INVESTIGATION INTO THE SUSCEPTIBILITY OF CORROSION RESISTANT ALLOYS TO BIOCORROSION

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Technical Report No. 2
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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

It has long been recognized that sulfate reducing bacteria (SRB) found in natural and industrial waste waters promote microbiologically influenced corrosion (MIC) of certain metals and alloys. Corrosion may be enhanced biologically, through direct enzymatic action of the bacteria, or abiotically, as a result of reaction with metabolic byproducts or changes in the local conditions (for example, pH) brought about by bacterial activity. In this study, X-ray photoelectron spectroscopy (XPS) is utilized in conjunction with conventional microbiological and quantitative chemical analytical techniques to analyze the effects of localized environmental conditions similar to those found near the surface of the passive stainless steel on the behavior of SRB, and to determine the ability of these bacteria to alter local environmental conditions in such a way as to create conditions that accelerate corrosion. Specifically, the interactions of Fe,Cr,Ni and Mo ions with Desulfovibrio sp. under anodic conditions were studied in order to determine the influence of passive dissociation products on the extent of sulfate reduction and to determine the resulting speciation of the metal ions and sulfur. In all cases ,XPS revealed the presence of multiple reduced sulfur species, as well as reduction of both molybdate and ferric ions.

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Spectroscopic Study of Sulfate Reducing Bacteria-Metal Ion Interactions Related to Microbiologically Influenced Corrosion (MIC)

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ABSTRACT: It has long been recognized that sulfate reducing bacteria (SRB) found in natural and industrial waste waters promote microbiologically influenced corrosion (MIC) of certain metals and alloys. Corrosion may be enhanced biologically, through direct enzymatic action of the bacteria, or abiotically, as a result of reaction with metabolic byproducts or changes in local conditions (for example, pH) brought about by bacterial activity. In this study, X-ray photoelectron spectroscopy (XPS) is utilized in conjunction with conventional microbiological and quantitative chemical analytical techniques to analyze the effects of localized environmental conditions similar to those found near the surface of a passive stainless steel on the behavior of SRB, and to determine the ability of these bacteria to alter local environmental conditions in such a way as to create conditions that 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 influence of passive dissociation products on the extent of sulfate reduction and to determine the resulting speciation of the metal ions and sulfur. In all cases, XPS revealed the presence of multiple reduced sulfur species (SO_3^{2-}, elemental S and S^{2-}), as well as reduction of both the molybdate and ferric ions. Localized reduction in pH due to SRB metabolic activity was presumed to play a role in the formation of stable molybdenum disulfide and ferrous species.

KEYWORDS: sulfate reducing bacteria (SRB), X-ray photoelectron spectroscopy (XPS), microbiologically influenced corrosion (MIC)

Introduction

Sulfate reducing bacteria (SRB) are ubiquitous to both natural and industrial waters. Nearly sixty years ago Wolzogen and Van der Vlught [*1*] considered the influence of SRB

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on microbiologically influenced corrosion (MIC). More recent studies have established strong evidence that SRB are involved in the corrosion process of some metals [2]. However, to establish the causal relation between SRB activities and corrosion of metals, one must be certain to find a link between corrosion sites and the presence of a definable corrosion cell involving a biogenic agency. The components of such a cell may include a biofilm and liquid and solid metabolic products. Since corrosion is not always associated with such biofilms it is necessary to determine the critical conditions which are required to initially accelerate localized corrosion. To initiate a study to determine the nature of such conditions, it is necessary to analyze and catalogue the constituents of a biofilm in close proximity to a corroded site and to formulate a rationale for the production of such constituents. Consideration must be given to the possibility of both biotic and abiotic formation of sulfur compounds. Traditional models of SRB-driven corrosion have generally failed to consider these differences. Furthermore, because of the possible action of enzymes, thermodynamic data alone cannot predict the nature of the sulfur products [3]. Of special interest would be any evidence of sulfur products, for instance, which can reliably be predicted not to form abiotically but otherwise can be formed by SRB. Such a case has recently been made for the formation of mackinawite by SRB [4], although in this case abiotic synthesis is not entirely excluded [5].

The work of Mohagheghi [6] seems to illustrate that metal ions bound to cell walls of SRB are more reactive with bacteriogenic sulfur than the same ions involved in an equivalent abiotic process occurring on a metal surface exposed to a solution containing sulfur. The formation of sulfides in biomasses must be considered a potential source of cathodic activation of the corrosion cell [7–10]. Additionally, Newman, et al. [11], have reported that $Na_2S_2O_3$, $Na_2S_4O_6$, KSCN, Na_2S, Na_2SO_3 and H_2S in neutral chloride solutions are all capable of promoting the pitting of 304 stainless steel.

Characterization of corrosion products associated with MIC presents considerable constraints on the nature of the analytical system which can be used. For example, the importance of low atomic number species limits the usefulness of such techniques as energy dispersive X-ray analysis. Similarly, few techniques can provide determination of speciation. By contrast, X-ray photoelectron spectroscopy (XPS) provides a method for determination of the chemical state of high and low atomic number species on the surface of both inert biological material [12–15] as well as the surfaces of living cells [16–19]. In this paper we demonstrate the use of XPS in determining the extent to which SRB may alter local environmental chemistry. We have considered the effects of Fe, Cr, Ni and Mo ions on the activity of *Desulfovibrio sp.* in a modified Postgate's medium C.

The nature of metal ion-bacteria interactions is important to understand since metal ions released from stainless steels, even under passive conditions, may bring about nutrient or toxic conditions. The concentration and types of metal ions released from a stainless steel under passive conditions (10^{-5} A/cm^2) were considered in this study [20].

Experimental Procedure

Culture

Desulfovibrio sp. 1CA3 was isolated from waste disposal leachate in Postgate's medium B. Upon isolation, the bacterium was maintained in Postgate's medium C. The bacterium is a gram negative vibrioid rod, desulfoviridin positive, which produces acetic acid as a major end product when grown in the presence of lactic acid and vigorously reduces sulfate with hydrogen sulfide gas production.

Medium

Modified Postgate's medium C consisting of (g/L deionized water): 1 NH_4Cl, 2.25 lactate; 0.06 $MgSO_4 \cdot 7H_2O$; 4.5 Na_2SO_4; 1 yeast extract; 0.5 KH_2PO_4; 0.06 $CaCl_2 \cdot 2H_2O$; 0.004 $FeSO_4 \cdot 7\ H_2O$, pH adjusted to 7.8 with KOH. The modification consisted of the addition of lactate as lactic acid instead of sodium lactate. The medium was prereduced by boiling and purging with ultra-high purity nitrogen, and 40 mL was dispensed into each 60 mL serum bottle in a nitrogen filled glove box as described previously [*21*]. The bottles were capped with butyl-rubber stoppers, sealed and autoclaved at 121°C, 20 psi for 20 min. The final pH of the medium after sterilization was 7.4. The bottles each contained 900 μmoles lactate and 1800 μmoles sulfate.

Metals

Ultra high purity (UHP) nitrogen purged solutions of iron (III) chloride ($FeCl_3 \cdot 6\ H_2O$), sodium molybdate (Mo(VI)) ($Na_2MoO_4 \cdot 2\ H_2O$), chromium (III) chloride ($CrCl_3 \cdot 6\ H_2O$) and nickel (II) chloride ($NiCl_2 \cdot 6\ H_2O$) were separately added to the sterile medium in the glove box for a final concentration of 0.2 mM. The solutions containing the metal ions were added to the sterile medium after filtration through a 0.22 μm filter. The pH was adjusted to 7.4 after addition with sterile acid or base. Sodium molybdate was also added to a series of bottles for a final concentration of 0.2, 1, 10 and 20 mM molybdate. Specific sample bottles were reserved without metal additions as controls. Metal concentrations were measured by atomic absorption spectrophotometry.

Inoculation

One milliliter of a 24 h culture of *Desulfovibrio sp.* 1CA3 was added to each bottle and incubated in the dark at 26 ± 1°C. Uninoculated samples and inoculated samples without metals were prepared and used as controls.

Microbiological and Chemical Analyses

After incubation for five days the following analyses were performed: (1) total gas production by using an analog pressure gauge (Marcsh Co.) attached to a 22 gauge needle, (2) lactic acid consumption and acetic and propionic acid production by high performance liquid chromatography (HPLC) with a UV/VIS (Spectra-Physics) and refractive index (Shimadzu) detector after filtration through a 0.22 μm filter [*22*], (3) the pH, (4) sulfate reduction spectrophotometrically by precipitation with barium chloride [*23*], and (5) metal remaining in solution by atomic absorption spectrophotometry on a filtered, acidified aliquot. Turbidity was not used as a measure of growth because of the formation of metal sulfide precipitates. The biomass along with any precipitate was recovered by centrifugation at 10 000 rpm for 15 min in 40 mL acid-washed, anaerobically sealed centrifuge tubes. The cell pellets (bacterial cells and sulfide precipitate) were placed in a desiccator and allowed to dry under anoxic conditions for two days.

Sample Preparation for XPS Analysis

The dried cell pellets were stored under nitrogen and transferred to an argon-purged glove box. The cell pellets were then crushed onto indium foil, mounted onto the XPS

sample holder and transferred to the spectrometer, all in a glove box under an argon atmosphere to prevent surface chemical oxidation and contamination.

XPS Analysis

All XPS measurements were performed with a modified V.G. Scientific ESCA 3 Mark II spectrometer controlled by a VGX 900 computer-based data acquisition system, located in the Department of Materials Science and Engineering at the State University of New York at Stony Brook. Special features of the XPS unit include an environmental cell, multiple injection ports and a probe with heating and liquid 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 $\times$ 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. Further details on XPS instrumentation, particularly for biological applications, can be found elsewhere [*24–29*]. A summary of the advantages and limitations of the use of XPS for the study of MIC is given in Table 1.

In all cases, the incident radiation was $AlK\alpha_{1,2}$ X-radiation from a non-monochromatized source operated at 400 W. All XPS measurements were carried out at a high take-off angle (50°) measured with respect to the plane of the sample. The entrance and exit slit widths for the hemispherical analyzer were 4 mm, which resulted in a half angle for photoelectron emission of 0.095 radians. In each case, 1000 eV survey scans were run to locate the most intense peaks. These peaks were repeatedly scanned to improve the sensitivity and signal-to-noise ratio. The metal and sulfur peaks were first identified and then separate narrow scan peaks were obtained. A 20 eV pass energy was used for all narrow scan analysis providing a full width at half maximum (FWHM) for the $Au4f_{7/2}$ singlet of 1.35 eV. For reference, the binding energy of the $Au4f_{7/2}$ singlet was found to be 83.8 eV and that of the $Cu2p_{3/2}$ singlet was found to be 932.4 eV. All binding energies were corrected for charge shifting by referencing to the C1s line from the adventitious carbon at 284.6 eV. Details of the curve fitting procedures and data analysis routines may be found elsewhere [*30*]. Standards data for all peak parameters and sensitivity factors were developed from work done on compounds in this laboratory. Powdered standards were crushed into the surface of indium foil under argon and transferred under argon to the spectrometer to ensure a clean surface for analysis. Sulfur 2p and sulfur 2s level photoelectron binding energies determined in this way are presented in Table 2.

TABLE 1—*Advantages and limitations of the application of XPS to the study of MIC.*

ADVANTAGES
• Sensitivity to a wide range of elements (all except H and He)
• Provides data on speciation of all elements present
• Non-destructive technique
• Relatively straightforward method of data analysis
LIMITATIONS
• All analysis must take place in a UHV environment, hence sample must be dry and biological samples must be liquid nitrogen cooled to prevent outgassing or decomposition
• Severe sample charging may occur; in some cases use of a low energy electron flood gun may be necessary
• Complex C1s photoelectron signal may not be useful for charge referencing
• Fairly complex apparatus for analysis

TABLE 2—*Sulfur 2s and 2p X-ray photoelectron binding energies.*

Compound	Species	S2s B.E., eV[a]	S2p B.E., eV
FeS	S^{2-}	225.8	161.9
Pure S	S	228.8	164.2
Na_2SO_3	S^{4+}	231.3	166.1
$FeSO_4$	S^{6+}	232.8	169.2

[a] Note: All binding energy values are ±0.1 eV.

The ion bombardment operation that is typically done (with 2.5 kV argon ions) to remove surface contaminants was found to reduce sulfur species (Fig. 1) and therefore has limited value in a study of this kind. Contaminants found in the vacuum system were attributed to the egress of certain sulfur compounds (especially elemental sulfur and ferrous sulfide, FeS) within the biomass when under vacuum. Sample cooling with liquid nitrogen greatly reduced the severity of outgassing.

Results and Discussion

Microbiological Analysis

Gas production and sulfate reduction by *Desulfovibrio sp.* 1CA3 in the presence of Fe, Cr, Ni and Mo ions are presented in Table 3. The pH dropped slightly in inoculated samples

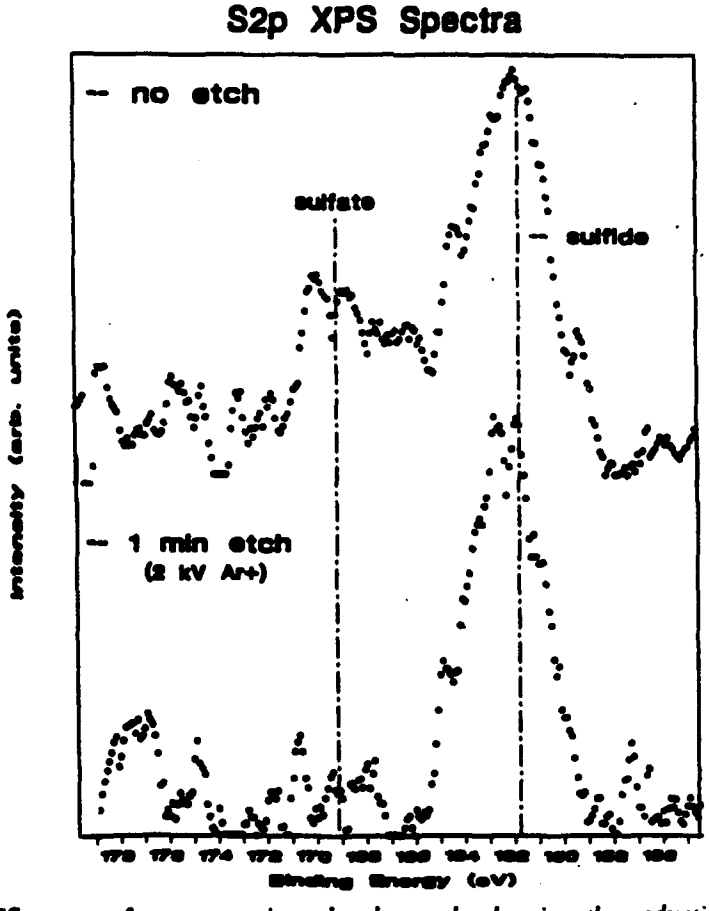

FIG. 1—*S2p XPS spectra from a non-inoculated sample showing the reduction in photoelectron intensity from sulfate following a one minute etch with 2kV Ar^+ ions.*

TABLE 3—*Effect of metal ions on the activity of sulfate reducing bacteria.*

Treatment	pH	Gas Produced, ml	Sulfate Reduced, %	Metal Remaining in Solution, %	Organic Acids (μmoles)		
					Lactic	Acetic	Propionic
No metal added							
Uninoculated	7.46 ± 0.06	ND[a]	ND	ND	855 ± 49	ND	ND
Inoculated	6.96 ± 0.05	6.8	15	ND	ND	941 ± 25	283 ± 7
Chromium							
Inoculated	6.95 ± 0.05	6.8	30	90	ND	773 ± 22	274 ± 5
Iron							
Inoculated	7.00 ± 0.01	9.5	23	3	ND	816 ± 111	174 ± 72
Molybdenum							
Inoculated	6.86 ± 0.01	6.8	39	<1	ND	704 ± 51	265 ± 11
Nickel							
Inoculated	7.10 ± 0.01	6.8	24	4	ND	1050 ± 26	88.6 ± 4

[a] ND = none detected.

due to acid production. Gas production (CO_2 and H_2S) was greatest in the presence of Fe, presumably due to increased metabolic activity by *Desulfovibrio* in response to the abundance of available Fe. *Desulfovibrio* has an exceptionally high requirement for inorganic iron [3]. However, sulfate reduction was greatest in the presence of molybdate, with almost 40% of the added sulfate reduced. All the metals, except chromium (III), were removed from solution. Addition of 0.2 mM Fe, Cr, Ni and Mo ions to *Desulfovibrio sp.* 1CA3 did not inhibit microbial activity (Fig. 2) as indicated by acid production, sulfate reduction and gas production (Table 3).

XPS Analysis

The XPS technique involved the irradiation of the cell pellet sample by a soft X-ray beam which induced the emission of photoelectrons from the core atomic levels in the outermost layer of the sample surface. The kinetic energies of the emitted electrons were analyzed to determine their binding energies in the species of interest according to the following relationship:

$$h\upsilon = E_{kin} + E_{bc} + \phi \quad (1)$$

where $h\upsilon$ is the energy of the incident X-rays, E_{kin} is the kinetic energy of the emitted photoelectrons, E_{bc} is the electron binding energy, and ϕ is the work function of the spectrometer. Peaks in the deconvoluted spectra were associated with specific elements and particular valence states or compounds of those elements by comparison to standards.

The XPS S2p spectra from the inovulated SRB are referred to in an earlier study [31]. In all cases, sulfur exists in the original sulfate form and as three reduced states: sulfide, elemental sulfur and sulfite. The amount of sulfate reduced as determined by analysis of sulfate remaining in solution after incubation in the presence of various metals is presented in Table 3. The sample inoculated with molybdate showed the maximum amount of sulfate reduction. The XPS sulfur spectral data from the biomass (Fig. 2) confirmed this finding. The presence of low levels of iron sulfide in the biomass is due to its high vapor pressure and hence volatility in the vacuum system of the XPS unit.

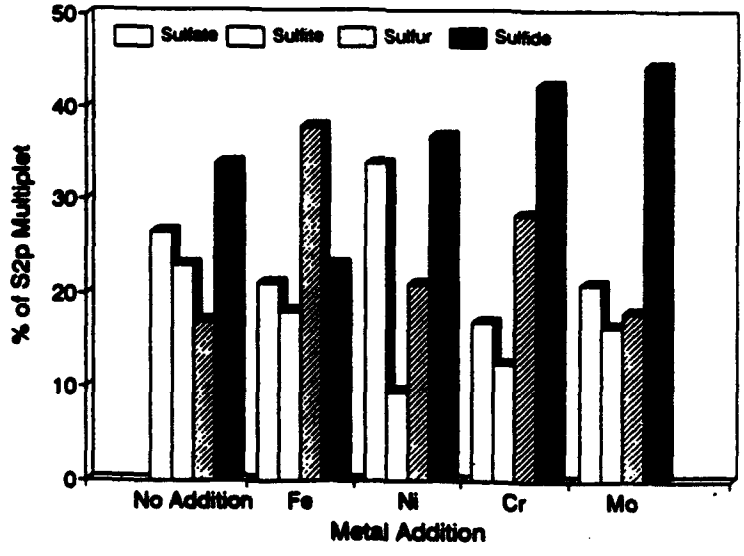

FIG. 2—*XPS analysis of sulfur species in biomass of* Desulfovibrio sp. *incubated in the presence of Fe, Ni, Cr and Mo.*

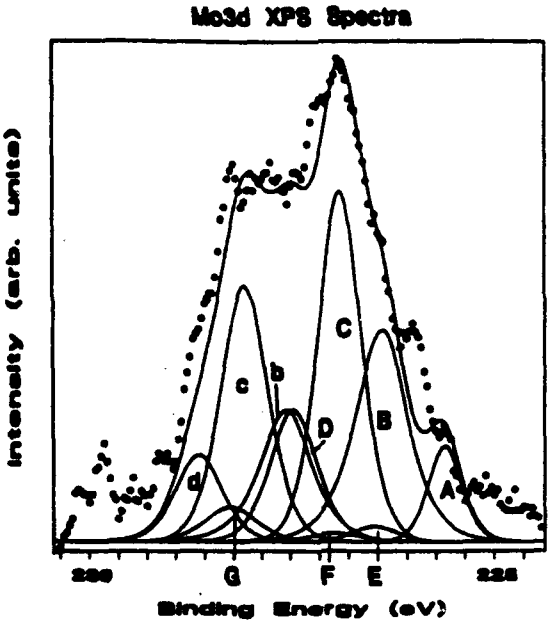

FIG. 3—*Mo3d (and overlapping S2s) XPS spectra from* Desulfovibrio sp. *biomass exposed to 0.2 mM sodium molybdate. Peak identification:* (A) S^{2-} *(from* MoS_2*),* (B) Mo^{4+} $3d_{5/2}$ *(from* MoS_2*),* (b) Mo^{4+} $3d_{3/2}$ *(from* MoS_2*),* (C) Mo^{5+} $3d_{5/2}$*,* (c) Mo^{5+} $3d_{3/2}$*,* (D) Mo^{6+} $3d_{5/2}$ *(from molybdate),* (d) Mo^{6+} $3d_{3/2}$ *(from molybdate),* (E) *S* (F) SO_3^{2-} (G) SO_4^{2-}.

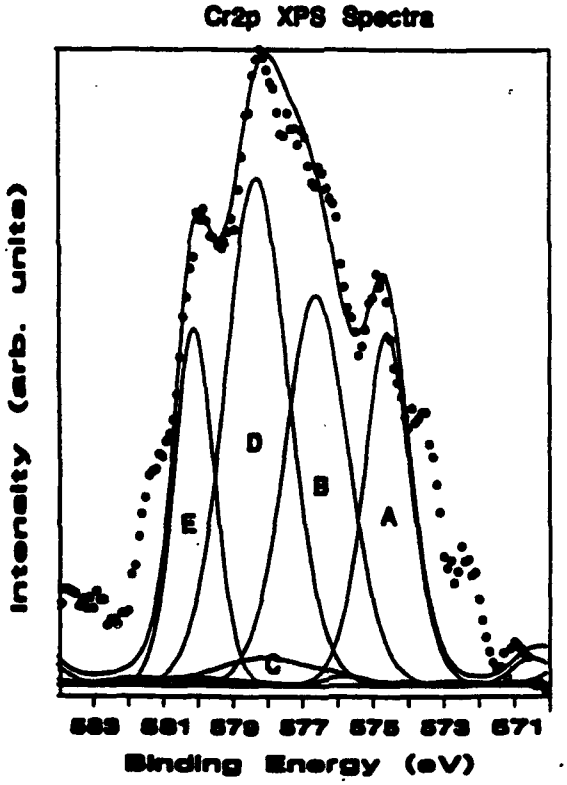

FIG. 4—$Cr2p_{3/2}$ *XPS spectra from* Desulfovibrio sp. *biomass exposed to 0.2 mM chromic chloride. Peak identification:* (A) Cr^{3+} *(from* Cr_2S_3*),* (B) Cr^{3+} *(from* $CrOOH$ *or* $Cr(OH)_3$*),* (C) *satellite from* $Cr2p_{1/2}$ *photoelectron peak,* (D) Cr^{3+} *(from* $CrCl_3$*), and* (E) Cr^{3+} *(from* $Cr_2(SO_4)_3$*).*

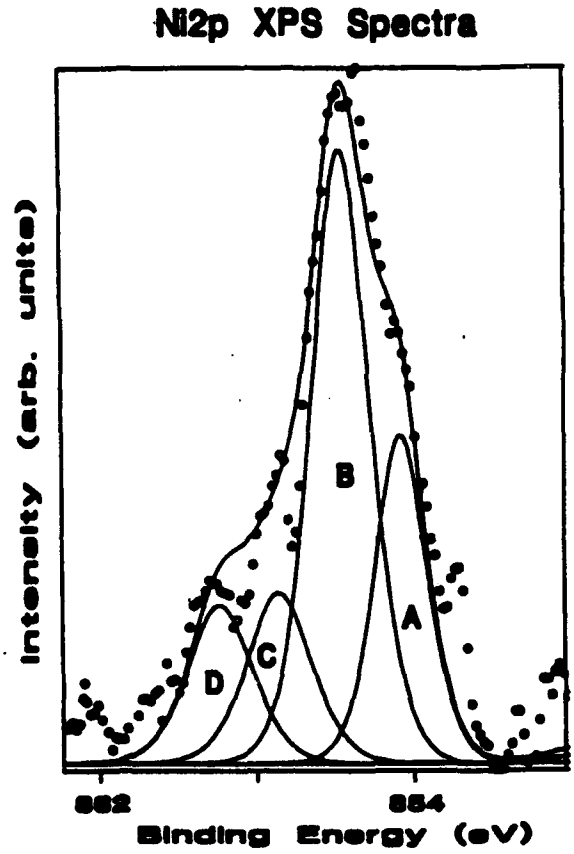

FIG. 5—*Ni2p$_{3/2}$ XPS spectra from* Desulfovibrio sp. *biomass exposed to 0.2 mM nickelous chloride. Peak identification:* (A) *Ni^{2+} (from NiS),* (B) *Ni^{2+} (from $Ni(OH)_2$),* (C) *Ni^{2+} (from $NiCl_2$), and* (D) *Ni^{2+} (from $NiSO_4$). (Note—No satellite from $2p_{1/2}$ component included in multiplet due to larger value of spin-orbit splitting.)*

The XPS metal spectra appear in Figs. 3 through 6. Photoelectron spectra were analyzed for the Fe2p, Ni2p, Cr2p and Mo3d core levels for the samples inoculated with the respective metal ions. The Mo3d spectra (Fig. 3) is complicated by its overlap with the S2s core photoelectron spectra. The formation of metal sulfides from cationic (Fe, Cr and Ni) metal complexes in a neutral pH medium was not surprising, but evidence of molybdenum disulfide was. One possible explanation is that molybdenum disulfide may form by direct reaction of molybdate with hydrogen sulfide, but this requires acidic conditions [*32*]. 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. It is of interest to note that molybdate has been found to play an important role as an electron acceptor in the outermost layer of passive films formed on Mo-bearing stainless steels and hence any reduction of molybdate to lower valent species would adversely affect the ability of the passive film to inhibit ingress of anionic species such as Cl^- [*33*]. In addition, we noted that the Mo3d photoelectron spectra show a strong signal from a pentavalent reduction product, possibly a precurser to disulfide formation or part of a molybdenum oxy-hydroxide complex, or both.* Further clarification is currently being sought.

All other metal spectra indicated the presence of the respective chloride in the cell pellet as well as the metal sulfides and sulfate compounds. The Cr2p, Ni2p and Fe2p spectra also

* D. G. Kim, Ph.D Thesis, SUNY Stony Brook, NY, 1993.

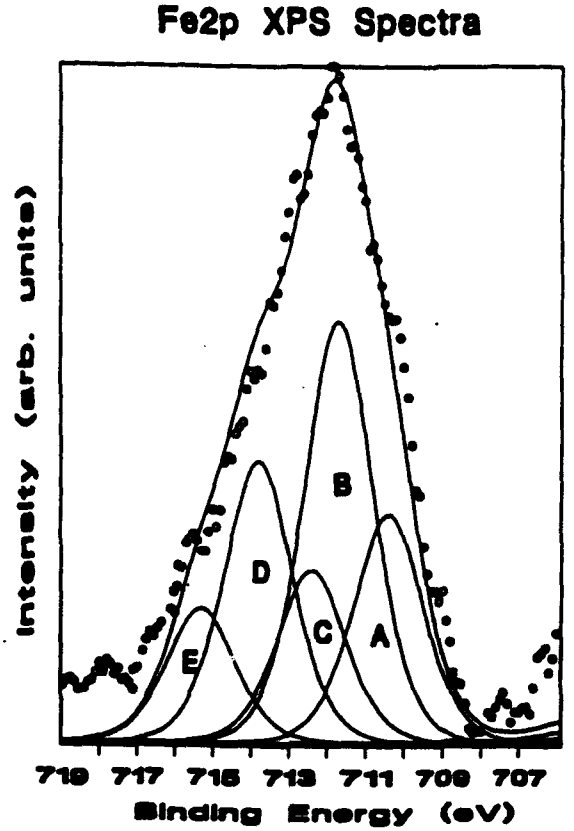

FIG. 6—*Fe2p$_{3/2}$ XPS spectra from* Desulfovibrio sp. *biomass exposed to 0.2 mM ferric chloride. Peak identification:* (A) *Fe^{3+} (from Fe_2O_3 or FeOOH or $Fe(OH)_3$),* (B) *Fe^{3+} (from $FeCl_3$),* (C) *Fe^{2+} (from FeS),* (D) *Fe^{2+} (from $FeSO_4$), and* (E) *Fe^{3+} (from $Fe_2(SO_4)_3$). (Note—No satellite from $2p_{1/2}$ component included in multiplet due to larger value of spin-orbit splitting.)*

indicated the formation of oxy-hydroxides or hydroxides of the respective metal ions, most likely a result of the interaction of the aliquot with the metal species during the process of sulfate reduction. By analogy this would further suggest the presence of stable pentavalent molybdenum as part of an oxy-hydroxide complex. Both the Cr and Ni spectra exhibited only a single valent state; however, there is evidence of both ferric and ferrous compounds in the Fe2p photoelectron spectra (Fig. 6). According to the Pourbaix diagram for the iron-water system [*34*], a shift to lower pH due to SRB metabolic activity creates a condition favorable for the reduction of the ferric (III) ion to the ferrous (II) ion. The Fe2p XPS spectra, in fact, show evidence of not only ferric (III) chloride but also ferrous (II) sulfide and sulfate. The Cr(III) and Ni(II) ions are predicted to be stable in acidic environments, and hence no reduction to lower valent states (unlikely in any case) is expected.

Conclusions

1. Exposure of *Desulfovibrio sp.* to solutions containing 0.2 mM concentrations of Fe, Cr, Ni and Mo metal ions was not found to inhibit the activity of the microorganism as indicated by lactic acid consumption, acetic acid production, sulfate reduction and gas production.
2. Of the four metal ions tested (Fe(III), Cr(III), Ni(II) and Mo(VI)), the highest rate of sulfate reduction by *Desulfovibrio sp.* occurred in the presence of molybdate.
3. XPS revealed the presence of metal sulfides in the biomass that had been inoculated by both cationic (Fe, Cr and Ni) and anionic (Mo) metal ions. 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 may be facilitated by the microbial production of hydrogen sulfide gas, acetic and propionic acids which may sufficiently lower the pH at localized sites. In addition, enzymatic reduction of Mo(VI) by *Desulfovibrio sp.* may also occur. In any case, both the production of sulfides and the reduction of molybdate may limit the protective capabilities of passive films formed on stainless steels.

4. Fe2p XPS spectra revealed evidence of iron(II) which may result from the localized reduction in pH due to SRB activity.
5. XPS appears to be an invaluable tool in elucidating the possible mechanisms and processes involved in corrosion in the presence of sulfate reducing bacteria, such as *Desulfovibrio sp.* Though limitations exist, primarily due to the ultra-high vacuum environment and the possibility of high sample charging, corrections can be made and techniques utilized which enable XPS to determine quantitative chemical information for a variety of biological samples.

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