Microbiologically influenced corrosion (MIC) studies deal with the role microorganisms have on electrochemical processes leading to corrosion.\(^1\)\(^-\)\(^3\) As bacteria and fungi interact with the metal surface and its environment, they are able “to initiate, facilitate or accelerate the corrosion reaction without changing its electrochemical nature.”\(^4\)\(^-\)\(^6\) The ubiquitous nature of some microorganisms can present a challenge to MIC studies, where contamination with species of diverse metabolic capabilities can alter the result of the experiments. Therefore, minimizing contamination from foreign microorganisms to maintain a microbial community that properly reflects the desired microbial composition (i.e. these being an environmental sample or a specifically defined community) is of critical importance in MIC research.

Most of the components used in an MIC study (i.e. glassware, reactors or electrochemical cells, and solutions) can be sterilized following standard sterilization procedures\(^5\) used by microbiologists; however, there is no consensus regarding sterilization procedures for metal samples in the literature. In the ideal situation, the sterilization methodology should kill all microorganisms and spores on the metal, but it should not alter its surface nor affect the inherent susceptibility of the metal to corrosion while adequately deactivating biological activity. However, there is no consensus in the literature regarding such procedures due to, in part, the lack of a universally accepted methodology. This investigation evaluates various sterilization methods for carbon steel concerning practicality, efficacy, and effects on the electrochemical response of the metal. Three sterilization procedures using i) dry heat, ii) ethanol, or iii) glutaraldehyde as sterilizing agents were evaluated. Even though all sterilization approaches were equally effective in eliminating microorganisms and spores from the metal surface, dry heating at 170°C in an inert atmosphere was identified as the most convenient sterilization method regarding practicality and consistency in the electrochemical response of the metal. Sterilization of carbon steels in 75 vol% ethanol and glutaraldehyde, as well as alcohol followed by flaming, is discouraged given the large dispersion in corrosion response caused by the exposure to the sterilization media.

Minimizing contamination of control treatments in microbiologically influenced corrosion (MIC) studies is of critical importance. Metal sterilization procedures should not alter the surface nor affect the inherent susceptibility of the metal to corrosion while adequately deactivating biological activity. However, there is no consensus in the literature regarding such procedures due to, in part, the lack of a universally accepted methodology. This investigation evaluates various sterilization methods for carbon steel concerning practicality, efficacy, and effects on the electrochemical response of the metal. Three sterilization procedures using i) dry heat, ii) ethanol, or iii) glutaraldehyde as sterilizing agents were evaluated. Even though all sterilization approaches were equally effective in eliminating microorganisms and spores from the metal surface, dry heating at 170°C in an inert atmosphere was identified as the most convenient sterilization method regarding practicality and consistency in the electrochemical response of the metal. Sterilization of carbon steels in 75 vol% ethanol and glutaraldehyde, as well as alcohol followed by flaming, is discouraged given the large dispersion in corrosion response caused by the exposure to the sterilization media.

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Table I. Summary of sterilization methodologies in MIC studies involving plain carbon steels. Methods are described as they appear in the literature. Over 200 papers dealing with MIC in carbon steels were surveyed. This list only includes papers describing sterilization procedures in enough detail to be reproduced by other laboratories.

| Authors (year)                      | Alloy                                              | Metal sterilization/preparation procedures (after polishing, if applicable)                                                                 |
|------------------------------------|----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Beech and Gaylarde (1991)          | BS970 (MS-II), SAE1020 (MS-I), BS316 (SS-II), AISI304L (SS-I) | Stubs were autoclaved in watertight containers; before exposure to bacterial cultures, steels were immersed in 70 vol% alcohol, flamed, placed inside sterile universal bottles and allowed to cool |
| Castañeda and Beneton (2008)       | SAE-1018 carbon steel (UNS G10180) coupon           | Coupons were sterilized in 70 vol% ethanol solution for 30 min and maintained under sterile laminar air flow before the immersion in the electrochemical cells until use |
| de França and others (1996)        | AISI 304 stainless steel (UNS S30400)               | Coupons placed in field conditions were not sterilized; however, to test the direct effect of seawater, metal coupons were placed inside flasks containing 100 mL of seawater and sterilized at 120°C for 20 min |
| Dorsey and others (2005)           | Multielectrode array sensor probes and UNS G10180 monitoring coupons in flow loop | Flow loop was “sterilized” by circulating 2000 ppm chlorine solution for 30 minutes, followed by treatment with non-oxidizing biocide (glutaraldehyde) overnight. A small amount of glutaraldehyde was also added to the water following sterilization to provide additional protection from contamination during the first days of testing |
| Eckert and others (2006)           | Coupons machined from API 5L X42 grade pipe steel   | Coupons were ultrasonically cleaned in absolute ethanol and finally rinsed in acetone. After cleaning, the coupons were packaged in individual polyethylene bags in a dry nitrogen environment to prevent contact with moisture. The coupons were not exposed to air until immediately before installing in the test system. |
| Edyvean and others (1992)          | AISI 316 stainless steel (UNS S31600) as part of modified Robbins devices (MRD) | MRD were sterilized by filling them with 2.5 vol% hypochlorous acid (commercial bleach) and allowed to stand for 2 hours; then, they were rinsed with sterile deionized water. |
| González-Rodríguez and others (2008) | AISI 1018 (UNS G10180) mild steel coupons           | Cleaned coupons were sterilized with ethanol before exposure to the experimental media |
| Gu and others (1998)               | AISI 316 (UNS S31600) stainless steel coupon as a part of EIS cells | The internal and external surfaces of the EIS cells were sterilized with 70 vol% ethanol and dried at room temperature in a laminar flow sterile hood. |
| Li and others (2001)               | SAE 1018 (UNS G10180)                               | Coupons were degreased with alcohol and then rinsed with sterilized, deionized water. Electrodes were sterilized in 70 vol% alcohol for 2 hours and then stored in clean bench until use |
| Lugauskas and others (2009)        | Low carbon steel, composition (wt%): 0.05–0.12 C, 0.003–0.10 Cu, and <0.07 P | Coupons were treated with a fine suspension of Mg(OH)₂ and high purity acetone to minimize initial contamination of the surface with nutritive substances from the environment. |
| Miyanaga and others (2007)         | Carbon steel coupons composition (wt%): 99.71 Fe, 0.03 C, 0.01 Si, 0.19 Mn, 0.013 P, 0.0017 N, 0.026 Al | The coupon surface was disinfected by transilluminator for 5 min. |
| Peng and Park (1994)               | Steel coupons composition (wt%): 0.01 Si, 0.01 P, 0.19 Mn, 0.01 S, and 99.78 Fe | coupons were degreased with 100 vol% ethanol, rinsed with acetone and distilled water, dried with N₂ blower, and stored in a desiccator before use; to inactivate microorganisms, glutaraldehyde was added to the non-inoculated solution. |
| Rodin and others (2000)            | Mild steel                                         | Clean coupons were placed in test tubes that were sterilized at 170°C for 3 hours. Wires were immersed in baths of acetate and 1.5 N HCl for 15 min. Cleaned wires were threaded through butyl rubber stoppers and the protruding regions on both sides were coated with an anticorrosive lacquer. The stopper/wire system was then fitted to serum bottles with sterile medium and purged with N₂. |
| Royer and Unz (2002)               | Music spring quality steel wire (0.216 mm diameter, ASTM A228/A228M) | Coupons were sterilized by filling them with 2.5 vol% hypochlorous acid (commercial bleach) and allowed to stand for 2 hours; then, they were rinsed with sterile deionized water. |
| Stadler and others (2008)          | Pure iron (Armco), carbon steel ST37, stainless steel AISI 304 (UNS S30400) | Cleaned coupons were sterilized in 70 vol% ethanol solution for 30 min and maintained under sterile laminar air flow before the immersion in the electrochemical cells until use. |
| Stranger-Johannessen (1987)        | Painted steel plates                               | Plates were sterilized by dipping in 0.1 vol% hydrogen peroxide prior to inoculation |
| Tanji and others (2002)            | Carbon steel coupons composition (wt%): 99.71 Fe, 0.03 C, 0.19 Mn, 0.017 S, 0.013 P, and 0.01 Si, mounted in epoxy resin | Before being attached to the epoxy resin, polished coupons were cleaned ultrasonically in acetone for 15 minutes, air dried, and stored in a desiccator. |
| Valencia-Cantero and others (2003) | High-carbon steel (1 wt% C) and plain carbon steel (0.015–0.020 wt% C) | High-carbon steel coupons were cleaned by soaking in acetone for 30 min and then they were brushed with a toothbrush, using a modification of the method of Haruta et al. (1991). The carbon steel coupons were cleaned using a slight modification of the method of Bryant et al. (1991), subjected to ultrasonication in citric acid (5 wt%) for 5 min, and then rinsed in distilled water for 1 min. The coupons were flame and weighed, then placed in closed culture tubes containing salt-rich culture medium V9. |
| Wen and others (2006)              | C1018 (UNS G10180) cylindrical coupon used as working electrode in bioreactor | The bioreactor was autoclaved before each run. |
| Yuan and Pehkonen (2007)           | Stainless steel AISI 304 (UNS S30400)              | Coupons were rinsed with deionized water thrice, followed by degreasin with acetone, then sterilized by immersion in 70 vol% ethanol for 8 hours and dried aseptically in air. Newly prepared samples were immediately exposed to the test medium. |

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selected because of known damaging effects of moist heat on carbon steel, and because isolating the coupon on a sealed container before autocloving could reduce the efficacy of the method by limiting steam-microorganism contact, which is necessary for optimal sterilization by autocloving. Similarly, the use of oxidizing agents such as chlorine compounds (e.g. sodium hypochlorite solutions) or hydrogen peroxide solutions was avoided for similar reasons.

As shown in Table III, one of the selected methods involved using dry heat (i.e. drying oven). Even though the main disadvantages of dry heat for sterilization purposes are the slow heat penetration and microbial killing rates, it is a reliable sterilization method commonly used in healthcare facilities. The most commonly used time-temperature relationships are 170 °C for 60 minutes, 160 °C for 120 minutes, and 150 °C for 150 minutes; warming up and cooling down times should be added to the total time. For this study, samples were treated at 170 °C for 3 hours (i.e. including warming up and cooling down times) based on the temperature profile of the oven. Although the gas composition of the atmosphere at which the coupon is exposed does not influence the killing efficacy of the method, it might affect the surface properties of the specimen when heated, possibly affecting the corrosion response of the sample. Moreover, heat itself can promote the formation of surface oxides. Therefore, specimens for electrochemical testing were dry heated under two different atmospheric conditions: i) filtered air and ii) an inert argon atmosphere.

To perform dry heat sterilization, 100 mL heat resistant glass flask containing the polished, clean coupons were flushed for about one hour. Flushing was performed through a hole in the flasks’ heat resistant screw threaded cap, which had PTFE-silicone septa that were thermally stable up to 180 °C. The temperature profile of the oven was established before sterilization. The temperature profile was obtained using thermocouples placed inside the flasks, positioned at the center of the vial. During dry heat sterilization, samples were located within the plateau of the oven. After sterilization, flasks remained sealed until testing.

Another sterilization method consisted in using an ethyl alcohol solution in water as a chemical disinfectant. In healthcare facilities, ethyl alcohol solutions in water (60–90 vol%) are rapidly bactericidal, tuberculocidal, fungicidal, and virucidal. Water solutions have been found more efficient in denaturing proteins than 200-proof ethyl alcohol solutions in water (60–90 vol%) are rapidly bactericidal, tuberculocidal, fungicidal, and virucidal.

Table III. Sterilization methods.

| Table II. Alloy nominal composition (wt %). |
|--------------------------------------------|
| UNS designation  | AISI designation  | C  | Mn  | P (max) | S (max) |
|------------------|------------------|----|-----|---------|---------|
| G10100           | 1010             | 0.08–0.13 | 0.30–0.60 | 0.040 | 0.050 |
| G10180           | 1018             | 0.15–0.20 | 0.60–0.90 | 0.040 | 0.050 |

Efficacy of sterilization methods.—The effectiveness of all sterilization procedures was determined based on the ability to cultivate organisms after treatment of the coupons as follows. In the direct method, sterilized samples were incubated for 3–5 days under aerobic conditions at room temperature in a sterile rich medium (Luria-Bertani, hereafter LB, broth containing per liter: 5.0 g of yeast extract (YE), 10.0 g of tryptone, and 10.0 g of NaCl, pH 7.4), which was inoculated with 105 colony forming units (cfu) of aerobic bacteria, with the exception that no C sources were included. YE was not added to the electrolytes used in electrochemical tests given that YE has been shown to affect corrosion measurements. The composition of the solution was (per liter): 3.0 g of NaNO3, 0.7 g of KH2PO4, 0.3 g of MgSO4.7H2O, 0.01 g of CaCl2.2H2O, 0.02 g of FeSO4.7H2O, 0.5 g of NaCl, 0.01 g of MnCl2.4H2O, and 2 mL of trace element solution. The composition of the trace element solution was (per liter): 0.75 g of MnSO4.7H2O, 0.15 H2BO3, 0.08 g of FeCl3.6H2O, 0.08 g of CoCl2.6H2O, 0.075 CuSO4.5H2O, and 0.05 g Na2MoO4.2H2O. The pH of the solution remained between 4.50–4.70 during testing.

The third and final sterilization method consisted of using a glutaraldehyde solution. According to Rutala and others, activated aqueous glutaraldehyde solutions (i.e., alkaline solutions) have gained acceptance as a disinfectant and chemical sterilant in healthcare settings because of their microbiocidal properties and non-corrosive action on equipment. Usually, 2 vol% glutaraldehyde solutions buffered to pH 7.5–8.0 are effective in killing microorganisms; however, long exposure times are required. Additionally, special safety measures are recommended due to the known health hazards associated with glutaraldehyde exposure.

Therefore, for this method, coupons were immersed in 2 vol% glutaraldehyde (pentane-1,5-dial) aqueous solution, pH 8.0, for 15 hours. After immersion, coupons had to be used immediately for electrochemical testing. As with ethyl alcohol, exposure times were selected to replicate an “overnight” exposure.

Another sterilization method consisted in using 2 vol% glutaraldehyde solution. According to Rutala and others, activated aqueous glutaraldehyde solutions (i.e., alkaline solutions) have gained acceptance as a disinfectant and chemical sterilant in healthcare settings because of their microbiocidal properties and non-corrosive action on equipment. Usually, 2 vol% glutaraldehyde solutions buffered to pH 7.5–8.0 are effective in killing microorganisms; however, long exposure times are required. Additionally, special safety measures are recommended due to the known health hazards associated with glutaraldehyde exposure. Therefore, for this method, coupons were immersed in 2 vol% glutaraldehyde (pentane-1,5-dia) aqueous solution, pH 8.0, for 15 hours. After immersion, coupons had to be used immediately for electrochemical testing. As with ethyl alcohol, exposure times were selected to replicate an “overnight” exposure.

For a second independent confirmation of the sterilization efficacy, an indirect, reverse sterilization approach was used. Deliberately contaminated clean, as-polished coupons were sterilized according to the same methods described above. Before sterilization, coupons were immersed in an *Escherichia coli* ATCC25540 culture grown overnight aerobically in LB broth. After sterilization, coupons were incubated and tested for bacterial growth following the direct method procedure. For this approach, controls included flasks containing (i) sterilized medium and no coupons (control 1), and (ii) sterilized medium and non-sterilized coupons (control 2). Glassware as well as all other instrumentation was sterilized before use by autoclaving and by ethanol immersion followed by flaming immediately prior to use.

After incubation, microbial growth was determined using two independent techniques: i) spectrophotometry and ii) fluorescent microscopy. For spectrophotometry, absorbance was determined at a wavelength of 600 nm for all sterilization treatments and controls. Additionally, transmitted light was used in conjunction with DAPI (4',6-diamidino-2-phenyindole) staining on the incubated media from each treatment and controls for observation of cells under a fluorescent microscope. This blue-fluorescent stain (excitation maximum at 358 nm and emission maximum at 461 nm), widely used in fluorescence microscopy, stains DNA specifically.

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Electrochemical methods.—Solutions for electrochemical testing.—The synthetic medium used for testing the corrosion response of the metal after sterilization procedures had a chemical composition similar to solutions used for growth of common fouling bacteria, with the exception that no C sources were included. YE was not added to the electrolytes used in electrochemical tests given that YE has been shown to affect corrosion measurements. The composition of the solution was (per liter): 3.0 g of NaNO3, 0.7 g of KH2PO4, 0.3 g of MgSO4.7H2O, 0.01 g of CaCl2.2H2O, 0.02 g of FeSO4.7H2O, 0.5 g of NaCl, 0.01 g of MnCl2.4H2O, and 2 mL of trace element solution. The composition of the trace element solution was (per liter): 0.75 g of MnSO4.7H2O, 0.15 H2BO3, 0.08 g of FeCl3.6H2O, 0.08 g of CoCl2.6H2O, 0.075 CuSO4.5H2O, and 0.05 g Na2MoO4.2H2O. The pH of the solution remained between 4.50–4.70 during testing.
A separate set of samples was tested in 0.1 M NaCl pH 4.50 to compare the corrosivity of the synthetic medium to that of an electrolyte commonly used in corrosion investigations.

Electrochemical tests.—Electrochemical measurements were conducted using a conventional 3-electrode array. A platinum mesh was used as counter electrode, and a saturated calomel electrode (SCE) was used as a reference electrode. The reference electrode was connected to the solution through a Luggin capillary that was 1 mm in diameter and which was positioned between 2–3 mm from the sample. All tests were performed using a conventional potentiostat. A minimum of four samples was used per sterilization method, and four extra coupons were used as non-sterilized controls (control 5). An indirect assessment of the changes in surface condition caused by each sterilization treatment was obtained by comparing the corrosion response between sterilized and non-sterilized samples.

Electrochemical tests included:

Potentiodynamic polarization tests - Tafel slopes.—Potentiodynamic polarization tests were performed to determine Tafel slope values, necessary for corrosion rate (C.R.) calculations. Tests were performed after reaching a stable, i.e. ±10 mV, open circuit potential (Eoc), which usually occurred after 1 hour of exposure, at a scan rate of 0.167 mV/s. The potential was scanned from −200 mV to +200 mV vs. Eoc.

Potentiodynamic polarization resistance measurements.—Potentiodynamic polarization resistance measurements were performed according to the ASTM G59 specification with the following modifications. Tests were conducted after free corrosion potential stabilization, which typically took 60 minutes. Potentiodynamic polarization resistance measurements were taken at a scan rate of 0.167 mV/s. In this regard, the scan rate of 0.167 mV/s recommended by Mansfeld and Kendig and Townley. The potential range was +/−25 mV vs. Eoc. Polarization resistance (Rp) values were obtained as the slope of the potential versus current density plot at i = 0. RP values were then used to calculate corrosion rates via the Stern-Geary equation. Tafel slopes were used as inputs for this calculation; more details about these calculations are presented in the Results section.

Non-electrochemical techniques.—Electrical Resistance (ER) probes.—An ER instrument was used to estimate the corrosion rate of carbon steel in ethanol and glutaraldehyde solutions. The studies were conducted using a UNS G10100 (AISI 1010) carbon steel probe with a wire-loop geometry. Probes were exposed for 20 days. The anticipated response time, i.e. the minimum time in which a reliable, measurable change takes place, of the probe was between 2 and 6 days, depending on the anticipated corrosion rate. Changes in electrical resistance were converted to corrosion rate values using the recommendations of the manufacturer.

Measurement of mass changes during isothermal heat treatments.—Isothermal heat treatments were performed to determine the oxidation kinetics of the material. Rectangular UNS G10100 (AISI 1010) carbon steel specimens with an approximate area of 8 cm2 were used. Five different temperatures were evaluated: 180, 300, 400, 500 and 600 °C. All tests were conducted in duplicate. Specimens were introduced into a furnace at room temperature. The furnace was then heated to the desired temperature, and it was maintained for 2 hours. The furnace was subsequently turned off, and the specimens were allowed to cool down in the furnace. Samples were weighed in an analytic balance to the nearest 0.1 mg to determine weight loss or gain due to the different heat treatments.

Reproducibility and statistical analysis.—Tests to evaluate the efficacy of the various sterilization methods were conducted in triplicates (direct approach) and duplicates (indirect approach).

All electrochemical measurements were repeated at least in quadruplicates. An additional test was carried out when a large dispersion in corrosion rate values was observed (e.g. ethanol).

Given that potentiodynamic polarization resistance measurements can either under- or over-estimate polarization resistance depending on the direction of the polarization, each potentiodynamic polarization resistance measurement was repeated 6 times per sample, 3 in the forward direction and 3 in the backward direction, verifying minimum hysteresis between scans. For each sample, reported Rp values are an average of these 6 measurements. Heat treatments to determine oxidation kinetics of plain carbon steel were performed in duplicate.

The range was used to estimate the variability in the results given that the number of replicate independent values, n, was small, i.e. n < 12. The range, w, is defined as the difference between the maximum and minimum values in a set of replicate data values. As explained in ASTM G16, w makes no assumption about the distribution of error given its non-parametric nature. Because in all instances n < 12 and assuming a standard distribution, the standard deviation, S, was approximated as:

$$S = \frac{w}{\sqrt{n}; n < 12}$$

Results

Table IV summarizes all control cases described throughout this study. Controls 1–4 are the control conditions used to evaluate the efficacy of the different sterilization procedures. Control 5 represents control conditions for all electrochemical tests.

Results of the direct sterilization procedure are shown in Figure 1. Figure 1a shows mean absorbance values measured at 600 nm for samples sterilized following the direct sterilization approach, whereas Figure 1b shows those values for the reverse sterilization method. In both cases, samples were grouped by sterilization method.

Figure 2 compares potentiodynamic polarization resistance diagrams taken in the forward direction for the main sterilization procedures against the control (control 5). The average polarization resistance of six consecutive measurements, three in the forward direction and three in the reverse direction, is also included in each figure for comparison.

Polarization resistance values in $\Omega \cdot \text{cm}^2$ were obtained using conventional corrosion analysis software, as:

$$R_p = \left( \frac{\partial i}{\partial \epsilon} \right)_{E=E_{oc}}$$

where $\epsilon$ is $E_{oc}$ in volts.

Figure 3 compares anodic and cathodic polarization curves for the main sterilization procedures against control 5. For each replicate, Tafel slopes were estimated using conventional corrosion analysis software. Anodic Tafel slopes were determined at least 50 mV away from the oxidation kinetics of the material.
from E_{DC}, which gave a maximum error of approximately 7%.\textsuperscript{50} Anodic Tafel slope values were in line with values reported by Stern and Weisert.\textsuperscript{51} Since cathodic curves were under mass-transfer control in all cases, Figure 3, cathodic Tafel slopes were approximated as $\beta_c \rightarrow \infty$. Given that the Stern-Geary coefficient, $B$:

$$B = \frac{\beta_a \cdot \beta_c}{2.303 \cdot (\beta_a + \beta_c)}$$

is dominated by the smaller of the two Tafel slopes, $B$ reduces to $B = \beta_a / 2.303$ under cathodic mass transfer control.\textsuperscript{46,52}

$$\epsilon = E - E_{DC} (V)$$

The corrosion current density, $i_{corr}$, in $\mu A/cm^2$ was subsequently determined as:

$$i_{corr} = \frac{B}{R_p}$$

and converted to corrosion rates in mm/y using:\textsuperscript{53}

$$CR = 3.27 \cdot 10^{-3} \cdot \frac{i_{corr} \cdot EW}{\rho}$$

where EW is the equivalent weight of the corroding species in grams per equivalent and $\rho$ is the density of the alloy in g/cm$^3$. EW and $\rho$ were approximated as 27.97 g/eq and 7.87 g/cm$^3$, respectively.\textsuperscript{54}

The solution resistance, $R_s$, was measured on a separate set of specimens exposed to either synthetic growth medium or 0.1 M NaCl.

Figure 1. Mean absorbance values measured at 600 nm for samples used in (a) direct and (b) reverse sterilization efficacy confirmation. Error bars correspond to standard deviation values. The deviation is smaller than the symbols for points that do not have error bars. Arrow points to treatment where bacterial growth was observed after incubation. Results grouped by sterilization method.

Figure 2. Typical UNS G10180 potentiodynamic polarization resistance curves for the main sterilization procedures, taken in the forward direction: (a) control 5—dashed line—and dry heat (Ar) and (b) ethanol (18h) ethanol (flame), and glutaraldehyde. $R_p$ values shown in the figures represent the average of six consecutive scans, three in the forward direction and three in the backward direction. All tests conducted in synthetic medium.
The corrosion rate of UNS G10100 (AISI 1010) in 75 vol% ethanol and 2 vol% glutaraldehyde as measured by a wire-loop ER probe is shown in Figure 5. In Figure 5, a vertical dashed line indicates the response time of the probe. Since the response time of an ER probe depends on the anticipated corrosion rate, a conservative response time was calculated by assuming an average corrosion rate of 0.05 mm/y. Data points taken below the probe’s response time are indicated by dashed symbols. Finally, Figure 6 summarizes weight change as a function of temperature.

Discussion

Sterilization efficacy.— All sterilization methods deactivated microorganisms on the metal surface. Solutions from direct sterilization treatments showed no difference in optical density (OD) when compared to the sterilized medium containing no coupons (control 1,
Table V. Corrosion rate measurements obtained by the potentiodynamic polarization resistance method.

| Treatment                      | AVG OCP (V_SCE, SCE) | STD E_{OC} (mV_SCE) | AVG Anodic Tafel Slope, \( \beta_a \) (V/decade) | AVG Cathodic Tafel Slope, \( \beta_c \) (V/decade) | Mean C.R. (mm/y) | Max. C.R. (mm/y) | Min. C.R. (mm/y) | C.R. Range, \( w \) (mm/y) | C.R. STD, S (mm/y) |
|--------------------------------|----------------------|---------------------|-----------------------------------------------|-----------------------------------------------|-----------------|-----------------|-----------------|---------------------|------------------|
| Control 5                      | −0.693               | 8.1                 | 0.093                                         | \( \infty \)                                 | 0.797           | 0.847           | 0.715           | 0.132               | 0.0660           |
| Dry heat (Ar)                  | −0.678               | 3.3                 | 0.092                                         | \( \infty \)                                 | 0.692           | 0.751           | 0.586           | 0.165               | 0.0825           |
| Dry heat (air)                 | −0.674               | 7.4                 | 0.083                                         | \( \infty \)                                 | 0.578           | 0.762           | 0.403           | 0.360               | 0.1800           |
| 75 vol% Ethanol, 15 hours      | −0.687               | 10.7                | 0.107                                         | \( \infty \)                                 | 0.644           | 0.822           | 0.495           | 0.327               | 0.1635           |
| 75 vol% Ethanol, 18 hours      | −0.677               | 70.64               | 0.111                                         | \( \infty \)                                 | 0.461           | 0.879           | 0.030           | 0.849               | 0.379            |
| 75 vol% Ethanol, Flame         | −0.679               | 56.7                | 0.108                                         | \( \infty \)                                 | 0.323           | 0.631           | 0.106           | 0.524               | 0.234            |
| 2 vol% Glutaraldehyde          | −0.664               | 4.4                 | 0.099                                         | \( \infty \)                                 | 0.449           | 0.593           | 0.208           | 0.385               | 0.1925           |
| 0.1 M NaCl pH 4.50             | −0.548               | 13.6                | 0.057                                         | \( \infty \)                                 | 0.234           | 0.284           | 0.136           | 0.148               | 0.074            |

Figure 1a). Furthermore, microorganisms were not detected by microscopy. Only solutions from non-sterilized controls (control 2, Figure 1a) showed increased optical density; the presence of microorganisms was later confirmed visually under the microscope.

Reverse sterilization tests, in which microorganisms were intentionally grown on the surface, provided further evidence of the efficacy of the different treatments used in this study. No difference in growth medium optical density was observed in almost all sterilization methods when compared to flasks containing sterilized medium exclusively (control 3, Figure 1b). The absence of microorganisms was later confirmed under the optical microscope. The only treatment that showed a slight increase in optical density was dry heat. No microorganisms were detected by microscopy, but rather the presence of debris from the oven-dried biofilm that had formed during the deliberate contamination that led to a slightly higher OD in LB medium.

As expected, contaminated controls (control 4, Figure 1b) had increased optical density, associated with microbial contamination, which was later confirmed by optical microscopy.

Because in the direct sterilization approach specimens were immersed in a rich growth medium, it is plausible that microorganisms that cannot be cultured in this commonly used broth might have survived the sterilization process. In that case, the direct approach would not have detected the presence of such microorganisms. However, the efficacy of the sterilization methods was also assessed following a reverse procedure, determining their effectiveness in killing living bacteria (i.e. *E. coli* ATCC25404.) The results presented herein suggested that all sterilization methods eliminated living bacteria from the metal surface when intentionally contaminated. Furthermore, contamination did, in fact, occur in the untreated control case but did not in the sterilized samples, implying that all sterilization methods were capable of eliminating not only living microorganisms but also, at a minimum, all those that can be cultured in the LB broth. The use of different growth media and complimentary detection techniques such as culture independent approaches to detecting activity could give additional information about sterilization efficacy, which was outside the scope of this work.

**Electrochemical tests.**—Understanding the effects of sterilization treatments on the corrosion behavior of the alloy is critical to MIC research. The ideal sterilization treatment will effectively kill all living microorganisms and spores on the surface without altering the surface condition of the sample, assessed here by the electrochemical response of the material. Corrosion rates, therefore, have to be similar on average, with a dispersion (estimated using the range) within the variability of the untreated control. In this regard, a large corrosion rate...
corrosion rates represent 20% and 60% of the accepted corrosion rate of carbon steel in aerated, quiet (i.e. moving at less than 0.6 m/s) seawater.57,58.

**Influence of the electrolyte composition.**—In addition to the effect of the sterilization treatment, the influence of the electrolyte used for corrosion testing has to be considered. MIC research is typically conducted using complex electrolytes that add a significant number of components necessary to promote microbial growth. As discussed by Webster and Newman,59 the corrosivity of these nutrient-rich synthetic media can be quite different from that of the actual service environment, e.g. fresh- and seawater.

Figure 4 shows that the corrosion rate of carbon steel (control 5) in the complex solution used herein was, on average, 3.4 times faster than that in a 0.1 M NaCl solution tested at the same pH value of 4.50. Although short-term laboratory experiments in simulated environments cannot be used to predict long-term corrosion rates in natural environments, it is illustrative to compare the short-term behavior of carbon steel in the synthetic electrolyte employed in this investigation with the long-term behavior in fresh- and sea-water.37,39 Corrosion rates in the sterile synthetic medium were 8 times faster than reported mean corrosion rates of carbon steel in fresh water.55,56 and 6.35 times faster than reported values for carbon steel in natural seawater.57,58 Comparing corrosion rates from a MIC investigation against typical corrosion rates in service could be misleading. A negative control, i.e. a control without microorganisms, in the actual sterile synthetic medium is necessary to separate the effect of microorganisms from that of the inorganic and organic species added to the electrolyte. In this regard, some compounds can either enhance corrosion (e.g. Fe3+ and NO3−) by acting as added oxidizing agents or slow/inhibit dissolution kinetics by the formation of surface protective films (e.g. phosphates).61,62 YE additions have also been shown to affect corrosion results.29

**Oxidation kinetics.**—The evidence presented above suggested that dry heat was the most convenient sterilization procedure for carbon steel. However, prolonged heating at moderately elevated temperatures could potentially alter the surface properties and microstructure of the steel. Additional tests were performed to determine the maximum allowable temperature for dry heat sterilization.

As seen in Figure 6, even when exposed to air, oxide formation was minimal during dry heat sterilization given the relatively low temperatures of the procedure. Likewise, Figure 6 shows that weight gain due to oxidation was measurable with an analytical balance only at temperatures above 400°C. Weight gain during carbon steel and iron oxidation is sensitive to the amount of cold work and sample preparation methods like electropolishing or mechanical grinding.63 Despite this, weight gain of samples exposed to temperatures above 400°C was in reasonable agreement with the value expected using parabolic growth law with coefficients reported in the literature.64–66 For samples exposed to 400°C or above, the corrosion rate in the test solution exhibited a drastic decrease (not shown), probably due to the formation of protective oxides. Oxide growth should not be an a priori matter of concern for sterilization of carbon steel when conducted at 150–180°C.

**Use of dry heat sterilization on other alloys.**—Based on the results presented herein, it may be tempting to assume that dry heat sterilization in inert atmospheres could be used on other alloys. Even though no microstructural changes on carbon steel due to sterilization treatments at 150 to 180°C are expected based on the kinetics of the Fe-C-Mn system, it is well known that prolonged heating at moderately elevated temperature can produce grain growth and aging of precipitation hardenable alloys, such as wrought aluminum alloys.67

While dry heat sterilization in an inert atmosphere should work equally well on carbon and low alloy steels as on most stainless steels and nickel-based alloys, heating in the 150–180°C range could result in precipitation hardening of, e.g., most wrought aluminum alloys used in aerospace applications.67 The effect of possible microstructural
changes due to the sterilization procedure must be taken into account in MIRC research.

Conclusions

Based on the evidence presented herein the following conclusions were drawn:

- All sterilization methods were equally effective in eliminating microorganisms and spores from the metal surface as determined by linear polarization measurements. This publication presents a methodology that could be followed by other laboratories to evaluate the effect of alternative sterilization treatments in specific laboratory settings.
- Dry heating at 170°C for 60 minutes (plus heating and cooling times) in an inert atmosphere was the most convenient sterilization method for carbon steels regarding practicality and consistency in the response of the metal to its application.
- While dry heat sterilization in an inert atmosphere should be equally adequate for all carbon, low alloy, and most stainless steels as well as nickel-based alloys, prolonged heating at 150−180°C could lead to microstructural changes in some age-hardenable alloys.
- Sterilization of carbon steels in 75 vol% ethanol and 2 vol% glutaraldehyde as well as alcohol followed by flaming is discouraged given the large dispersion in electrochemical response caused by the exposure to the sterilization media.

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