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The Application of XPS to the Study of MIC

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ABSTRACT

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 utilized 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 thermo-mechanical treatments on the MIC resistance of stainless steels.

Keywords. X-ray Photoelectron Spectroscopy (XPS), Microbiologically Influenced Corrosion (MIC), Sulfate Reducing Bacteria (SRB), stainless steel, corrosion product characterization, bacteria—metal ion interactions.

INTRODUCTION

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 H_2S 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 H_2S in natural environments.

Traditional models for the mechanism of SRB corrosion, such as that by von Wolzogen and Van der Vlught[2], do not take into consideration the differences between biotic and abiotic sulfide formation. Furthermore, the nature of sulfur species formed cannot be predicted from thermodynamic data alone.[3] For example, Macinawite is a sulfide phase that is thermodynamically unlikely to form under ambient conditions. Yet, MacNeil and Little[4] have observed Macinawite to form biotically and MacDonald[5] has observed Macinawite under abiotic conditions. The physico-chemical properties of the bacteria cell wall as well as the sulfide must also be considered.

The selective adsorption of certain metal ions by SRB and subsequent sulfide formation has not been fully investigated. The retention of metal ions on cell walls by the formation of intermediate organometallic and metal-organic complexes provides a unique means for metal ions to react with bacteriogenic sulfur. Mohagheghi, et al.,[6] have demonstrated that metal ions bound to bacterial cell walls are more reactive with sulfide than are metal ions in solution. This illustrates that the biotic environment is far more complex than the simple abiotic analog of sulfur reacting on a metal surface to form a sulfide in a biotic environment. However, there clearly exists considerable opportunity for corrosion to result from the direct contact of sulfur with a metal surface, especially in the presence of a sulfide that is acting as a cathodically active surface.[7-10] Newman, et al.[11] have demonstrated that Type 304 is more susceptible to pitting in neutral, chloride-containing solutions when sulfur species ($Na_2S_2O_3$, $Na_2S_4O_6$, KSCN, Na_2S, Na_2SO_3, and H_2S) are added.

One reason for the limited characterization of corrosion products associated with MIC is that the nature of the chemical species within the biomass could not be precisely determined. This may be due to one or more of the following reasons:

1. Limits of detection for the technique(s) employed (e.g. SEM Energy Dispersive X-ray Analysis cannot determine the valence state of an element).
2. The small quantity of corrosion products that were analyzed (such as those that are typically present in a pit),
3. Inadequate preservation of the biological and/or chemical integrity of the sample.

Determination of the precise chemistry of corrosion products in a biomass may help to determine the specific role of bacteria in a corrosion process.

XPS is a well established technique for the study of the surfaces of inert biological materials [12-15] as well as the surface of living cells [19-22]. In the present 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 Fe, Cr, Ni, Mo ions with

Desulfovibrio sp. in a modified Postgate's medium C was examined. The concentration and type of metal ions utilized represent those that might be present at a clean, passive (approximately 10^{-5} A/cm^2) stainless steel surface.[23] 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 thermo-mechanical 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[3]. 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, desulfoviridin positive, gram negative, vibrioid rod, produces acetic and propionic acids from lactic acid.

Medium. Modified Postgate's medium C consisting of (g/l): 1 NH_4Cl, 2.25 lactic acid; 0.06 $MgSO_4 \bullet 7H_2O$; 4.5 Na_2SO_4; 1 yeast extract ; 0.5 KH_2PO_4; 0.06 $CaCl_2 \bullet 2H_2O$; 0.004 $FeSO_4 \bullet 7H_2O$ and 1000 ml deionized water. (The modification consisted of the use of lactic acid instead of sodium lactate.) The medium contained 1800 μM sulfate and 900 μM lactic acid. The medium was pre-reduced, by boiling and purging with N_2, and dispensed in 60 ml serum bottles in an anaerobic glove box filled with 95% N_2 and 5% H_2, as described previously.[24] 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 ($FeCl_3 \bullet 6H_2O$), sodium molybdate ($Na_2MoO_4 \bullet 2H_2O$), chromic chloride ($CrCl_3 \bullet 6H_2O$) and nickelous chloride ($NiCl_2 \bullet 6H_2O$) 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 2. 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 2-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. (See Figure 1 and Tables 1 and 2 for results.) 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 (Marsh Co.) 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 minutes. 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 (Instrument Laboratories). Fe was analyzed by a colorometric method with ortho-phenanthroline and a Spectronic 20 (Bausch and Lomb). Organic acids were quantified by High Performance Liquid Chromatography (HPLC) (Spectra-Physics) with a UV/VIS detector and refractive index detectors (Shimadzu) as described previously.[25] Sulfate was determined spectrophotometrically by precipitation with barium chloride.[26] The pH was measured with a pH meter and combination electrode (Beckman).

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 V. G. Scientific ESCA 3 Mark II spectrometer controlled by a V. G. 1000 computer-based data acquisition system. Figure 2 schematically illustrates the X-ray Photoelectron Spectrometer at the Materials Science Department of the State University of New York at StonyBrook. An Al $K_{\alpha1,2}$ 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. Ultrahigh vacuum conditions (base pressure was 1 to 2 x 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.[27-32]

All XPS measurements were carried out at a high take-off angle (50°) measured with respect to the plane of the sample. 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 the signal-to-noise ratio. The metal and sulfur peaks were first identified and then separate narrow scan peaks were obtained. Details of the methodology for deconvolution have been previously reported.[33]

The ion bombardment operation that is typically done (with Krypton at 2 keV) to remove surface contaminants was found to reduce sulfur species. Contaminant 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 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 microbial growth, as indicated by acid production, sulfate reduction, and total gas production (Figure 1 and Table 1).

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, where possible, from the deconvoluted XPS spectra. Figure 3 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 iso-structural to sulfate, such as molybdate, are generally regarded as inhibitory to the growth of *Desulfovibrio sp.*[3] However, the molybdate concentration (0.2mM) that was used in this study, may have been too low to inhibit growth. The molybdate concentration utilized is representative of that which might be present at the surface of a clean, passive stainless steel (approximately 10^{-5} A/cm^2 current density).[23] Further work will demonstrate the critical concentration at which molybdate will affect the activity of sulfate reducing bacteria.

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.[34] 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 4. Unlike the sulfur spectra, the signal-to-noise ratios for the 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 one can deduce 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

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 microbial growth, as indicated by acid production, sulfate reduction and hydrogen sulfide gas pressure.
2. 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, acetic and propionic acids which may lower the pH at localized sites.
3. 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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REFERENCES

1. B. J. Little, P. A. Wagner, W. G. Characklis, and W. Lee, "Microbial Corrosion", in Biofilms, W. G. Characklis and K. C. Marshall (Eds.), Wiley Interscience, N. Y. 1990: pp. 635-670.
2. C. A. H. von Wolzogen and L. S. Van der Vlught, Water (The Hague) 18 (1934): p. 147.
3. J. R. Postgate The Sulfate Reducing Bacteria, 2nd Ed., Cambridge University Press, Cambridge, UK, 1984, p. 132.
4. M. B. McNeil and B. J. Little, 46, 7 (1990): p. 599.
5. D. D. MacDonald, B. Roberts and J. B. Hyne, Corrosion Science, 18 (1978): p. 411.
6. A. Mohagheni, D. M. Updegraff and M. B. Oldhaber, Geomicrobiology, 5 (1984): p. 153.
7. R. C. Newman, B. J. Webster and R. G. Kelly, ISIJ International, 31 (1991): p. 201.
8. P. Marcus and H. Talah, Corrosion Science, 29 (1989): p. 455.
9. J. S. Smith and J. D. A. Miller, Br. Corrosion J., 10 (1975): p. 136.

10. G. Schmidt, Corrosion, 47 (1991): p. 285.
11. R. C. Newman, H. S. Isaacs, B. Alman, Corrosion, 3 (1980): 8 p. 261.
12. Andrade, J. D. In "Surface and Interfacial Aspects of Biomedical. Polymers," Vol. 1, Chapter 5, Andrade, J. D., Ed. (Plenumm Press: New York, 1985), pp. 105-195.
13. Defosse, C. In Characterization of Heterogeneous Catalysts, Chapter 6, Delannay, F., Ed. (Marcel Dekker: New York, 1984), pp. 225-298.
14. Ratner, B. D., McElroy, B. J., In Spectroscopy in the Biomedical Sciences, Chapter 5, Gendreau, R. M., Ed. (CRC Press: Boca Raton, 1986), pp. 107-140.
15. Siegbahn, K., Phil. Trans. R Soc. London Ser A, 318 (1986): p. 3.
16. Baddiley, J., Hancock, I. C., Sherwood, P. M. A., Nature (London), 243 (1973): p. 43.
17. Meisenheimer, R. G., Fisher, J. W. Rehfeld, S., J. Biochem. Biophys. Res. Commun.68 (1976): p. 994.
18. Millard, M. M., Scherrer, R., Thomas, R S., Biochem. Biophys. Res. Commun. 72 (1976): p. 1209.
19. Magnusson, K. E., Johansson,L., Studia Biophys., 66, (1977): p. 145.
20. Millard, M. M., Bartholomew, J. C . Anal. Chem. 1977, 49): p. 1290.
21. Pickart, L., Millard, M. M., Beiderman, B., Thaler, M. M. Biochim. Biophys. Acta 1978, 544): p. 138
22. Van Haecht, J. L., Defosse, C., Vandenbogaert, R., Rouxhet, P., Colliods Surf. 1982, 4): p. 343.
23. Nash, B. K. and Kelly, R. G. "The Use of Ion Chromatography for the Study of Localized Corrosion," submitted to the J. Chromatography.
24. Francis, A. J. and Dodge, C. J., Applied and Environmental Microbiology, 54, (1988): p. 1009.
25. Francis, A. J. and Dodge, C. J., Gillow, J. B. and Cline, J. E., Radiochimica Acta, 52-53 (1991): p. 311.
26. Standard Methods for the Examination of Water and Wastewater, 14th ed., (American Public Health Assoc.-American Water Assoc., 1976), p. 496.
27. Briggs, D., Ed. Handbook of X-Ray and Ultraviolet Photoelectron Spectroscopy" (Heyden & Son, London, 1977).
28. Briggs, D., Seah, M. P., Eds. Practical Surface Analysis by Auger and X-Ray Photoelectron Spectroscopy, 2nd. ed, (John Wiley,: Chichester 1990).
29. Brundle, C. R., Baker, A. D., Eds. Electron Spectroscopy: Theory, Techniques and Applications, , Vol. 1, (Academic Press: New York, 1977).
30. Carlson, T A., Photoelectron and Auger Spectroscopy, (Plenum Press: New York, 1975).
31. Nefedov, V. I. , X-Ray Photoelectron Spectroscopy of Solid Surfaces, (VSP/BV: Utrecht, 1988).
32. Siegbahn, K., Nordling, C., Fahlman, A., Nordberg, R, Hamrin, K., Hedman, J., Johansson, G., Bergmark, T., Karlsson, S. E., Lindgren, I., Lindberg, B., ESCA: Atomic, Molecular and Solid State Structure Studies by Means of Electron Spectroscopy, Ser. IV, Vol. 20, (Nova Acta Regiae Soc. Sci.; Uppsaliensis Almqvist & Wiksells Boktryckeri AB: Uppsala, 1967).
33. Halada, G. P. and Clayton, C. R., J. Electrochemical Soc., 138, (1991); p. 2921
34. Cotton, F. A. and Wilkinson, G., Advanced Inorganic Chemistry, 5th ed., J. Wiley and Sons, Inc.: New York, 1980), p. 810.

TABLE 1—Effect of Metal Ions on Sulfate Reducing Bacteria Activity

Treatment	pH	Gas Produced (ml)	Sulfate Reduced (%)
No metal added Uninoculated (Control)	7.46 ±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	6.86 ± 0.01	6.8	39
Nickel Inoculated	7.10 ± 0.01	6.8	24

TABLE 2—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		None	Yellow[1]
	Inoculated	0.18 ± 0.02	90	None	Clear
Ferric	Control	0.37 ± 0.01		None	Yellow
	Inoculated	0.013 ± 0.007	3	None	Clear
Molybdate	Control	0.47 ± 0.01		None	Yellow
	Inoculated	<0.01	<1	Red-Brown	Clear
Nickelous	Control	0.51 ± 0.02		None	Yellow
	Inoculated	0.02 ± 0.01	4	None	Clear

[1] Yellow color is due to media ingredients peptone and yeast.

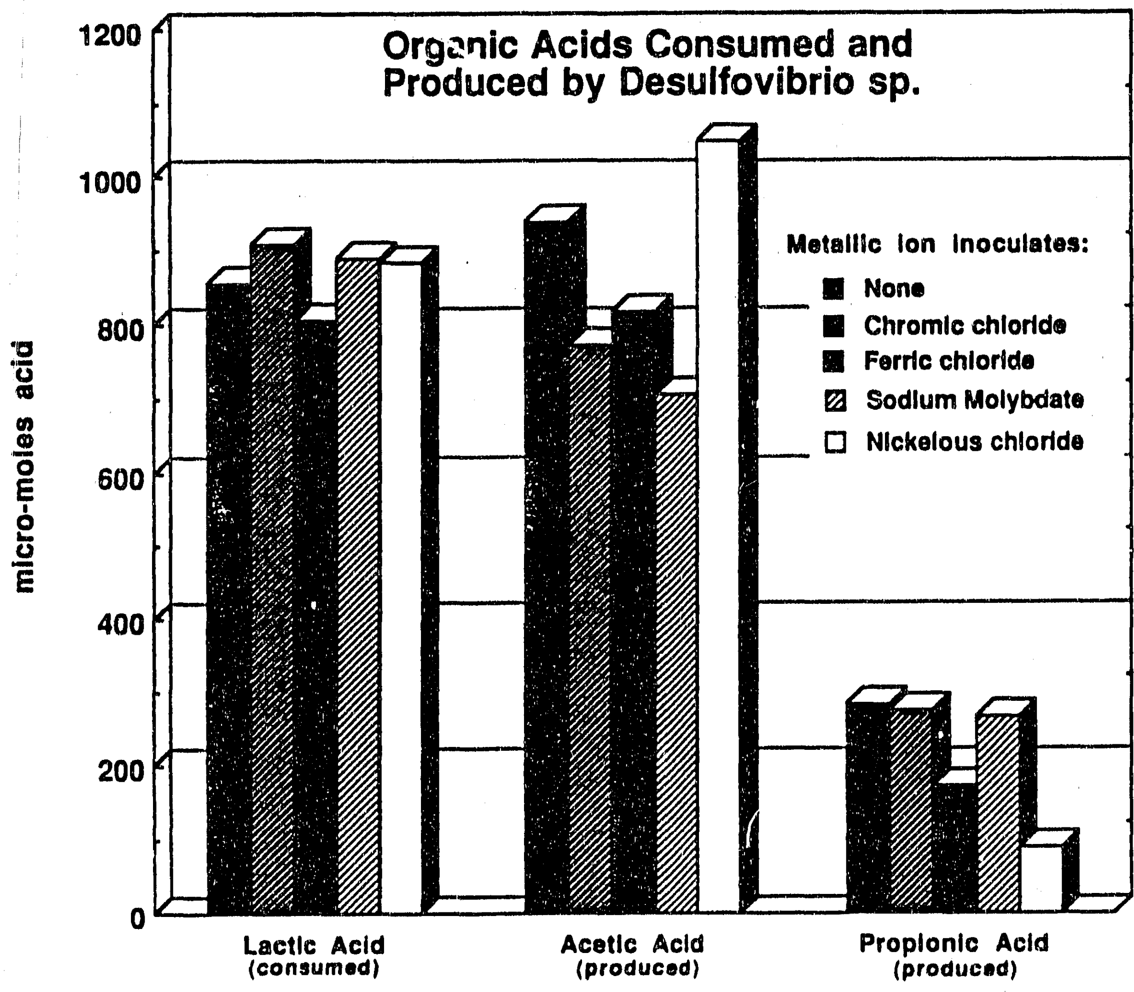

Figure 1—Organic acids consumed (lactic) and produced (acetic and propionic) by *Desulfovibrio sp.* as determined by HPLC.

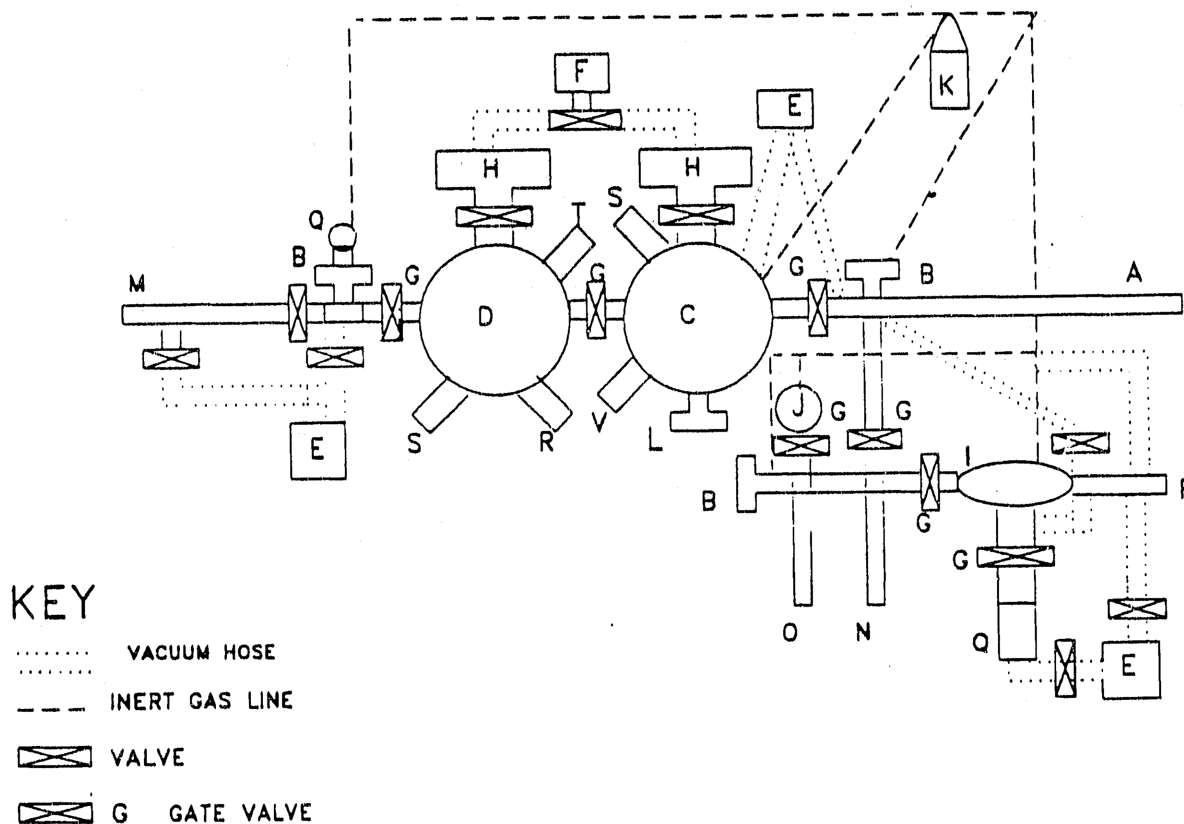

Figure 2—Schematic representation of an X-ray photoelectron spectrometer. Key: (A) Magnetically coupled probe, (B) Rapid entry port, (C) XPS/AES analysis chamber 1, (D) XPS/AES analysis chamber 2, (E) Roughing pump, (F) Backing pump, (G) Gate valve, (H) Vapor diffusion pump, (I) Surface processing chamber, (J) In-Situ electrochemical cell, (K) Inert gas tank, (L) Titanium sublimation pump, (M) Probe with heating and liquid nitrogen cooling capabilities, (N) Transfer assembly, (O) Transfer assembly, (P) Magnetically coupled probe, (Q) Turbo molecular pump.

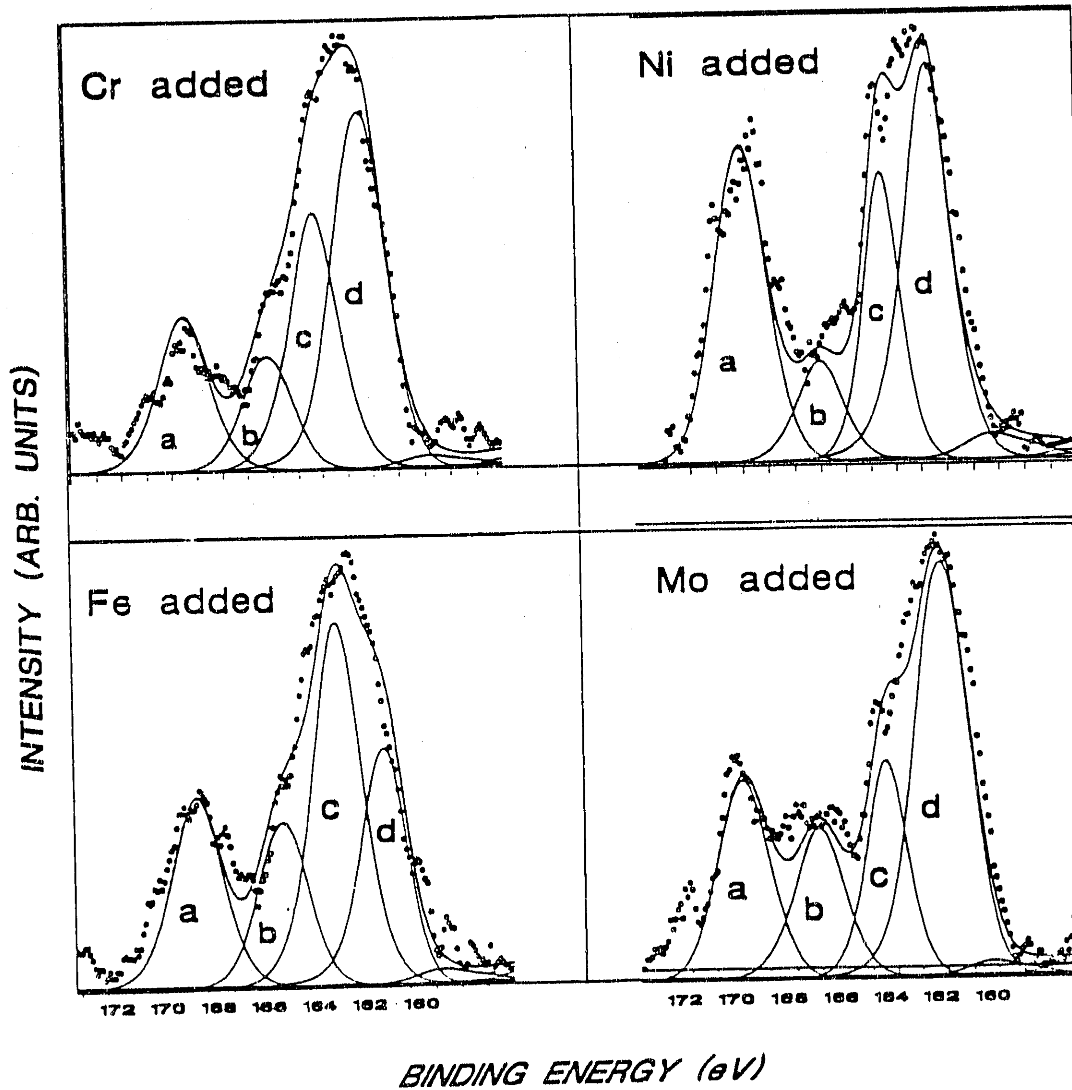

Figure 3—XPS spectra of Sulfur (S 2p) for biomasses consisting of *Desulfovibrio sp.* in Postgate's Medium C inoculated with Cr, Ni, Fe and Mo metal ion species. Species indicated: (a) SO_4^{2-}, (b) SO_3^-, (c) S, (d) S^{2-}.

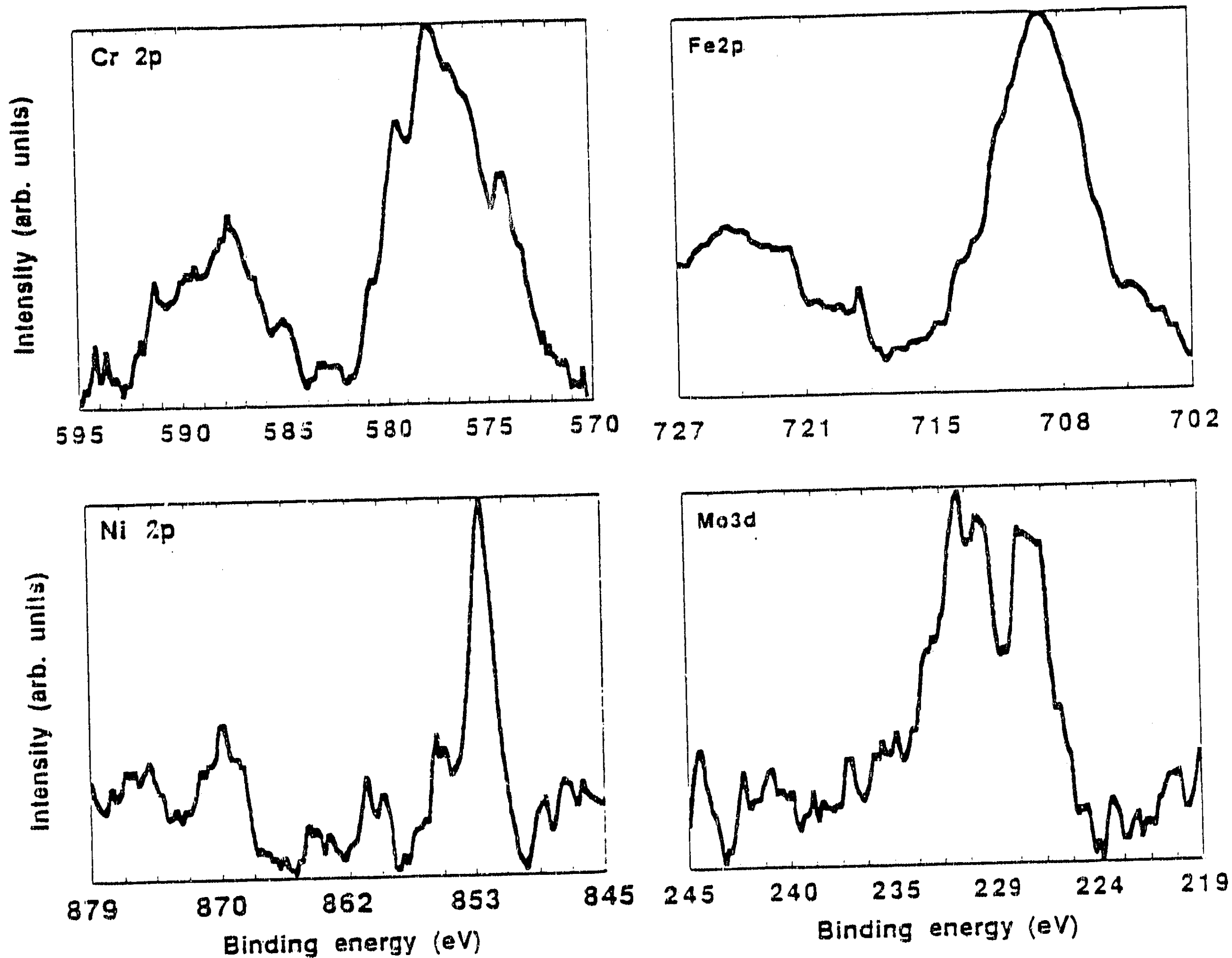

Figure 4—XPS spectra of metals (Cr 2p, Ni 2p, Fe 2p, and Mo 3d) for bio-masses consisting of *Desulfovibrio sp.* in Postgate's Medium C inoculated with Cr, Ni, Fe and Mo metal ion species.

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