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MICRON-SCALE MIC OF ALLOY 22 AFTER LONG TERM INCUBATION IN SATURATED NUCLEAR WASTE REPOSITORY MICROCOSMS

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ABSTRACT

The effects of potential microbiologically influenced corrosion (MIC) on candidate packaging materials for nuclear waste containment are being assessed. Coupons of Alloy 22, the outer barrier candidate for waste packaging, were exposed to a simulated, saturated repository environment consisting of crushed rock from the repository site and a continual flow of simulated groundwater for periods up to five years. Coupons were incubated with YM tuff under both sterile and non-sterile conditions. Surfacial analysis of the biotically-incubated coupons show development of both submicron-sized pinholes and pores; these features were not present on either sterile or untreated control coupons. Quantification of these effects will help define the overall contribution of MIC to the integrity of the containment system over a period of 10,000 years.

Keywords: MIC, Alloy 22, repository, nuclear waste, corrosion, Yucca Mountain

INTRODUCTION

The U.S. Dept. of Energy is currently designing a long term, underground, high-level nuclear waste repository at Yucca Mountain (YM), NV to contain both spent nuclear fuels and high-level defense waste. The current repository design specifies a two-layer waste package consisting of an outer layer of Alloy 22, a nickel-based metal, as the primary corrosion barrier. It has already been established that a wide array of microorganisms reside within the repository horizon rock structures, and that the main limiting factor to their growth is water availability[1,2]. Here, we present results of a long-term study involving analysis of Alloy 22 coupons after incubation in simulated saturated repository environments (or microcosms). The study includes both non-sterile and sterile control microcosms (in which YM rock has been sterilized) to better assess the contribution of microorganisms in biotic experiments.

EXPERIMENTAL PROCEDURES

Microcosm Reactor Components

The microcosm apparatus (Figure 1), a continuous flow-through system, consists of a borosilicate glass vessel (500 mL) fitted with an inlet for introducing simulated YM ground water from a 1 liter reservoir, and an outlet for draining the spent media. The influx and efflux rates were maintained at an equal and constant rate (2 mL/hr) with the aid of peristaltic pumps, to maintain a volume of 250 mL of ground water in the vessel (total residence time was approximately 5 days). In-line media break tubes were incorporated into the outlet tubing to prevent back-contamination, and in-line filters (0.2 µm) were included on the inlet tubing to assure sterility of introduced simulated ground water. All components of the system were pre-sterilized, and the sterility of those system elements upstream of the microcosm vessel were maintained and monitored by live plating techniques whereby sterile media was monitored for the presence of contaminating bacteria by plating on Nutrient Broth gar (Difco). Reactors containing sterile and non-sterile YM rock (discussed below) were incubated for a total of 43 and 57 months, respectively, at ambient laboratory temperature (approximately 22 ^{o} C), or at an elevated temperature of 30 ^{o} C.

Simulated ground water (10XJ13 media) was formulated to represent a ten-fold concentration of YM ground water from well J13 in the YM region[3] using *The Geochemist's Workbench*[4] (Table 1). The ionic strength of the simulated groundwater was increased ten-fold to mimic the anticipated increased concentration of ground waters entering the proposed repository. To prevent precipitation of minerals due to concentrating the reported ground water composition, maintain electric neutrality, and control initial pH, the concentrations of calcium, sodium, and carbonate were decreased (with respect to the reported composition of J13), resulting in near-saturation conditions with respect to mineral phases. Additionally, the 10XJ13 media was supplemented with 0.1% glucose (5.55 mM), a minimal organic carbon source; the pH was 7.6. Previous studies had shown that this formulation supported microbial growth of organisms contained in YM rock[1,2]. Available forms of soluble silica (metasilicates) significantly increase the pH of the solution, therefore no silica was added to the simulated 10XJ13 formula; silica was thus only provided from dissolution of the added YM rock (below).

YM volcanic welded rock tuff was aseptically collected from the drift walls of the Exploratory Study Facility at the repository horizon. Collected tuff was then crushed and sieved (particle size fraction 1-4 mm) using sterile techniques, and stored under sterile conditions, to prevent contamination with microorganisms not associated with post-construction repository conditions. YM tuff composition[5] is shown in Table 2. To assess the effects of abiotic corrosion, in parallel experiments, aliquots of tuff were sterilized (3-4 Mrad gamma irradiation, using a ^{60}Co gamma source) and dosed with antibiotics (rifampicin, streptomycin, amphotericin B, and geneticin at final concentrations of 250 ug/mL, 200 ug/mL, 12.5 ug/mL, and 500 ug/mL, respectively) were added to maintain sterility. Microcosms experiments were performed in duplicate.

In addition to simulated ground water, each microcosm contained 20 test coupons of Alloy 22 (Table 3) [3.23 cm^2 surface area per coupon, total coupon surface area was 64.5 cm^2 per microcosm reactor candidate waste package material] imbedded vertically in crushed YM rock (100 g); coupons were placed to prevent physical contact between them. The test coupons incubated at 22^{o} C. were prepared by stamping with identification numbers, wet-polished with abrasive paper progressively to 240-grit, and cleaned with distilled water and acetone. The test coupons incubated at 30^{o} C. were milled to root mean square (RMS) 32 factory finish and cleaned as above. Coupons were then weighed for eventual weight-loss analysis before being sterilized (3-4 Mrad total dose)[5], and emplaced in assembled microcosms using sterile techniques. In addition to the metal coupon-containing microcosms, reactors without coupons were assembled to assess the effects of YM rock and indigenous microorganisms on ground water chemistry.

Sample Preparation

Test Coupons. At each sampling time point, Alloy 22 coupons were removed from their respective microcosms using sterile techniques. One coupon from each microcosm was immediately fixed to preserve the bacteria and biofilm adhered to the surface for analysis while the remaining coupons were cleaned following a protocol developed at LLNL that combines ASTM procedure G1, designations C6.1 and C7.4 (Figure 2). Solution temperatures and immersion times were modified to minimize cleaning effects. Alloy 22 foil controls were implemented to monitor metal loss during cleaning. Gravimetric analysis of these controls indicated that the procedure could be performed twice, if required, on the same sample without detectable loss of mass.

Bulk Solution. Periodically, aliquots of bulk aqueous solution effluent from microcosm reactors were collected at the reactor outlet. The aliquots were filtered (0.2 mm) for pH analysis, inductively coupled plasma-mass spectrometry (ICP-MS), and ion chromatography (IC). Samples analyzed by ICP-MS were acidified to pH 2 with ultrapure HNO_3.

Analytical Techniques

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were used for imaging the surfacial features and to identify the elemental composition of deposits, respectively. Atomic force microscopy (AFM) was also implemented to quantify pore dimensions. Unreacted/unexposed coupon surfaces were analyzed in parallel with those withdrawn from microcosms to allow comparison with corrosive effects under experimental microcosm conditions.

SEM/EDS. Test and unreacted/unexposed coupon surfaces were characterized with an FEI Quanta 200 ESEM equipped with an EDAX Genesis Analysis System for imaging and elemental analysis, respectively. Typically, image analysis was performed at an accelerating voltage (AV) of 15 kV but was adjusted to 10 kV for low vacuum analysis of non-conductive particulates. Imaging parameters included a working distance of 8 mm and spot size 4, and elemental analysis parameters were 20 kV accelerating voltage, working distance 11 mm, spot size 6, and 50% dead time. The electron beam penetration depth on Alloy 22 was $\leq$1 µm at 15 kV AV. The beam was cropped or operated in spot mode during elemental analysis to minimize scattering. Test coupons were not coated prior to analysis.

AFM. Pit dimensions and surface roughness measurements (RMS) of test and unreacted/unexposed coupons were made with a Dimension 3100 AFM operated in tapping mode. For RMS determinations, scans were collected on 100 mm^2 areas to quantify gross topographical features and on 10 mm^2 areas to quantify fine features.

ICP-MS. Solubilized metals originating from Alloy 22 coupons (Table 3), tuff (Table 2), and 10XJ13 growth media (Table 1) in bulk aqueous microcosm reactor effluents were analyzed using ICP-MS (Activation Laboratories, Ontario, Canada; Perkin Elmer ELAN 6100 ICP-MS). For elements that exceeded the upper boundary limits of quanitification using mass spectrometry (generally, Na and K), ICP-Optical Emission Spectrometry was performed. Elemental analysis was also performed on solution samples collected from no-metal control microcosms to assess elemental concentrations originating from simulated 10XJ13 water or YM rock components.

IC. Anions were analyzed with ion chromatography (Activation Laboratories, Ontario, Canada, DX120 Ion Chromatograph). Although nitrate and chloride concentrations were of particular interest, a full panel analysis was performed to monitor concentrations of anions from the rock tuff and growth medium.

RESULTS AND DISCUSSION

Surfacial and Aqueous-Phase Chemical Analyses of Alloy 22 Coupons Incubated at 30^{o} C.

Surface Analysis. Alloy 22 coupons from microcosms incubated at 30 ^{0}C were withdrawn, cleaned, and imaged using SEM surfacial analysis. An unreacted Alloy 22 coupon was also cleaned and imaged in parallel for comparison. Coupons incubated in the non-sterile, microcosm reactors showed the development of pinholes, primarily along the ridges formed by polishing, while coupons incubated in sterile microcosms and those that were not reacted in microcosms showed no evidence of pinhole formation after one cleaning cycle (Figure 3). The micropits appeared uniform in shape except where they had grown together and ranged in size from 200-700 nm in diameter.

Cleaning Effects. To assess whether the cleaning process had an impact on the coupon surface, an alternate non-sterile coupon was sampled and imaged prior to cleaning. Although the surface appeared mottled, no pinholes were observed (Figure 4). Elemental analysis of the surface did not indicate the presence of a silica scale that could potentially coat and obscure surface features. This same coupon was then cleaned and pitting on the ridges appeared, similar to the micropits noted previously on non-sterile coupons (Figure 4). Furthermore, the pinholes did not contain fill material (fines or re-precipitated mineral phases) as was expected, suggesting that the pores were introduced during cleaning. Because this phenomenon was not observed on the unreacted and sterile-treated samples, the cleaning procedure was again tested on an unreacted coupon. After one cleaning cycle, no pitting was observed. The cleaning cycle was repeated once and SEM analysis indicated the genesis of pinhole formation, albeit these features were subtle. Further cleanings confirmed this finding, and propagation of the micropits continued after three cleaning cycles (Figure 5). Preliminary interpretation of these results indicates that the coupon surface is more susceptible to pitting during cleaning after exposure to YM tuff microorganisms. Ridges are typically weaker than underlying surface material and mechanically inferior; microbial colonization and activity may exacerbate this condition. No further cleaning cycles were performed, therefore it is inconclusive if pitting due to cleaning on the unreacted coupon would have eventually resembled the pits developed on the non-sterile surface, where the micropits were approximately uniform in shape, size and distribution. Gravimetric analysis of reacted/cleaned and unreacted/cleaned coupons were inconclusive because the loss of material was minimal compared with coupon masses.

Despite these unexpected artifacts associated with serial cleaning, it should be emphasized that when unreacted, sterile-reacted and non-sterilely-reacted coupons are compared after a single cleaning (Figure 3) micropitting is not observed on the unreacted and sterile-reacted coupons, but only on those exposed to YM microorganisms. It is not, however, evident that the actual pitting was *directly* caused by microbial activity, but the data support a hypothesis that microbial activity has altered the coupon surface in such a way that it is more susceptible to chemical corrosion than coupons that have not been exposed to YM microorganisms. Follow-on testing will include determining whether continued serial cleaning might simulate the non-sterile micropitted surface and repeating serial cleaning on a reacted, sterile coupon(s). Quantification of surface roughness, pit distribution, and pit dimensionsare being examined using AFM analysis. Chemical alterations to the non-sterile coupon surface may be detected by x-ray photoelectron spectroscopy where surface and near-surface compositions can be determined. Comparison of unreacted-not cleaned and non-sterile–not cleaned coupons may also show speciation differences that could explainthis observed phenomenon.

Effluent Chemistry. Chemical analysis of bulk aqueous solutions was undertaken to determine the concentration of solubilized elements of interest. Solubilized metal from coupons and contributions from the tuff and growth media were identified with ICP analysis. A seven to sixteen fold elevation in Mn concentration was detected in the non-sterile microcosm reactors containing tuff and metal coupons (exceeding 600 ppb), compared to the background media, non-sterile no-metal control reactors containing tuff (86 ppb), and sterile control reactors containing coupons and tuff (38 ppb) (Figure 6). Manganese is a component of both Alloy 22 and YM tuff, therefore the Mn concentration in the no-metal control reactors reflects Mn solubilized solely from tuff in the presence of YM bacteria (Mn composition of YM tuff is 0.05-0.06 weight percent (500-600 ppm). Sterile control reactors which contain Alloy 22 coupons and tuff contained small amounts of solubilized Mn (Mn composition of Alloy 22 is 0.26 weight percent, 2600 ppm Mn) in the absence of microbial activity. Since the Mn concentration in the no-metal control reactors was 86 ppb and 38 ppb in sterile control reactor effluent, it is evident that the majority (>80%) of solubilized Mn in the non-sterile reactors arose from the Alloy 22 coupons due to microbial activity.

Molybdenum in non-sterile, coupon-containing microcosm reactor effluent was also elevated above background and control values, but the absolute concentration was low (10 ppb). The no metal, non-sterile control was measured just above detection limits (0.4 ppb; Figure 6) showing that very little to none of the Mo originated from tuff. The sterile control reactors also contained Mo at levels just above detection indicating that the solubilized Mo in non-sterile reactor effluent must be due to microbial activity. Ion chromatography to identify soluble salts, including analysis of nitrate concentrations, did not show any remarkable changes between influent and effluent concentrations.

Based on the effluent chemistry alone, it is unknown whether dealloying of Alloy 22 or generalized, homogeneous dissolution with subsequent precipitation, adsorption, or use by bacteria of other alloying elements is occurring. End point analysis of precipitated and adsorbed particulates in the reactor will assist in identifying speciation and deducing dissolution mechanisms.

Surfacial and Aqueous-Phase Chemical Analyses of Alloy 22 Coupons Incubated at 22 ^{o}C

Surface Analysis. In contrast to Alloy 22 non-sterile coupons incubated at 30^{o} C., coupons withdrawn after long-term incubation from biotic microcosms incubated at 22^{o} C. showed markedly different patterns of micropitting. Again, small micropores (generally less than one micron) were apparent on the surface, but their distribution and frequency was extensive, covering all regions of the coupon, and not restricted to polishing ridges. Further, the micropores were less uniform in shape and size compared with those observed on the 30 ^{o}C incubated coupons (Figure 7). Coupons analyzed prior to cleaning contained a micropore density similar to those that were cleaned, micropores were so pervasive that cleaning effects, if any, were not observed. Unreacted coupons and those incubated under sterile conditions did not show micropore formation (Figure 7). Coupons incubated biotically at room temperature for shorter durations (17 months, 32 months) also demonstrated pores (Figure 8) showing that the pores were generated at the earlier sampling time points. Gravimetric analysis was again inconclusive because many of the pores were filled with deposited or precipitated minerals. Energy dispersive spectral analysis of pitted coupons showed that the micropits were often filled with siliceous material, which apparently originated from dissolved and re-precipitated silica, or particulate fines from the YM rock[6] (Figure 9). Pit dimensions have not yet been measured, therefore overall metal loss has not presently been established. Once effective procedures for removing deposits from pits (without further damaging the metal surface) are found, pit dimensions (using AFM) and pit distribution (pits/unit area, using SEM) measurements will be made and used to quantify metal loss as a result of micropitting.

Atomic force microscopy indicated that the overall roughness of the surface of coupons incubated in non-sterile microcosms at ambient temperature decreased contributing to a flattening of the coupon surface, even as micro-scale roughness increased due to pore formation. Root mean square analysis was used to compare the non-sterile and unreacted coupons at 100 mm^2 and show a decrease in measured roughness (Figure 10). Photomicrographs (SEM) also indicate that the surface is smoothing; the polishing ridges have flattened and only the gross features, such as surface gouges, remain (Figure 11).

Effluent Chemistry. Chemical analyses (ICP/IC) performed on aliquots of the bulk aqueous solution in microcosms incubated at room temperature did not indicate elevated concentrations of metals and dissolved salts above background and control concentrations, indicating that the metal lost from the surface has precipitated or been adsorbed.

It is not yet clear why the distribution and frequency of pores differs between coupons incubated in biotic environments at room temperature and 30^o C. Clearly, the environments differ enough to create a sharply contrasting pattern of micropitting. Differences in the microbial communities may have caused the differential pattern of pitting observed. To further probe this possibility, we are currently undertaking analysis of the microbial communities, those pelagic organisms in the solution phase, as well as those adhered to coupons, in biotic room temperature and 30^0 C. microcosms. Further, analysis of the biofilms including redox state, pH, relevant chemical species, and dissolved oxygen concentration is essential to understanding the mechanisms of MIC.

CONCLUSIONS

During long term incubation of Alloy 22 coupons under non-sterile conditions in contact with crushed YM tuff, simulated ground water, and a carbon source, microbially induced alteration of the coupons' surfaces was deduced by comparison with both sterile-reacted and unreacted control samples. Incubation studies showed:

- At 30 ^{o}C. surface alteration by microbial activity was evidenced as an enhanced susceptibility of the surface to -pitting after subsequent cleaning. This susceptibility was not observed on sterile and unreacted control coupons. Surface alterations were comprised of micropits on polishing ridges. Chemical analysis of microcosm effluent showed microbially-induced solubilization of Mn and Mo from the coupons, which is consistent with chemical alteration of the surface.

- At ambient temperature (22^{o}C.) surface alterations by microbial activity was evidenced by development of numerous micropits that covered the surface of the coupon and were partially filled with siliceous material derived from crushed tuff. The overall surface roughness of the coupon was significantly reduced by microbial activity. Microbial alteration of the metal surface was more pervasive and occurred sooner when compared to coupons incubated at 30^o C. Micropitting was not observed on sterile-incubated and unreacted coupons incubated at ambient temperature.

- Despite a seemingly minor difference in temperature, the nature of the metal surface alterations differed in significant ways between coupons incubated at a constant temperature of 30^o C. and those incubated at ambient temperature. Given that the abiotic chemical compositions in the systems incubated at both temperatures were identical, it seems likely that differences in microbial activities were responsible for the observed results. These differences could be explained by either; 1) different microbial species with dissimilar activities dominating at the two temperatures, or 2) the same species dominated but had dissimilar activities at the two incubation temperatures. Surface spectroscopic analysis and microbiological community analysis will be undertaken to further examine these possibilities.

Although these preliminary results show that YM microorganisms have an effect on Alloy 22, these results do not suggest catastrophic failure of the waste package over 10,000 years of storage. The effects observed thus far are minor, and well within containment design parameters for long-term storage of nuclear waste.

REFERENCES

1. Horn, J., C. Carrillo, and V. Dias. 2003. Comparison of the Microbial Community Composition of Yucca Mountain and Laboratory Test Nuclear Repository Environments. Natl. Assoc. of Corrosion Engineers (NACE), Corrosion 2003, Paper No. 03556, 16-20 March, San Diego, CA.

2. Horn, J., B.A. Masterson, A.J. Rivera, A. Miranda, M.A. Davis, and S.I. Martin. 2001. Bacterial growth dynamics, limiting factors, and community diversity in a proposed geological nuclear waste repository environment. Lawrence Livermore Natl. Lab, Report UCRL-JC-145155, Livermore CA.

3. Delany, J.M. 1985 Nov. Reaction of Topopah Spring Tuff with Water: A Geochemical Modeling Approach Using the EQ3/6 Reaction Path Code. Livermore, CA: Lawrence Livermore National Laboratory. Report UCID-53631.

4. Bethke, C.M. 1994 . The Geochemist's Workbench, Version 2.0: A Users Guide to Rxn, Tact, React, and Grplot, Hydrogeology Program, University of Illinois.

5. Broxton, D.E., D.L. Bish, R.G. Warren, Clays and Clay Minerals, 35(1987): p. 89.

6. Horn, J., S. Martin, B. Masterson, and T. Lian. 1999. Biochemcial contributions to corrosion of carbon steel and alloy 22 in a continual flow system. Natl. Assoc. of Corrosion Engineers (NACE), Corrosion 99, Paper No. 162, 25-30 April, San Antonio, TX.; Report UCRL-JC-136521, Lawrence Livermore Natl. Laboratory, May 2000.

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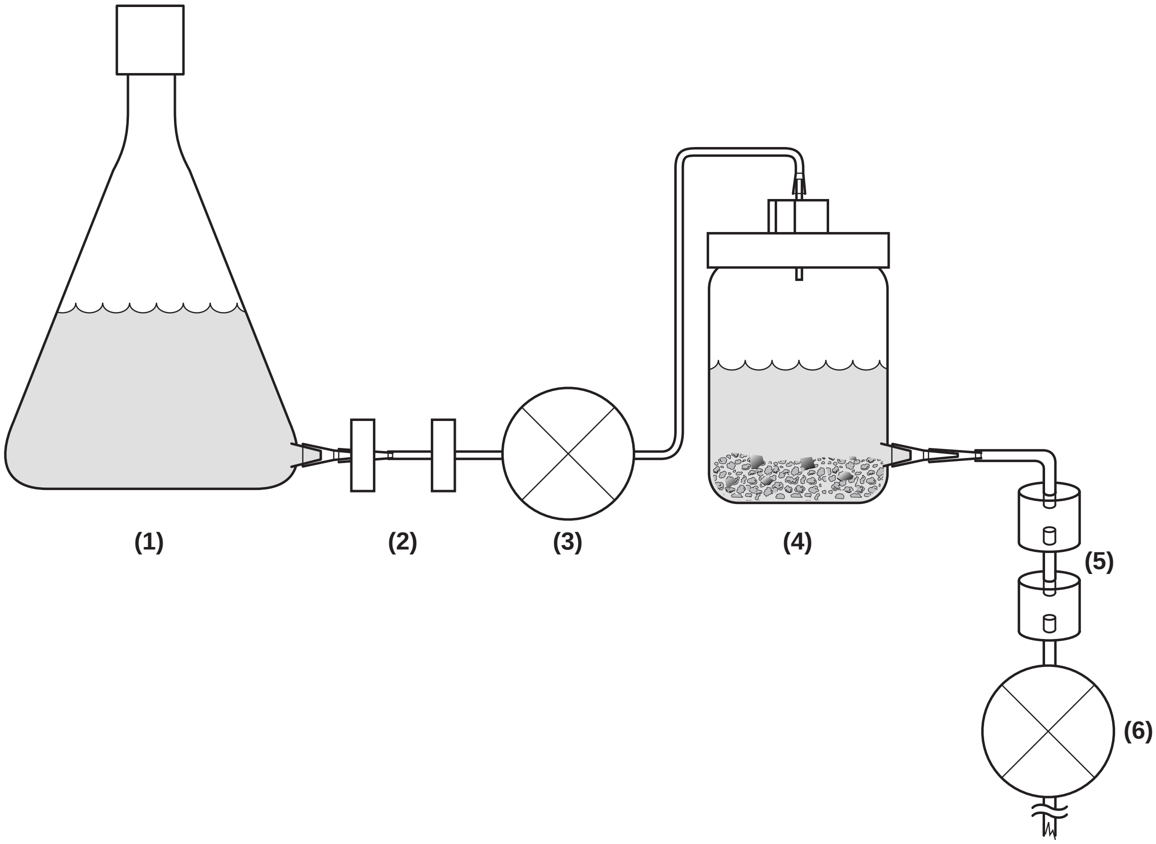


FIGURE 1. Configuration of microcosms. Each microcosm was composed of a 1 liter reservoir containing sterile media (1), that is fed through two 0.2 µm in-line filters (2), by means of a peristaltic pump (3), into a 500 mL modified spin flask that contains crushed YM tuff and coupon material (4). Media break tubes (5) on the outflow were used to prevent back-contamination, and the outflow rate was regulated by a second peristaltic pump (6).

TABLE 1.
COMPARISON OF NATURAL J13 GROUND WATER (CONCENTRATED 10-FOLD) WITH SIMULATED J13 WATER (CONCENTRATED 10-FOLD)

Component	**10X J13* ground water**	**10X J13 simulated water**
	concentration, mM	*concentration, mM*
Na	19.1	6.14
K	1.31	1.31
Ca	3.12	2.51e-2
Mg	0.790	0.790
NO_3	1.55	1.55
Cl	1.950	1.95
CO_3	20.50	0.527
SO_4	1.95	1.95
Li	0.061	0
Sr	0.004	0
Al	0.004	0
Fe	0.001	0
Si	9.61	0
F	1.16	1.16
Glucose	0	5.55

*This column represents a ten-fold concentration of elements reported by J.M. Delany, “Reaction of Topopah Spring Tuff with J-13 Water: A Geochemical Modeling Approach Using EQ3/6 Reaction Path Code,” UCID-53631, Lawrence Livermore National Laboratory, (1985).

TABLE 2.
BULK-ROCK COMPOSITIONS FOR TOPOPAH SPRING TUFF AT YUCCA MOUNTAIN, NEVADA*

	Unaltered lower vitrophyre	Altered lower vitrophyre
Element	**concentration, wt%**	**concentration, wt%**
Si	72.2	69.6
Ti	0.07	0.10
Al	14.3	19.5
Fe^{3+}	0.78	1.07
Mn	0.05	0.06
Mg	0.45	1.33
Ca	0.69	3.73
Na	6.43	3.65
K	4.98	0.91
P	0.01	0.02

*D.E. Broxton, D.L. Bish, R.G. Warren, Clays and Clay Minerals, 35(1987): p. 89.

TABLE 3.
COMPOSITION OF ALLOY 22 COUPONS

C (wt%)	***Mn (wt%)***	***P (wt%)***	***S (wt%)***	***Fe (wt%)***	***Co (wt%)***	***Mo (wt%)***	***Cr (wt%)***	***W (wt%)***	***V (wt%)***	***Ni (wt%)***
0.002	0.260	0.012	0.001	3.95	0.510	13.4	21.6	2.82	0.150	bal

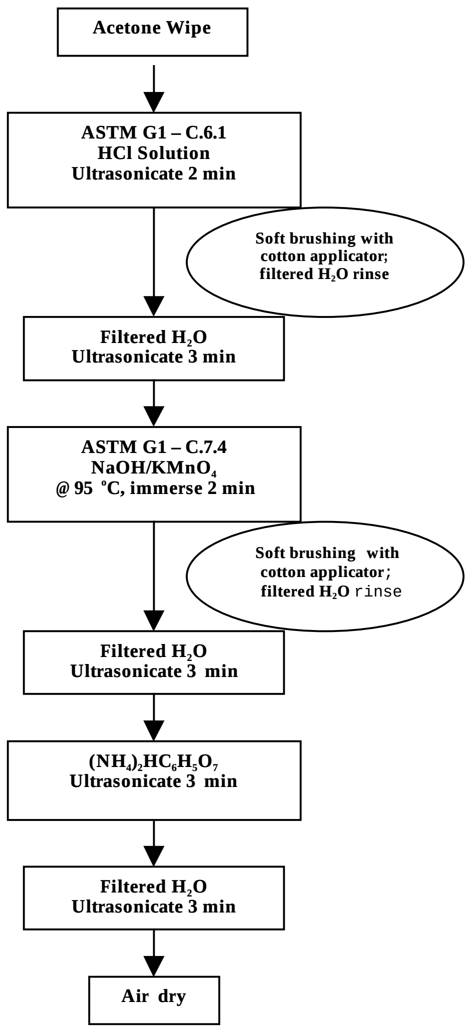
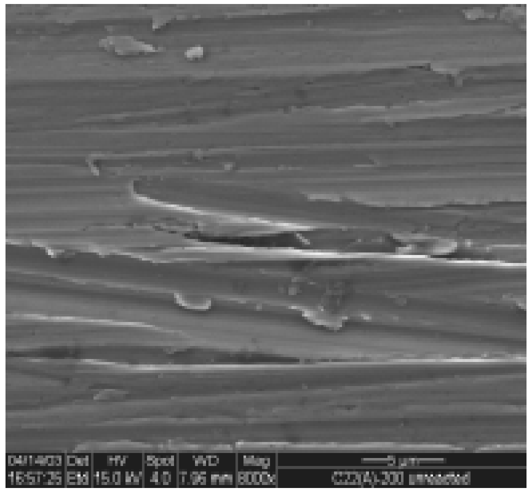


FIGURE 2. Cleaning procedure for Alloy 22 coupons. Procedure is a combination of two ASTM G1 methods for cleaning nickel and nickel alloys, and stainless steels.


Det HV Spot WD Mag
15.0 kV 4.0 7.96 mm 8000x
5 µm