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March 1995

**Presented at the International Conference and Corrosion Meeting for
the National Association of Corrosion Engineers (NACE)**

Miami, FL
March 27-31, 1995

Under Contract No. DE-AC02-76CH00016 with the U.S. Department of Energy.

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ABSTRACT

A Scanning Auger Microprobe analysis was performed on the corrosion products of an austenitic AISI type 304 ss after a potentiostatic polarization of one volt for ten minutes in a modified Postgate's C media containing sulfate reducing bacteria. The corrosion products were characterized and mapped in local regions where pitting was observed. A critical evaluation of the applicability of this technique for the examination of microbially influenced corrosion (MIC) is presented.

Key words: microbially influenced corrosion (MIC), sulfate reducing bacteria (SRB), auger electron spectroscopy (AES), scanning auger microprobe (SAM), stainless steel, potentiodynamic polarization, potentiostatic polarization.

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INTRODUCTION

In addition to being one of the oldest bacterial life forms on earth,¹ sulfate reducing bacteria (SRB) are commonly implicated in the localized corrosion of stainless steels.^{2,3} Given their strictly anaerobic nature the ubiquity of SRB is astonishing. Incorporation of SRB within a biofilm containing a plethora of microbial species presents a system of great complexity for scientific study. Therefore, a simplified system containing only SRB is examined in this study.

An analytical technique capable of characterizing heterogeneous surfaces of microbially active systems is of great value. Although a few attempts at utilizing Auger Electron Spectroscopy (AES) for analysis of microbially influenced corrosion (MIC) have been reported,^{4,5} the potential of this surface analytical technique has yet to be fully exploited. Presently, the corrosion scientist does not possess a satisfactory model revealing the relationship between SRB interactions and a corroding metal surface. As illustrated by several researchers,^{6,7,8,9} an important step toward developing an understanding of MIC would undoubtedly include a relationship between: 1) metal surface conditions, 2) near surface aqueous regions and 3) a microbial sphere of influence⁽²⁾. Traditional scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) have been utilized to characterize surface heterogeneities in biologically active specimens. However, these techniques require an elaborate preparation procedure for microbiological specimens that can alter the metal/biofilm interface.¹⁰ These procedures commonly include: 1) sample fixation, 2) dehydration and 3) application of a thin conducting coating. AES experiments require only a dehydration in either air or an inert atmosphere and liquid nitrogen cooling. No fixation is required and a conductive coating will obscure surface analysis. Insulating regions will experience an increase in the surface concentration of electrons termed, 'charging'. Charging difficulties are a major concern in AES experiments. Spectral analysis is therefore performed only in local conducting regions which must be present if corrosion is to occur. AES is a surface analytical technique where data is accumulated from the outermost layers which are nanometers from the surface. Whereas, EDS probes the outermost layers which are one micron from the surface. A summary of the advantages and limitations of AES when applied to biologically active systems is given in Table 1.

EXPERIMENTAL

Materials

Type 304 ss strips cut into 10 x 10 x 1 mm coupons were annealed at 1080°C for 1 hour in a pre-evacuated quartz vessel followed by water quenching. After heat treatment the samples were abraded, polished to a final surface finish of six microns and ultrasonically degreased in acetone. Enough samples were prepared for a triplicate analyses.

Microbiological Culture

The culture used in this study was *Desulfovibrio desulfuricans* (ATCC 7757): a vibrioid rod, desulfovibridin positive and gram negative bacterium. The bacteria produce propionic and acetic acid

(2) The microbial sphere of influence is introduced so as not to discount any possible effects due to bacterial exopolymers or respiratory products as well as any direct interaction between microbial cells and/or enzymes.

when exposed to lactic acid. Cultures were maintained in a modified Postgate's C medium¹¹ consisting of (g/l): 0.50 KH_2PO_4 , 1.00 NH_4Cl , 0.06 $\text{CaCl}_2 \bullet 2\text{H}_2\text{O}$, 0.06 $\text{MgSO}_4 \bullet 7\text{H}_2\text{O}$, 0.002 $\text{FeSO}_4 \bullet 7\text{H}_2\text{O}$, 2.26 NaSO_4 , 0.30 Na-citrate, 1.00 yeast extract, 3 ml 80% lactic acid and deionized water added to one liter. All ingredients except lactic acid were added and then the medium was deaerated by boiling and purging with high purity nitrogen for 20 minutes. The cooled medium was placed in a nitrogen atmosphere glove box and lactic acid was added. NaOH (Approximately 1.3 g/l) was used to adjust the pH to 8.2. 150 ml of media was then dispensed into 250 ml serum bottles and autoclaved at 121°C and 20 psi for 20 minutes. After autoclaving and cooling the pH was checked and confirmed to be 7.2. A 5% (v/v) inoculum was added to the medium and allowed to incubate for 3 days at 30°C.

Electrochemical Polarizations

Polarization was conducted using a EG&G Versastat and 352 data acquisition software⁽³⁾ interfaced to a 1 liter Greene cell (as described in ASTM G5 standard) and a saturated calomel electrode (SCE) as a reference. The polished samples were mounted with a quick drying epoxy as described elsewhere.¹² The entire experimental apparatus was placed in a glove bag containing pre-purified nitrogen. Next the medium was poured into the electrochemical cell. The type 304 ss sample electrode was sterilized with ethanol, rinsed with deaerated deionized water and immediately placed into the cell. Once in the cell, the open circuit potential was allowed to stabilize for one half hour then potentiostatically polarized to 1 volt for 10 minutes. After polarization the samples were dried in the nitrogen filled glove bag then removed from the Plexiglas mounting block and stored in the laboratory until analysis.

Optical Microscopy

An OLYMPUS BH2 system microscope⁽⁴⁾ was used after potentiostatic polarization to view the sample surface. This proved to be a very useful technique to locate areas of interest in the SEM with greater efficiency, thus, saving valuable filament time and reducing the sample electron dosage.

SEM, AES and SAM

All AES analyses were completed in a customized ultra-high vacuum system with a V. G. CLAM 2 analyzer⁽⁵⁾ and a LEG 1000 electron gun⁽⁵⁾ slaved to a VGX900I data acquisition system⁽⁵⁾ operated in an integrated spectrum mode. The entrance and exit slit widths of the analyzer were set to 4 mm. The electron gun was operated at 10 kV and a target current of 3 nA, producing a spot size less than 0.5 μm . Samples were mounted onto a sample stub and loaded into the entry chamber equipped with a roughing and turbo pump. After roughing, the turbo pump was engaged and when the chamber pressure reached 1×10^{-4} Torr (1.3×10^{-2} Pa), the sample was cooled by direct contact with a copper block at liquid nitrogen temperature. All biological samples must be cooled to prevent evaporation, sublimation and desorption within the Ultra-High Vacuum (UHV) environment. Freezing to liquid nitrogen temperatures has not been observed to alter the sample surface drastically, although some cracking of surface features has been observed, none of these regions were analyzed in this study. Surface cracking was confirmed by optical examination of the sample before and after AES

(3) EG&G Parc, Princeton, NJ.

(4) OLYMPUS, Lake Success, NY.

(5) FISON Instruments, Uckfield, East Sussex, UK.

analysis. Once cooled, the sample was transferred into an ion pumped preparation chamber with a base vacuum pressure less than 1×10^{-9} Torr (1.3×10^{-7} Pa). The sample remained in this chamber for 15 minutes to confirm proper vacuum compatibility and cooling. Next the sample was transferred into the analysis chamber with a vacuum pressure less than 5×10^{-10} Torr (6.7×10^{-8} Pa) and a liquid nitrogen cooled V.G. HPT 105⁽⁵⁾ manipulator. The sample temperature was continuously monitored and maintained at -165°C during analysis.

Initially the sample was examined in an SEM mode. The target current was maintained below 10 nA to reduce sample charging difficulties, often rendering this technique unsuccessful for biologically active systems, (typical target currents are several hundred nA). The target current was accurately measured by placing the electron beam directly onto a gold plated clip used for sample mounting. Once a candidate region was located the magnification was increased to encompass the entire area while simultaneously monitoring the sample target current. An increase in target current was indicative of an area possessing a higher conductivity than the surroundings.

The most important and challenging aspects of implementing AES to the study of MIC is locating a suitable area for analysis. These regions must be conductive since sample charging will not permit an accurate determination of electron energies. Sample charging is observed in AES spectra in two ways: 1) peaks in the auger spectra shift as a result of charge build up on a sample surface leading to changes in the work function of an electron escaping from the sample surface and 2) false peaks will be seen as a result of a localized insulating regions charging and discharging thus emitting electrons with a broad range of energy that dominate the total spectra. Once a suitable region is located, data is acquired and the peaks are then smoothed and differentiated using a method introduced by Savitsky and Golay.¹³

SAM was performed in regions yielding acceptable AES data to gain a better understanding of the elemental surface distribution. The data was acquired in a peak minus background mode where each pixel of the digitized image is scanned twice for peak and background intensity determinations. The difference is plotted as a gray scale value, thus generating the image known as a chemical map.

RESULTS AND DISCUSSION

Electrochemical Polarizations

Figure 1 illustrates typical E-log I plots for type 304 ss in abiotic medium and inoculated medium. The plot of type 304 ss in abiotic medium has an open circuit potential of -320 mV (SCE), a well defined passive range and a transpassive region beginning at approximately 500 mV(SCE). The plot of type 304 ss in inoculated medium has an open circuit potential of -440 mV (SCE), a more distinct active passive region and pitting was initiated at 260 mV (SCE). An analysis based on approximations using Pourbaix diagrams¹⁴ for chromium indicates a transpassive region beginning at 500 mV (SCE). When exposed to inoculated medium the passive region becomes more active as illustrated by the increase in current density of approximately two orders of magnitude at -200 mV and pit initiation begins at 260 mV (SCE).

Optical Microscopy

A typical optical micrograph of type 304 ss after potentiostatic polarizations in abiotic medium is shown in Figure 2. The surface is relatively clean with no pits observed on a micron scale.

A typical optical micrograph of type 304 ss after potentiostatic polarization in an inoculated media is shown in Figure 3. There are large pits exceeding 100 μm in diameter on the surface. Note the presence of deposits that appear to be the characteristic black sulfides commonly associated with SRB within the pits. These are more easily seen in Figure 4 which displays the large pit seen in the center of Figure 3 with a variation in the depth of focus of 2 μm . A comparison of the two figures clearly indicates the high degree of roughness within the pitted region and that the black deposits exist as protrusions located within the pits.

SEM, AES and SAM

Auger electron emission and X-ray fluorescence are competing relaxation processes occurring in atoms of an excited state. Auger transitions have a greater probability of occurrence for lighter elements (The crossover point occurs at atomic number 32, Ge).¹⁵ The increased sensitivity, in comparison to EDS, of lighter elements makes AES an attractive technique to characterize biologically active systems containing a higher concentrations of carbon, oxygen, nitrogen, sulfur, sodium, chlorine and phosphorus.

The relative Auger sensitivity for all elements below Zr ($Z = 41$) is a function of the incident electron beam energy with 3 keV yielding a higher sensitivity than 10 keV.¹⁶ An incident electron beam energy of 10 keV was used to improve the electron gun operating conditions which outweighed sensitivity loss.

Figure 5 is an SEM micrograph of the large pit as seen in Figure 3. The large depth of field associated with the SEM micrograph permits a simultaneous examination of a greater number of surface features than the optical micrographs. Meanwhile, the smaller depth of field associated with optical microscopy permits a more accurate evaluation of surface roughness. The quality of the SEM image (Figure 5) is reduced by the use of low sample currents to avoid localized radiation damage and sample heating capable of altering the physical and chemical morphology. Tolerable doses are 10^3 C cm^{-2} for biopolymers and up to 10^2 C cm^{-2} for SiO_2 decomposition.¹⁷ Sample cooling will raise the tolerable electron doses. For the parameters used during data acquisition, i.e. smallest rastered area of $160 \mu\text{m}^2$ and a 3 nA target current, the electron dose is calculated to be $1.9 \times 10^3 \text{ C cm}^{-2}$. This was the highest electron dose the sample received. In SEM mode the sample was exposed to a higher target current, about 10 nA, but the rastered area was much larger thus yielding an even smaller dose. The acceptable doses combined with the liquid nitrogen cooling used in each experiment should not have altered the sample in any way. This was experimentally confirmed by both optical observation and the lack of changes in AES spectra as a function of time.

The numbered points in each micrograph represent the center of a square area rastered during analysis. The differentiated spectra are seen in Figure 6. To date, a complete set of standards needed to accurately determine the precise speciation of the elements has not been compiled. However, it has been shown that oxidation causes core-core-core (ccc) shifts of negative kinetic energy.¹⁸ Area 3 was recorded at an electron take-off angle of $80^\circ \pm 1^\circ$ whereas areas 1 and 2 were recorded at an arbitrary

angle yielding the greatest signal to noise ratio. A precise determination of the electron take-off angle is not known since the rough regions within the pit do not permit a straight forward determination based on the sample positioning. Theoretically, an average electron take-off angle can be calculated if one assumes the analyzed regions to be homogeneous and then takes the average inclination of each spot analyzed.

The spectra from area 1 indicates the presence of sulfur, chlorine, carbon, oxygen, iron and a trace quantity of phosphorus. The presence of phosphorus is confirmed by a more distinguished peak in integrated spectrum. The spectra from area 2 is indicative of sulfur, chlorine, carbon, oxygen but no evidence of iron. It should be noted that each peak in this spectrum is broader than the corresponding peak in spectrum 1 and 3. It is believed that this is due to a slight degree of sample charging. Supporting arguments for this interpretation are: 1) increased brightness in the SEM image, 2) lower observed target current and 3) broad spectra. Obviously, an effect such as this would decrease the value associated with a species identification due to chemical shifts. Nonetheless, it is believed that these spectra still provides accurate elemental identification. The spectra from area 3 indicates the presence of sulfur, chlorine, carbon, oxygen and iron. Immediately obvious is the reduction in the amount of sulfur present. A comparison of the iron peaks from areas 1 and 3 also reveals differences. Specifically, area 1 contains a greater proportion of multiple species as indicated from a shift generating peaks at approximately 720, 615 and 580 eV, although a decisive identification of the precise speciation is not available at this point. Also of interest is the lack of any nickel species. This would be seen by a peak in the differentiated spectra at approximately 848 eV. XPS analysis of samples undergoing similar microbiological exposures have revealed the presence of NiS.¹²

AES spectra from the surface of type 304 ss in abiotic medium, area 4, indicates the presence of carbon, sulfur, oxygen and trace quantities of iron. The presence of iron is confirmed by a set of peaks more easily seen in the integrated spectra. The dominance in the AES spectra of carbon and sulfur is consistent with the formation of an organic layer in which sulfur is either chemically or physically bound. A complete set of standards would provide a more decisive explanation, however, it is likely that the surface is composed of an organic film arising from lactic acid and yeast extract in which sulfate is physically bound.

Figure 7a is a chemical map of sulfur in the pitted region. The areas of lowest sulfur concentration coincide with bright regions as seen in Figure 5. The differentiated spectra in Figure 6a indicates a lower concentration of sulfur in area 3 than that of 1 or 2. However, the chemical map does not illustrate such deviations. This is believed to be due to sample roughness effects producing an unfavorable electron take-off angle for signals arising from sulfur and iron but not carbon and oxygen. An AES signal arising from a homogeneous surface layer of only a few atomic diameters will not possess this angular dependence. The chemical map was performed at an electron take-off angle of $80^{\circ} \pm 1^{\circ}$ yielding a high Auger electron signal in regions away from the pit. This region was polished to a planar surface as opposed to the rough region within the pit which presented few local areas of favorable electron take-off angle. The dark areas corresponding to low sulfur concentrations could be explained by shadowing effects resulting from the high surface roughness. There are two justifications: 1) as seen in Figure 4 and with much greater clarity directly on the microscope, protrusions within the pit are normal to the sample surface and the high electron take-off angle (80°) should eliminate most shadowing and 2) shadows produced by roughness are expected to appear in each chemical map and this is not evident in chemical maps for carbon and oxygen. Figure 7b is a chemical map of iron in the pitted region illustrating areas of lower concentration within the pit, but

not corresponding directly to the same regions of low sulfur concentration. Figures 7 c and d are chemical maps of carbon and oxygen, respectively. It is interesting to note the uniform distribution of each element on the sample surface. This is the result of: 1) incomplete rinsing after electrochemical polarization, 2) surface contamination during vacuum entry procedures (i.e. cooling in entry chamber) and 3) atmospheric exposure during storage.

CONCLUSIONS

1. AES is a useful surface analytical technique for examining metallic surfaces after exposure to SRB especially when implemented in conjunction with optical and electron microscopy as well as other surface analytical techniques such as XPS.
2. Chemical maps obtained in SAM mode are valuable in determining the elemental surface distribution but caution must be used when interpreting the results of a rough area.
3. Deposits formed when type 304 ss was potentiostatically polarized to one volt for ten minutes in medium containing SRB (*Desulfovibrio desulfuricans*) contained iron, sulfur, carbon, oxygen, chlorine, and trace quantities of phosphorus. No evidence of nickel or chromium was detected.
4. Potentiodynamic polarization studies of type 304 stainless steel in the presence of SRB (*Desulfovibrio desulfuricans*) began pitting at 260 mV (SCE) and possess a more distinct active passive transition than the control in abiotic medium.

ACKNOWLEDGMENTS

This work was sponsored by the Office of Naval Research (Dr. A. J. Sedricks contract officer) under contract number N0001485k0437. Type 304 ss was donated by Allegheny Ludlum Corporation. The authors wish to thank Dr. Gary Halada for many useful technical discussions.

REFERENCES

1. N. Pfennig, F. Widdel and H. G. Troupers, The Dissimilatory Sulfate-Reducing Bacteria, P. 926-940. In M. P. Starr, H. Stolp, H. C. Truper, A. Balows and H. G. Schlegel (editors) The Prokaryotes, Vol 1, Springer-Verlag, Heidelberg.
2. R. E. Tatnall, Crevice Corrosion of Stainless Steel in Recirculating Cooling Water, CORROSION/81 paper no. 130.
3. C. Ringas and F.P. A. Robinson, CORROSION vol. 44, No., 6 (1988) 386-395.
4. Jiann-Ruey CHEN, San-Der CHYOU, Shwa-Ing LEW, Chen-Jen HUANG, Chien-Shyong FANG and Wan-Sun TSE, Applied Surface Science 33/34 (1988) 212-219.
5. J. G. Jolley, G. G. Geesey, M. R. Hankins, R. B. Wright and P. L. Wichlacz.
6. B. Little, P. Wagner, F. Mansfeld, Electrochimica Acta, Vol. 37, No. 12 (1992) 2185-2194.

7. C. R. Clayton, G. P. Halada, J. R. Kearns, J. B. Gillow A. J. Francis, Microbiologically Influenced Corrosion Testing, ASTM STP 1232, J. R. Kearns and B Little Eds., American Society for Testing and Materials (1994) 141-152.
8. R. A. King and J. D. A. Miller, Nature. Vol. 233 (1971) 491-492.
9. R. C. Newman, B. J. Webster and R. G. Kelly, ISIJ International, Vol. 31, No. 2 (1991) 201-209.
10. B. Little, P. Wagner, R. Ray, R. Pope and R. Scheetz, Journal of Industrial Microbiology, (1991) 213.
11. J. R. Postgate, The sulfate-Reducing Bacteria, Cambridge University (1984) 32.
12. G. Chen, C. R. Clayton, R. A. Sadowski, J. R. Kearns, J. B. Gillow and A. J. Francis, These Proceedings, No 95217.
13. A. Savitsky and M. J. E. Golay, Anal. Chem. 36, (1964) 1627.
14. M. Pourbaix, Atlas of Electrochemical Equilibria in aqueous solutions, Pergamon Press Ltd. (1966).
15. D. Briggs and M. P. Seah, Practical Surface Analysis, (1983) 93.
16. L. E. Davis, N. C. MacDonald, P. W. Palmberg, G. E. Riach and R. E. Weber, Handbook of Auger Electron Spectroscopy, Second edition, Physical Electronics Industries, Inc. (1976) 13-15.
17. L. Frank Vacuum, Vol. 42 No 1-2, (1991) 147-150.
18. H. H. Madden, J. Vc. Sci. Technol., 18(3) April 1981, 677.

Table 1- Advantages and limitations of AES to the Study of Biologically active systems.

Advantages

- Sensitivity to all elements except H and He
- Provides data on speciation of all elements present
- Straightforward method of data analysis
- Planar Mapping of the elements present
- Atomic detection limit as low as between 0.02 and .2 atomic percent

Limitations

- All analyses take place in a UHV environment; hence, samples must be dry and biological samples must be liquid nitrogen cooled to prevent desorption or decomposition
 - Analysis requires conducting samples
 - Complex analytical system
 - Chemical mapping is time consuming and sensitive to angular variances
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Figure 1 - E Log I plot of type 304 ss in abiotic medium and inoculated medium.

Figure 2 - Optical micrograph of type 304 ss after a potentiostatic polarization of 1 volt for 10 minutes in abiotic medium. Numeral 4 corresponds to the analyzed region.

Figure 3 - Optical micrograph of type 304 ss after a potentiostatic polarization of 1 volt for 10 minutes in inoculated medium. Numerals 1, 2 and 3 correspond to analyzed regions.

(a)

(b)

Figure 4 (a) and (b) - The large pit as seen in the center of Figure 3 at higher magnification and a variation in focal depth of 2 microns.

Figure 5 - SEM image of the pitted region viewed in Figure 4.

Figure 6 (a)-(d) - Differentiated AES spectra. Numerals 1, 2, 3 and 4 correspond to analyzed regions.

(a)

(b)

(c)

(d)

Figure 7 (a)-(d) - SAM chemical maps of the region displayed in Figure 3. (a) Sulfur chemical map, (b) Iron chemical map, (c) Carbon chemical map and (d) Oxygen chemical map.