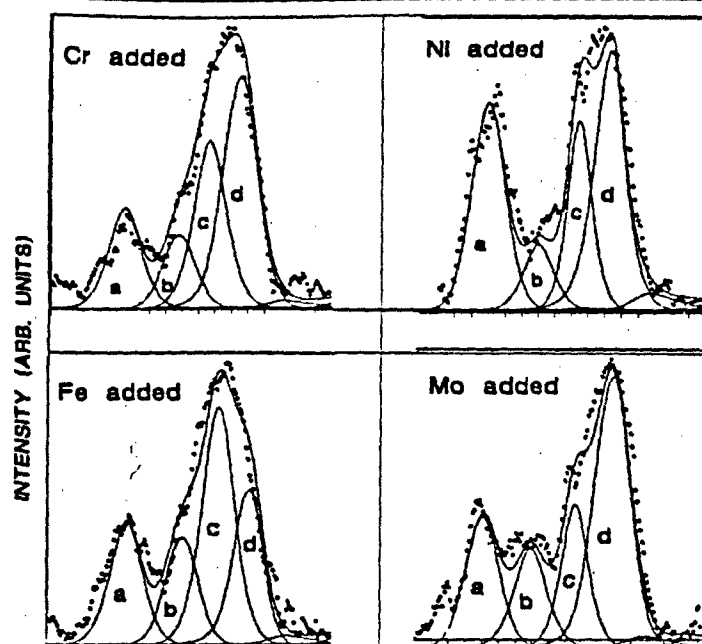


FIGURE 2

XPS spectra of sulfur (S 2p) for biomasses consisting of *Desulfovibrio* sp. in Postgate's Medium C inoculated with Cr, Ni, Fe, and Mo metal ion species. Species indicated: (a) SO_4^{2-} , (b) SO_3^{2-} , (c) S, (d) S^{2-} .

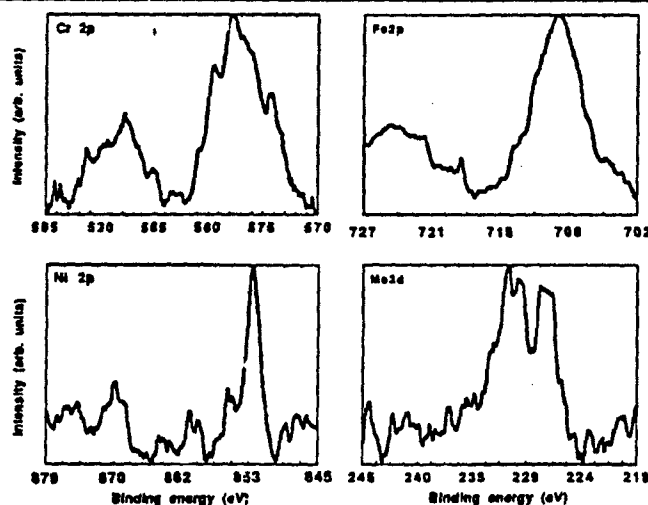


FIGURE 3

XPS spectra of metals (Cr 2p, Ni 2p, Fe 2p, and Mo 3d) for biomasses consisting of *Desulfovibrio* sp. in Postgate's Medium C inoculated with Cr, Ni, Fe, and Mo metal ion species.

metal spectra were too low to permit confident deconvolution. However, the overall shift of the Mo 3d spectra away from the molybdate binding energy strongly suggests that molybdate has become reduced in favor of a molybdenum sulfide compound. Further work will improve the signal-to-noise ratio so that more specific information can be obtained

on peak identification. The S 2s spectral region overlaps the Mo 3d region. Therefore, the Mo 3d deconvolution includes a contribution from the S 2s. Analysis of the Cr 2p spectra, the sulfide contribution to the Cr 2p spectra is less than that to the Mo 3d spectra. From this it can be deduced that there was greater transformation of the Mo than the Cr by

the bacterial species. The Fe 2p spectra of the Fe-inoculated sample revealed that the ferrous ion was the dominant species. The Ni 2p spectra of the Ni-inoculated sample showed the presence of nickelous ions.

Conclusions

- Exposure of *Desulfovibrio* sp. to solutions containing 0.2 mM concentrations of Fe, Cr, Ni, and Mo metal ions was not found to inhibit microbial growth, as indicated by acid production, sulfate reduction, and hydrogen sulfide gas pressure.
- XPS revealed the presence of metal sulfides in the biomass that had been inoculated by both cationic (Fe, Cr, Ni) and anionic (Mo) metal complexes. The formation of cationic metal sulfides in neutral pH medium was not surprising. However, the abiotic formation of molybdenum sulfide by reaction of hydrogen sulfide requires acidic conditions. The formation and stability of the sulfide is facilitated by the microbial production of hydrogen sulfide gas and acetic and propionic acids which may lower the pH at localized sites.
- The highest rate of sulfate reduction by *Desulfovibrio* sp. occurred in the culture inoculated with molybdate of the four metal complexes examined (Fe, Cr, Ni, and Mo).

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More information may be found in paper no. 178, presented at CORROSION/92 in Nashville, Tennessee.

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