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# MICROBIOLOGICAL AND CORROSION ANALYSIS OF THREE URINE PRETREATMENT REGIMES WITH TITANIUM 6A1-4V

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Interim Report



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One objective of the water recovery test (WRT) performed at NASA's Marshall Space Flight Center (MSFC) for the environmental control and life support systems (ECLSS) of Space Station *Freedom* is to determine the ability of the water recovery system to reclaim urine for crew reuse. In the process, raw urine is pretreated using a commercially available oxidant, Oxone (Dupont), and sulfuric acid (to reduce ammonia), and pumped into a urine processing subsystem. A combination of sodium hypochlorite and sulfuric acid has also been considered as an alternative pretreatment.

This study examined the ability of these pretreatments, plus a third pretreatment of ozone, to reduce microbial levels in urine generated during testing of the water recovery system at MSFC. In addition, the corrosion rate of weld and base metal specimens of titanium 6A1-4V, a candidate material for the water system of Space Station *Freedom*, was monitored in the presence of these pretreatments. Specimen surfaces were examined at completion of the 21-day test using scanning electron microscopy. Changes in pH, color, turbidity, and odor were recorded over the course of the test.

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## INTRODUCTION

The Vapor Compression Distillation (VCD) subsystem, used in the Water Recovery Test (WRT) being performed at NASA/MSFC for the Environmental Control and Life Support System (ECLSS) of Space Station Freedom, is intended to process urine for crew reuse. In the process, raw urine is pretreated, using a commercially available oxidant, Oxone (Dupont), and sulfuric acid (to reduce ammonia), and pumped into the subsystem. Recently, a combination of sodium hypochlorite and sulfuric acid has been considered as an alternative pretreatment.

In a previous study (1), the biological and corrosive effects of these pretreatment chemicals and a third pretreatment, consisting of ozone and sulfuric acid, were examined using 316L stainless steel, a candidate material for the water recovery system of Space Station Freedom. Both welded and base metal specimens were used. In this study, a second candidate material, titanium (Ti-6Al-4V), was examined under the same conditions used previously.

## MATERIALS AND METHODS

Approximately 8 liters of raw urine was collected from the Water Recovery Test at the Marshall Space Flight Center. Aliquots of 1600 ml were distributed into four polypropylene containers. The first container was treated with Oxone and sulfuric acid, the second with sodium hypochlorite and sulfuric acid, and the third with sulfuric acid only (in preparation for the addition of ozone). The fourth container received no treatment and served as a control. Table 1 lists the concentrations of treatment reagents used. Each container was inoculated with two microorganisms previously recovered from the VCD subsystem, a spore-forming bacterium, Bacillus insolitus, and a filamentous mold. Approximately $10^7$ bacteria and fungal spores were added to each container. After mixing, two 800 ml volumes were removed from each container and added to two glass corrosion cells (EG&G PARC), one of which contained a titanium base metal coupon, and the other a welded counterpart, giving a total of eight corrosion cells.

All coupons were pre-cleaned using trichloroethylene and absolute ethyl alcohol, aseptically fitted into nylon coupon holders (EG&G PARC), and inserted into the corrosion cells. In addition, each corrosion cell contained a needle sample port (incorporated to reduce extraneous microbial contamination during sampling), a reference electrode (EG&G PARC), a reference electrode bridge tube (EG&G PARC) and a working electrode (EG&G PARC). All components of the corrosion cell

were sterilized by autoclaving prior to use except the bridge tube, which contained a filter-sterilized solution of 0.1M sodium chloride. A 0.2um filter was attached to the working electrode body to prevent airborne contamination of the solution during the test.

Ozone was produced by the flow of compressed oxygen through an ultraviolet light source (185 nm). An ozone concentration in air of approximately 45 mg/hr was measured using Drager tubes and an oxygen flow rate of 0.5 standard cubic foot per hour. The ozone produced was split and bubbled into each acid-treated sample using a fritted glass sparger (EG&G PARC). Residual ozone concentrations were measured by the iodometric method (2), which is less accurate than the indigo dye method, but less susceptible to the colorimetric interferences present in these samples.

All corrosion cells were continuously mixed on a stirring plate using a teflon stirring bar. Corrosion rates, pH measurements, macroscopic observations (color,turbidity and odor) and microbiological analysis of the solutions and surfaces (SEM) were recorded. Corrosion rates were measured over a three week period by the DC polarization resistance technique using an EG&G Model 350A corrosion measurement device. The program POLCUR was used to calculate corrosion rates. In addition, average corrosion rates (avg. CR), expressed in mils per year (mpy), were calculated at the end of 144 and 480 hours of testing using the following formula (3):

$$\text{avg.CR(mpy)} = 0.1288 \times \text{Icorr} \times \frac{\text{Eq. wt.}}{\text{metal dens.}}$$

where Icorr equals the average of the measured corrosion current densities, Eq. wt. is the metal equivalent weight, and the denominator is the metal density.

Standard pH paper (0.5 increments) was used to determine pH values. Changes in color and turbidity were documented by photographic records.

Microbiological analysis of the solutions was performed over three weeks by collecting approximately 2 ml of liquid at each sample port using sterile evacuated tubes. The solution was diluted as necessary in sterile phosphate buffered saline (PBS), and spread plated onto R2A agar. The plates were incubated at 28C for 5 days. After enumeration, bacterial colonies exhibiting different colonial morphologies were subcultured to brain heart infusion agar in preparation for identification. The Minitek Identification System (BBL) and Biolog Identification System (Biolog) were employed for identification of bacterial isolates.

For scanning electron microscopy (SEM), base and welded metal specimens were aseptically removed from the coupon

holders at the end of the test, and were placed in sterile formaldehyde (3.7%) for 2 minutes. Coupons were then dried in absolute ethyl alcohol and freon for 2 minutes each. Fixed samples were sputter-coated with Au-Pb at 30-35mA for 30 seconds. Coupons were observed under a scanning electron microscope (Hitachi) with a field emission source (Quantum).

## RESULTS

### Macroscopic Observations

Photographs of corrosion cell solutions taken on day 1, day 4 and day 21 are shown in Figure 1a-c, respectively. By day 4, the ozonated solutions were colorless with a white, flocculent precipitate that remained throughout the test. The sodium hypochlorite solutions were brown in color at day 1, cleared somewhat by day 4, and eventually returned to the dark color seen initially. The turbidity increased in these samples after day 4. Oxone and control solutions remained light brown in color throughout the course of the test, with an increase in turbidity noted only in the control samples.

### Corrosion Current Measurements

Curves showing the variation of corrosion currents with time over the 3-week period are shown in Figures 2a-d. In each curve, a comparison of the corrosion currents for base and welded specimens was made. Corrosion currents were generally comparable for base and welded specimens except for Oxone treated and control coupons, where the currents were lower for the welded coupons. However, all corrosion currents remained quite low, with no significant differences observed between samples. Average corrosion rates for the first 6 days of exposure and for the entire 3-week period are presented in Table 2. The average percent change in corrosion rates of the 3-week period from those of the 6 day period is included. Average corrosion rates for the welded control and welded Oxone treated coupons were smaller than for their respective base metal counterparts. Average corrosion rates of base and welded coupons were comparable in both ozone and hypochlorite pretreatments. However, all corrosion rates were extremely low, with an average percent increase for the 3-week period over that for the first 6-day period of 18.6 percent, which is considered insignificant.

### Microbiological/Scanning Electron Microscopy Analysis

#### Sodium hypochlorite-sulfuric acid pretreatment

Figures 3a and 3b show that microbial numbers were higher in the weld and base metal hypochlorite solutions than for the other pretreatments during the course of the test, and coincided closely with levels seen in controls. Initial microbial levels were approximately $6 \times 10^7$ colony forming

units per milliliter (cfu/ml) for both corrosion cells, with numbers gradually increasing during the first 120 hours. One species, Escherichia coli, was isolated during this time. After approximately 200 hours, the samples had become much more turbid, and showed an order of magnitude increase in microbial numbers over counts at 120 hours. These were the highest counts obtained for any sample during the course of the test.

After 200 hours, two rod-shaped opportunistic pathogens of the family Enterobacteriaceae, Escherichia coli and Proteus mirabilis, were isolated. An increase in pH from 5.5 to 8.5 was probably due to the presence of P. mirabilis which hydrolyzes urea present in urine, producing ammonia. This organism was isolated with each saample for the remainder of the test. E. coli was not isolated after 120 hours. Its absence may have represented the decrease in microbial numbers seen after 200 hours. A coccoid bacterium, Streptococcus faecalis, was initially isolated after 312 hours and again at the end of the test.

Scanning electron microscopy (SEM) of the welded and base metal coupons revealed extensive biofilms on both specimens consisting primarily of coccoid bacteria. Figure 4A is a SEM photomicrograph of the base metal surface. Figure 4b shows the preferential attachment of bacteria in the welded region of the titanium coupon. At higher magnification, the extensive biofilm can be observed (Figure 4c).

Ozone-sulfuric acid pretreatment

Microbial levels in the base metal liquid sample from the ozone corresponded most closely with those in the Ozone base metal sample. Both samples had significantly lower microbial numbers than did hypochlorite pretreatment samples (Figure 3a). After 48 hours of continuous ozonation, microbes in the base metal corrosion cell numbered approximately 10 cfu/ml, and by 120 hours had dropped to 1 cfu/ml. Ozonation was discontinued after 192 hours when no bacteria (0 cfu/ml) were recovered from the liquid. Nor were bacteria recovered at any subsequent sampling time.

Microbial numbers in the welded metal liquid remained higher than in the ozone base metal liquid throughout the test, increasing from 30 to 150 cfu/ml prior to discontinuation of ozonation at 192 hours. These numbers were significantly lower than those of the parallel hypochlorite pretreatment. Microbial species shifted from Enterobacteria cloacae, which was isolated initially, to Pseudomonas maltophilia and Streptococcus faecium, which were then replaced by Clavibacter michiganensis. Microbial levels continued to increase following discontinuation of ozonation, with the replacement of previous bacterial species by a single isolate, Bacillus sp., at 312 hours. Ozonation of the weld metal corrosion cell was restarted and continued for 48

hours. At the end of this second period of disinfection, and for the remainder of the test, no bacteria were recovered. Figure 5 shows the difference in microbial numbers between the two corrosion cells in response to ozone treatment.

Scanning electron microscopy of the base and welded metals showed no bacteria on the surfaces. Instead, numerous crystalline structures were observed. Figures 6a-b show these structures on the welded specimen at increasing magnification. When X-ray fluorescence analysis of a white precipitate remaining in the corrosion cells was performed, potassium and calcium were among the elements found. The crystals observed under SEM were most likely chloride salts of these elements (e.g., potassium chloride and calcium chloride).

Oxone-sulfuric acid pretreatment

Microbial levels were reduced from approximately 100 cfu/ml at 24 hours to 1 cfu/ml by 72 hours for both base and welded metal samples (Figures 3a and 3b, respectively) exposed to the Oxone pretreatment. However, by the last day of testing, microbial levels in the fluid of the Oxone weld corrosion cell had increased to approximately 200 cfu/ml. The pH of the solution increased to approximately 5.0 and molds were occasionally seen during the course of the test in very low levels. Two bacteria were isolated from this sample, Proteus mirabilis and Streptococcus faecalis.

Scanning electron microscopy revealed primarily rod-shaped bacteria randomly distributed over the surface of the base metal coupon (Figure 7a). Established biofilms were observed on the welded metal coupon adjacent to weldment sites (Figure 7b). At higher magnification (Figure 7c), portions of the biofilm appeared to have sloughed off, which may account for the increased microbial levels in the fluid samples observed at the end of the test. Although predominantly rod-shaped bacteria were observed, coccoid forms were also present.

Control

After peaking 48 hours into the test at approximately $3X10^8$ cfu/ml, microbial numbers in the base and welded metal control fluids decreased to approximately $4x10^5$ cfu/ml. These numbers corresponded most closely with their respective hypochlorite counterparts (figures 3a and 3b), although the latter samples were at least one order of magnitude higher at both 120 and 192 hours. Of the 3 species isolated initially, only Proteus mirabilis was found throughout the test. The other two isolates, Enterobacter cloacae and Acinetobacter anitratus, were subsequently lost; their disappearance may account, in part, for the reduction in microbial numbers. Streptococcus faecalis was recovered on

the last day of testing. This organism and Proteus mirabilis, were the same species also seen in the oxone and hypochlorite samples on the last day of testing. The pH remained stable at 9.0.

Figure 8a is a scanning electron microphotograph which shows patches of biofilm on the base metal coupon surface. At higher magnification, these bacteria were found to be coccoid forms (Figure 8b). The presence of a well-established biofilm was much less evident on the welded coupon surface (Figure 8c) although microcolonies of bacteria, precursors to biofilm formation, can be observed in welded regions of the specimen. Both rod and coccoid forms were observed.

## DISCUSSION

The Vapor Compression Distillation subsystem operates by low pressure vaporization/distillation of a pretreated urine solution, resulting in processed water effluent. Rejected water (containing increasing brine concentrations) is reprocessed through the system until a determined brine concentration is reached, at which time the unit is temporarily brought off-line and the brine discarded. Parameters that are important to optimal operating efficiency, such as output rates, component life, and brine removal are largely dependent on the pretreatment solution employed. Presumably, the more completely oxidized the solution, the greater is the operating efficiency. By using decreasing turbidity as a measure of the oxidizing capability of the 3 pretreatments tested in this study, it was found that ozonation at approximately 45 mg/hr for 96 hours resulted in a significant decrease in the turbidity. In addition, the absence of color and odor of ammonia or urine is important to hygienic and aesthetic considerations. The turbidity in the hypochlorite and Oxone treated solutions did not appear to decrease during the test and a distinct odor of urine was noted. In addition, an elevated pH and the odor of ammonia were detected in both the hypochlorite and control samples, indicating the inability of the hypochlorite pretreatment to adequately control these parameters.

The raw urine used in this study was generated during the ECLSS water recovery test using a large number of donors, with a concomitantly large and diverse bacterial population. This may account for the inability to routinely recover the inoculated fungus, as bacteria probably outcompeted them under these test conditions.

Throughout the study, both hypochlorite and control fluid samples contained roughly the same numbers of bacteria which were significantly higher than in Oxone or ozone treated samples. Microbial numbers in the hypochlorite and control samples peaked early in the test, and decreased over time. Species diversity also decreased in these samples over time.

Only two bacterial species, Proteus mirabilis, a gram negative rod, and Streptococcus faecalis, a gram positive coccus, were isolated at the end of the test from the hypochlorite and control samples, indicating the ability of these bacteria to survive under these conditions.

Microbial levels in oxone treated liquid samples remained relatively low until the last day of testing when numbers in the Oxone weld fluid rose to approximately 200 cfu/ml. The same bacteria recovered from hypochlorite and control samples, P. mirabilis and S.faecalis, were recovered from this sample. One of these isolates, P. mirabilis, was found to significantly alter the chemical composition of the liquid by hydrolyzing urea present in the urine, resulting in the production of ammonia and increased pH. These are conditions undesirable for the water recovery system.

Bacterial levels in the ozone base metal solution averaged less than 2 cfu/ml for the course of the test, which was roughly seven orders of magnitude less than in hypochlorite and control samples. Microbial levels in the ozone weld solution increased following discontinuation of the ozonation process. This phenomenon has also been observed in previous studies using waste hygiene water (unpublished results) and is considered to be due to unequal distribution and/or mixing of ozone generated from a single source. A single bacterial species, Bacillus sp. was isolated. It is probable that, due to a suboptimal concentration of ozone in this sample, spores of this organism survived. Upon discontinuation of the initial treatment, the spores reverted back to a vegetative state. A second application of ozone destroyed the remaining bacteria, as examination of this sample 4 days following the second treatment indicated that no bacteria were present (0 cfu/ml).

Scanning electron micrographs of hypochlorite and oxone base and weld metal coupons showed evidence of more biofilm than on control specimens. These pretreatments were unable to completely eliminate bacteria from the liquid and may have "stimulated" biofilm formation which has been shown to protect bacteria exposed to stressful environments (4,5,6). Bacterial attachment was greatest on the hypochlorite coupons. This phenomenon is possibly attributable to both stimulation of the cells and the increase in cell numbers observed early in the test. Other investigators have shown an increase in cell adhesion during exponential growth, presumably due to increased cell wall hydrophobicity (7,8,9).

Although Oxone pretreatment appeared effective in reducing bacterial levels in the liquid, the presence of organisms on both base and weld metal coupon surfaces indicated that this pretreatment had not inhibited surface attachment. The bacteria appeared primarily as single cells on the base metal specimen, whereas established biofilms in close prox-

imity to the weldment sites appeared on the weld metal specimen. The apparent preferential attachment to and subsequent biofilm formation on the welded sites were also observed on the hypochlorite weld coupon. The affinity of biofilm for weld sites has been reported by other investigators as well (10,11). The patchy appearance of some of these biofilms on the oxone weld metal coupon indicated a sloughing of bacteria into the liquid, likely explaining the increase in microbial numbers on the last day of testing. The ability to recover these bacteria was presumably enhanced by the degradation of oxone over time. During testing of the VCD in the water recovery test, oxone concentrations remain fairly stable, and neither of these bacteria has been recovered (12). It is possible that only during periods of extended shutdown would bacteria such as these be isolated. Regardless, their presence on the surfaces represents a potential for contamination of the water.

The ozone base and weld metal coupons were devoid of bacteria. Instead, inorganic crystalline salts of elements commonly found in urine were observed. The increase in bacterial numbers observed in the weld metal liquid following the first ozonation treatment apparently allowed bacteria to attach to the specimen surface. The second treatment then eliminated the resulting biofilm, suggesting that ozonation may also be considered an effective remediation method for the removal of pre-existing bacterial surface contamination.

Although two morphologically distinct bacteria, a gram negative rod and a gram positive coccus, were recovered from the hypochlorite, control, and oxone weld liquid samples on the last day of testing, both types were not necessarily found on all specimen surfaces. Coccoid bacteria alone were observed on all but the control weld metal coupon which had both rod and coccoid types present. Rod-shaped bacteria were predominant on the coupons from Oxone-treated cells. This apparently selective attachment may represent differences in the ability of the various oxidants to alter the bacterial components of these distinct bacterial types involved in attachment processes.

Increases in the corrosion rates of Ti-6Al-4V were quite small for all pretreatments, indicating no apparent pretreatment-material incompatibilities during this 3-week test. Due to leakage of corrosion cell bridge tubes after extended use, the determination of material-solution interactions are generally limited to 3 weeks, and are considered adequate. However, this period of time does not necessarily allow for monitoring of the effects of biofilm-surface interactions, which may develop much more slowly. In the current test, this lack of longer exposure could have affected both Oxone and hypochlorite coupons.

## SUMMARY AND CONCLUSIONS

The presence of high pH in conjunction with the generation of ammonia, and bacteria (some of which were shown to exacerbate these conditions) in untreated samples reaffirms the need for some form of control of the biological and chemical constituents of raw urine. The turbidity of the solution is also of concern, especially in terms of overall subsystem performance.

Of the three pretreatments examined, ozone was clearly the most effective in eliminating microorganisms from liquid and surfaces and reducing the turbidity and odor of the raw urine. Oxone-treated samples remained turbid with an odor of urine at completion of the test. In addition, although bacteria in the oxone treated liquid maintained low numbers overall, their presence on the Ti-6Al-4V coupon surface indicated survival of some species which could recontaminate the solution. Weldment sites may have accelerated the recontamination process due to preferential attachment and subsequent biofilm formation. One of the species isolated, Proteus mirabilis, is an opportunistic pathogen capable of metabolizing urea, producing ammonia and an elevated pH. Samples receiving hypochlorite pretreatment remained turbid with an odor of urine and ammonia, the latter associated with the presence of P. mirabilis. Well-established biofilms were observed on both base and weld metal coupons receiving this pretreatment.

The hypochlorite and Oxone pretreatments appeared to influence the attachment efficiency of coccoid and rod-shaped bacteria, with cocci predominating the surface of hypochlorite treated coupons, while rods were predominant on Oxone treated specimens.

Corrosion rates were extremely low for all samples during the 3 week test. The influence of biofilms (observed on oxone and hypochlorite treated specimens) on these rates may require a longer test period.

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Table 1

Pretreatment Reagents and Concentrations Based on 1 liter Volume of Raw Urine

| | | | |
|---|---|---|---|
| Oxone | 5.0g | $H_2SO_4$ | 2.32g |
| Sodium Hypochlorite (5%) | 4.0ml | $H_2SO_4$ | 2.32g |
| Ozone | 45.0mg/hr | $H_2SO_4$ | 2.32g |

Table 2

Average Corrosion Rates for 6-Day and 20-Day Periods

| Treatment | 6-Day Average mils/year | 20-Day Average mils/year | Percent Change |
|---|---|---|---|
| Control | 0.00362 | 0.00417 | +15.2 |
| Control Weld | 0.00205 | 0.00189 | -7.8 |
| Oxone | 0.00341 | 0.00436 | +27.9 |
| Oxone Weld | 0.00280 | 0.00198 | -29.3 |
| NaHOCL | 0.00316 | 0.00435 | +37.7 |
| NaHOCL Weld | 0.00264 | 0.00358 | +35.6 |
| Ozone | 0.00247 | 0.00347 | +40.5 |
| Ozone Weld | 0.00345 | 0.00444 | +28.7 |

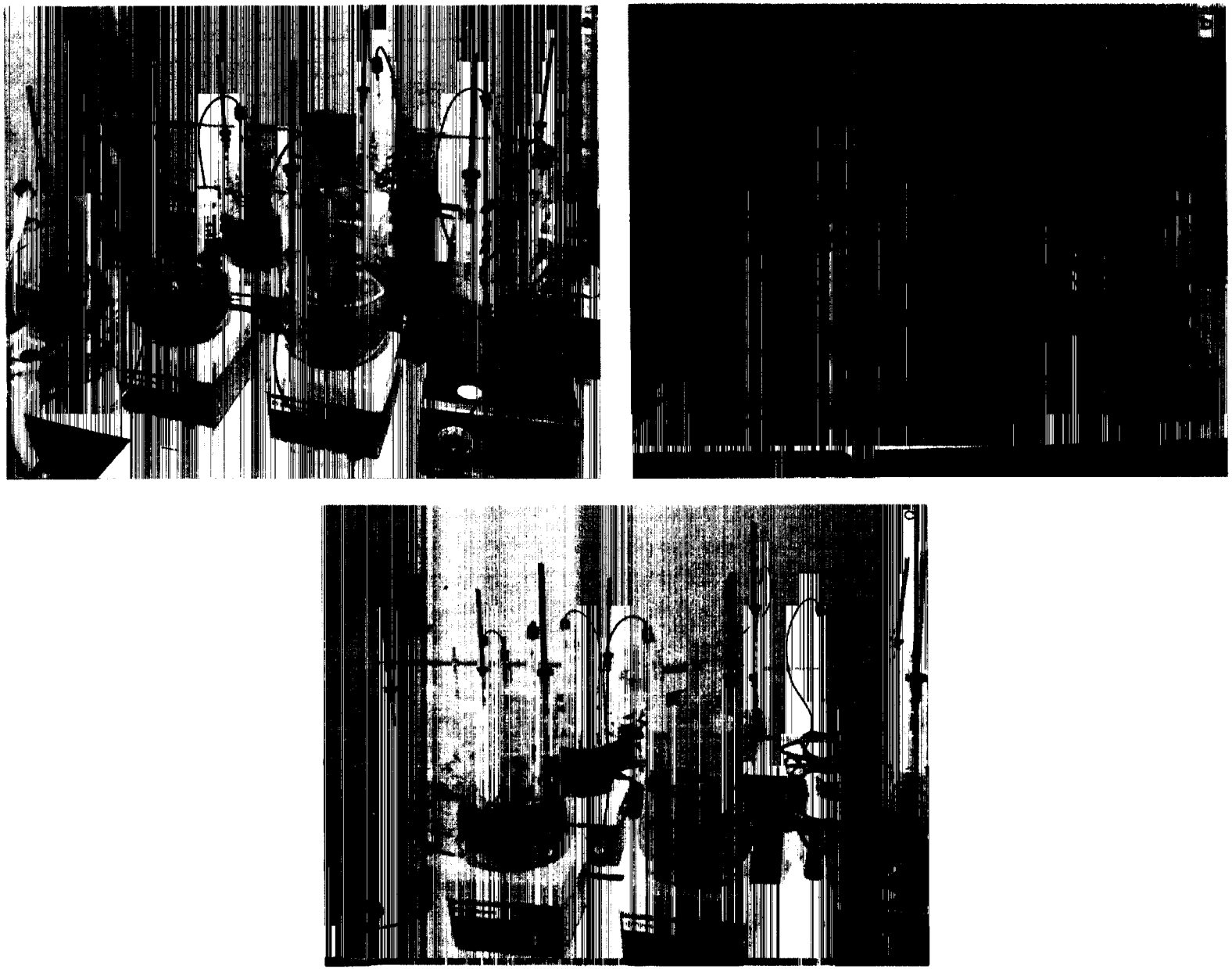


Figure 1. Changes in color and turbidity over a 3-week period. Corrosion cells are arranged left to right: ozone, hypochlorite, Oxone, control. Front row, base metal specimens. Back row, corresponding weld metal counterpart.
(a) Day 1, (b) day 4, and (c) day 21.

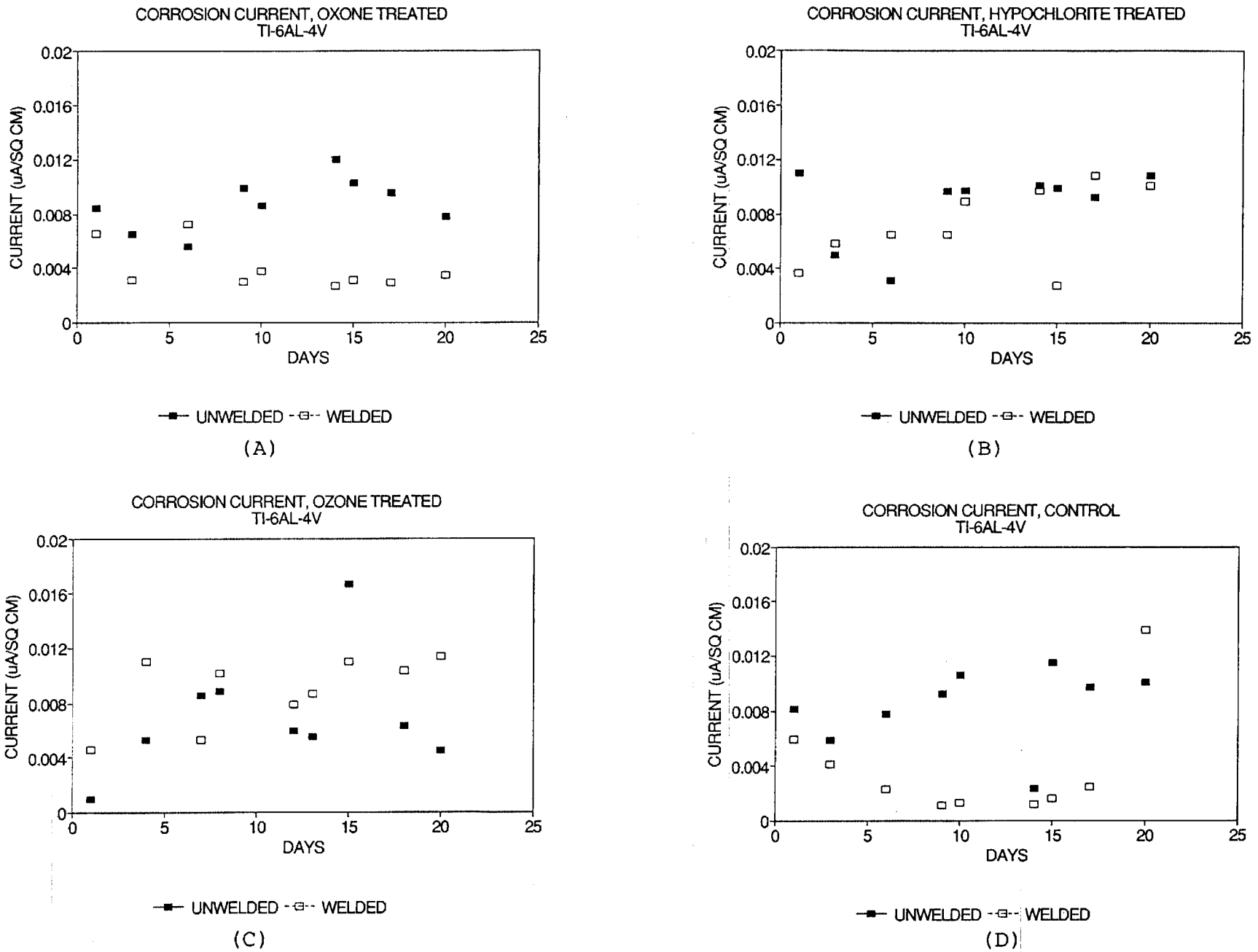
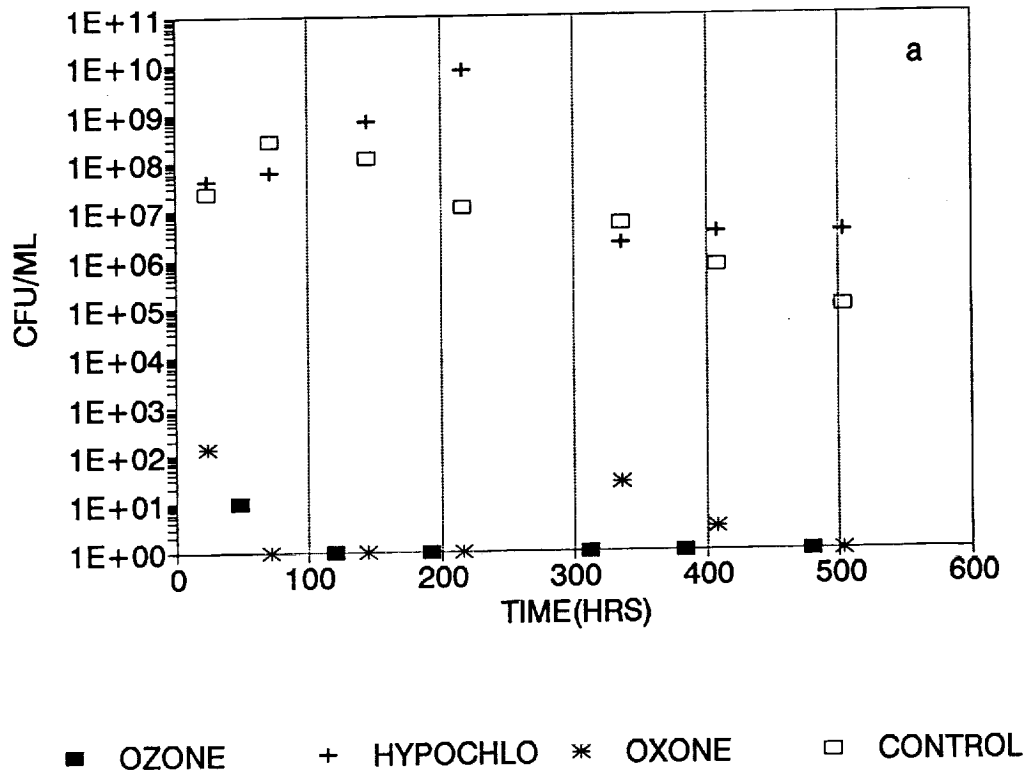



Figure 2. Variation of corrosion currents with time over the 3-week test period. (a) Oxone treated, (b) hypochlorite treated, (c) ozone treated, and (d) control.



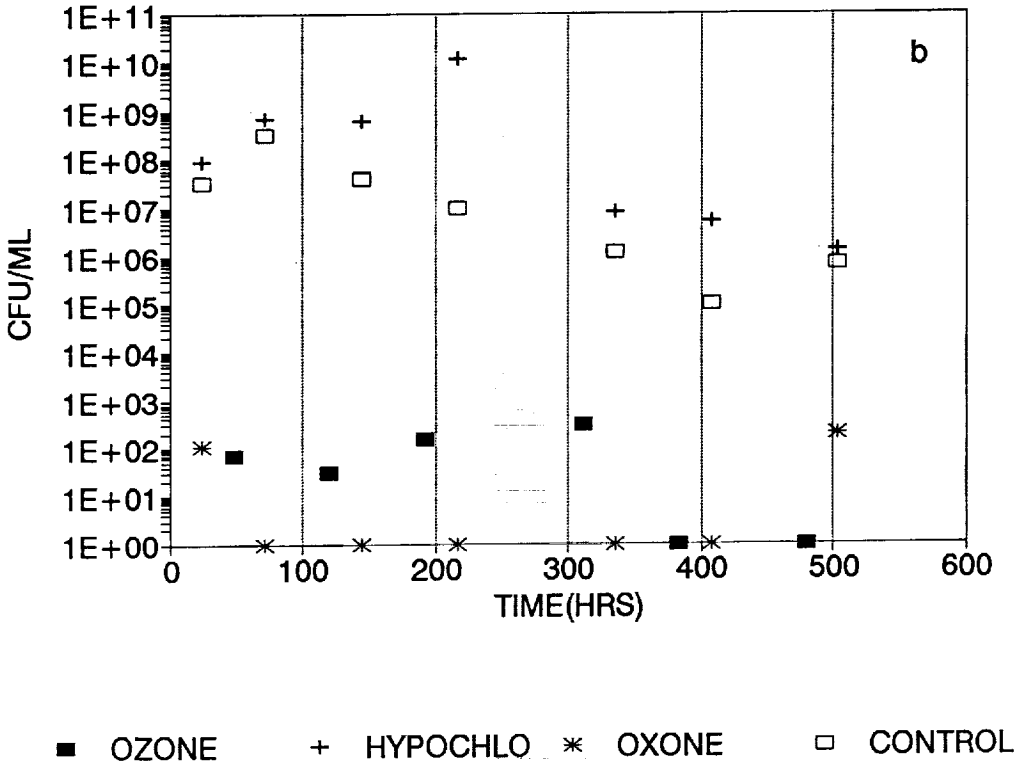




Figure 3. Changes in microbial numbers in control and pretreatment corrosion cells over a 3-week period. (a) Base metal samples, (b) weld metal samples.

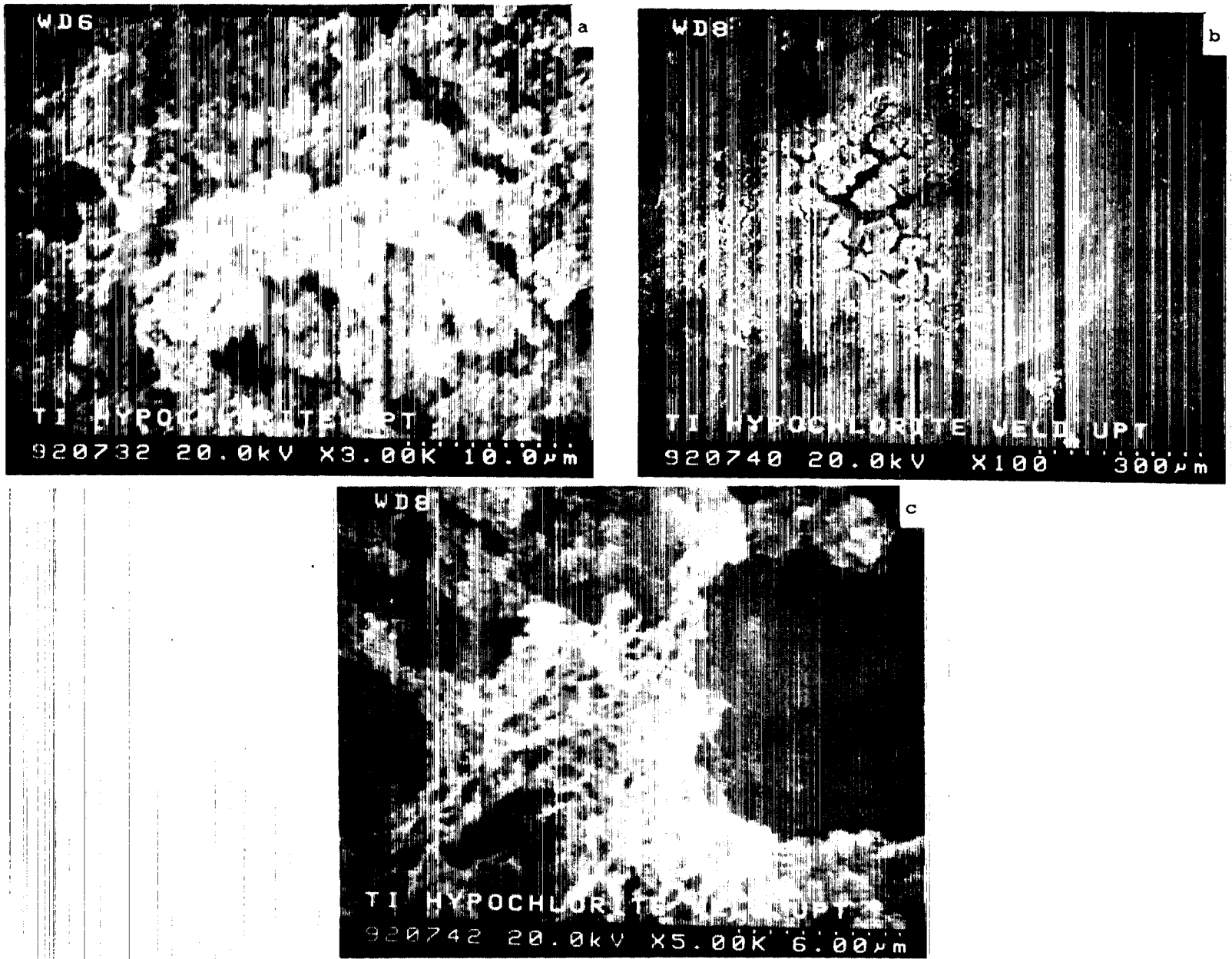



Figure 4. SEM photomicrographs of titanium 6-4 specimens exposed to urine treated with sodium hypochlorite. (a) Base metal, (b) weld metal, and (c) region on b, higher magnification.

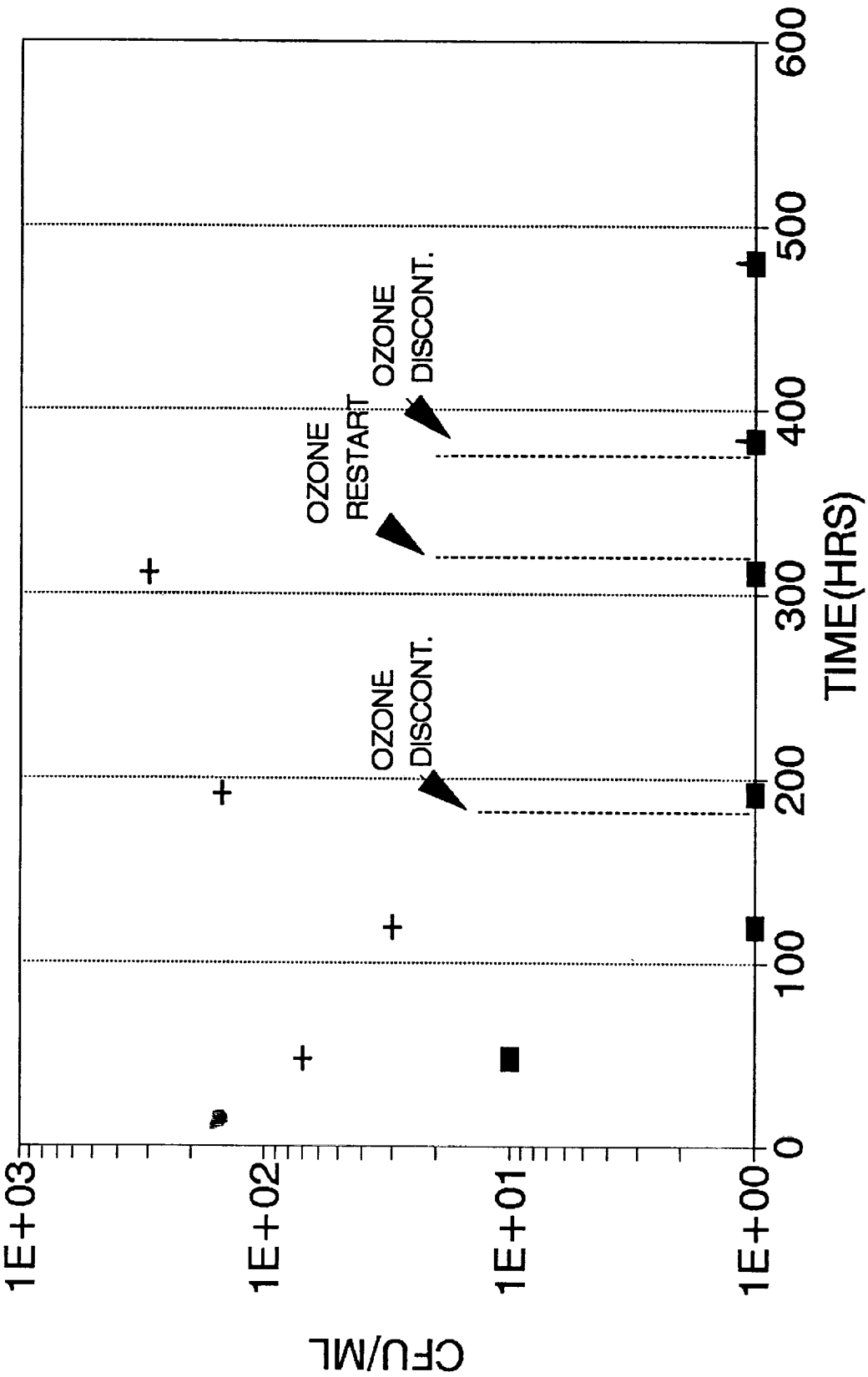



Figure 5. Comparison of microbial numbers in the ozone base and weld metal corrosion cells over a 3-week period.

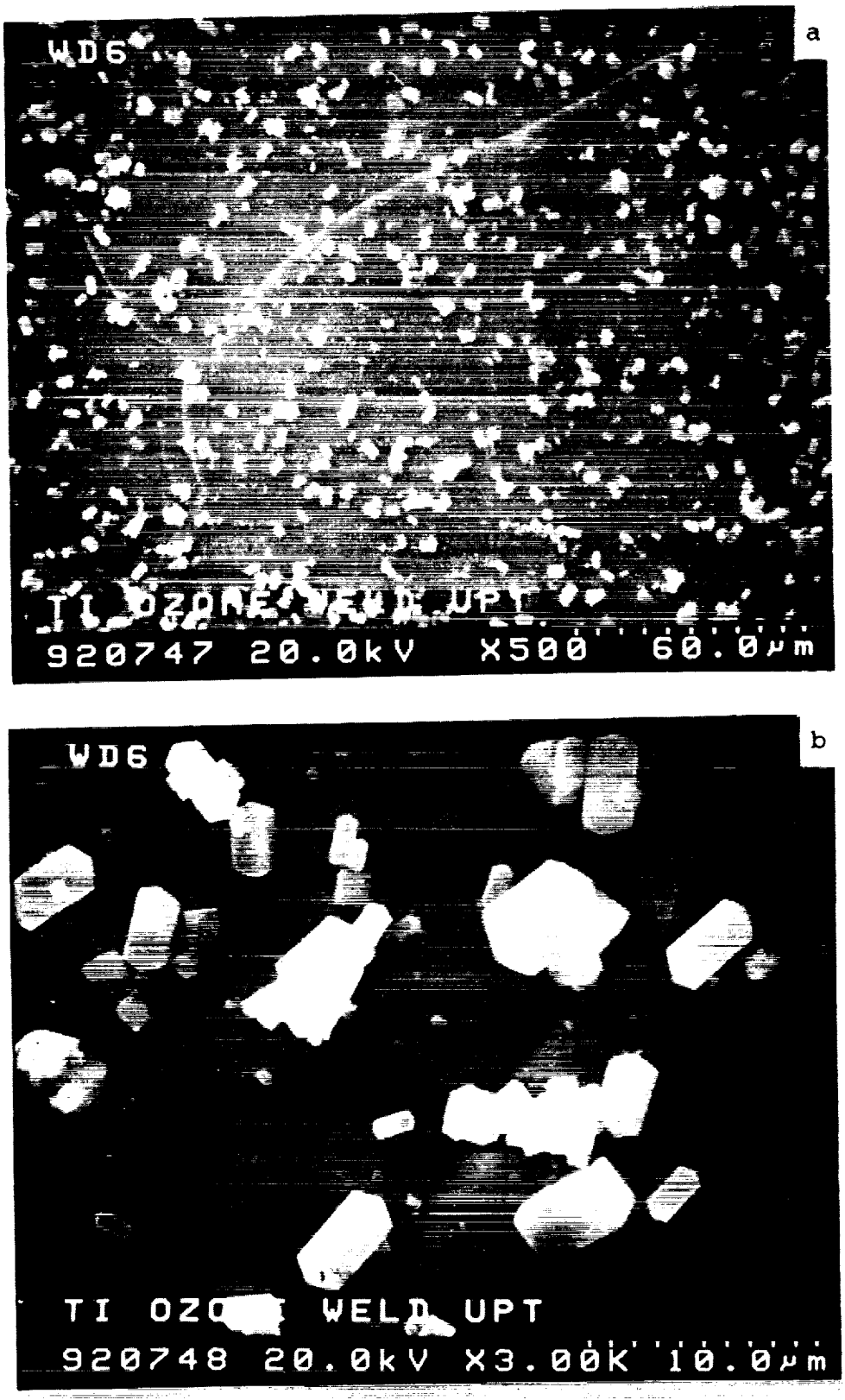



Figure 6. SEM photomicrographs of titanium 6-4 weld metal specimen exposed to urine treated with ozone. (a) Crystalline structures randomly dispersed over the surface, (b) region a, higher magnification.

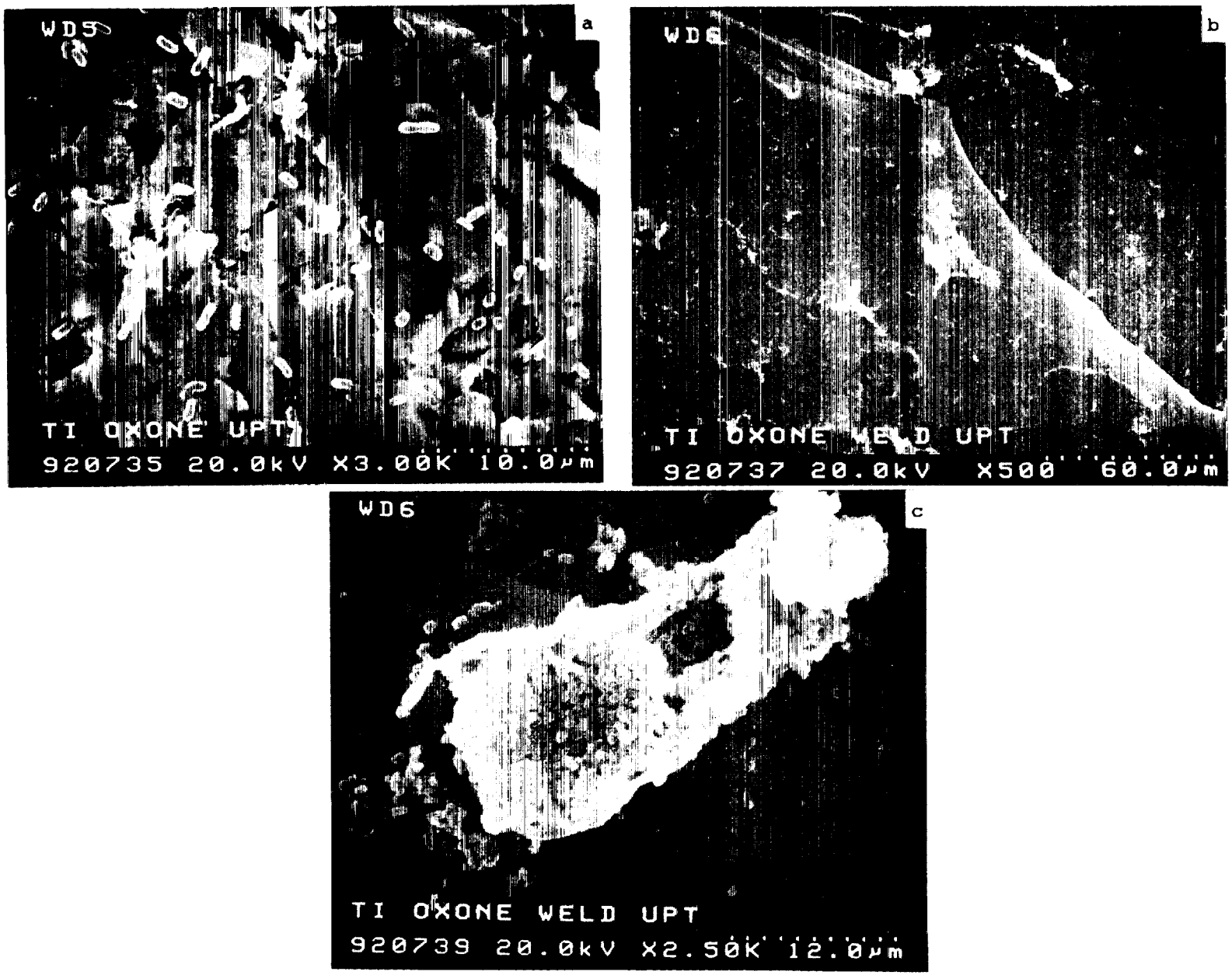



Figure 7. SEM photomicrographs of titanium 6-4 base and weld metal specimens exposed to urine treated with Oxone. (a) Base metal, (b) weld metal containing developed biofilms, and (c) region b, higher magnification.

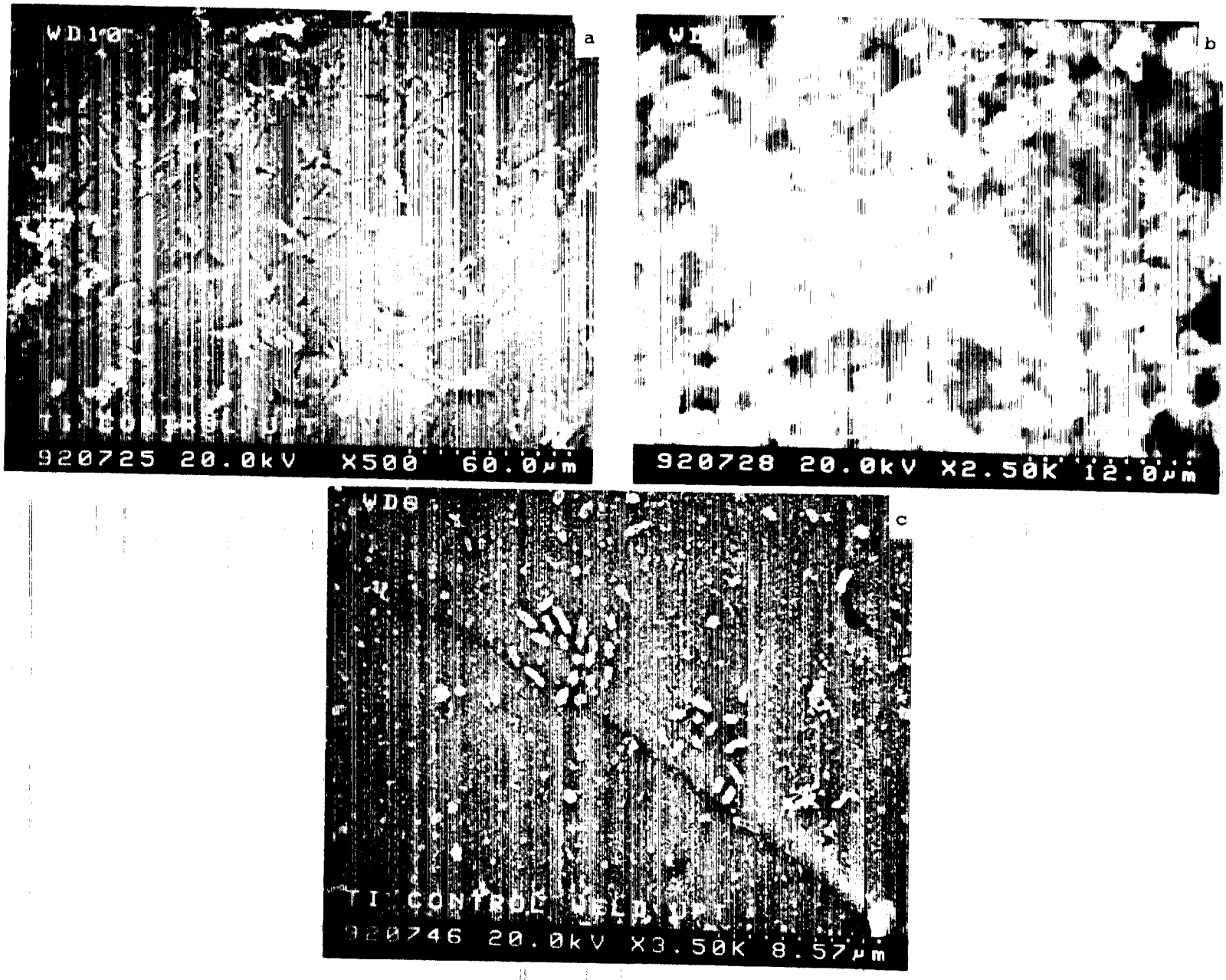



Figure 8. SEM photomicrographs of titanium 6-4 base and weld metal specimens exposed to raw urine. (a) Base metal, (b) region a, higher magnification, and (c) weld metal.

APPROVAL

MICROBIOLOGICAL AND CORROSION ANALYSIS
OF THREE URINE PRETREATMENT REGIMES
WITH TITANIUM 6Al-4V

By Timothy L. Huff

The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

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