Influence of Acetobacter Suboxydans on Aged 316l in Sea Water

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Abstract
The present study is pertaining to the influence of biofilm on pitting corrosion of UNS 31603 stainless steel in the sea water medium. These materials are welded and aged at 450°C for 10000 hours. The corrosion behavior of weld materials are compared with similarly aged base metal (BM). Corrosion studies were conducted using potentiodynamic studies in the presence and absence of bacteria G.rose at temperatures 40°C, 25°C and 10°C in sea water medium. Role of bacteria and its susceptibility to pitting were determined by its biofilm presence helps in corrosion protection to certain extent. But severe corrosion occurred when biofilm breakdown. The microbial adhesion was studied using Scanning Electron Microscope (SEM) and Fourier transform Infra Red Spectrometer (FTIR).

Keywords Pitting, FTIR, Biofilm, MIC

1. Introduction

Stainless steels are widely used in marine systems and technology because of their excellent resistance to seawater corrosion [1, 2], a characteristic that results from the formation of a stable passive layer on the metal surface. Nevertheless, this type of alloy remains susceptible to localized corrosion caused by chloride ions [3] and microbial activity [4,5]. Thus, microbiologically influenced corrosion (MIC) of stainless steel can be a serious problem in marine environments. Metal-depositing bacteria are microorganisms that cause MIC. These microorganisms are capable of depositing iron/manganese hydroxides (rust) at the extracellular level at a rate a hundred times higher than that in the abiotic process [6,7]. Hence, iron/manganese-oxidizing bacteria are amongst the most dangerous microorganisms from the perspectives of biofouling and corrosion. They create conditions favorable to localized corrosion, especially in the cases of metal passivity [8]. For stainless steel, the pitting corrosion (caused by microorganisms) in specific areas such as the grain boundary is reported to be caused by the segregation of elements. In fact, metal-depositing bacteria can induce subtle effects on the underlying metal surface, thereby mediating the chemical changes in the passive layer [9]. Such types of bacteria can be detrimental to the integrity of passive oxide films, and facilitate the local depassivation of the protective layer [10]. Therefore, the corrosive agents such as chloride ion into marine environments are promoted, causing localized corrosion on the metal surface [11]. Although, some reports have revealed the relationship between microbial corrosion and localized attacks [12-14], investigations on their mechanisms remain a challenge because these mechanisms can vary depending on microorganism and environmental conditions. Despite the technical importance of stainless steel and the important role of selective dissolution in determining its surface properties, little is known about the selective dissolution of this alloy; because the presence of numerous elements in the alloy also present challenges [15-18]. Thus, the present study aims to investigate the de-alloying of 316 stainless steel (316L SS) in the presence of a mixture of metal-oxidizing bacteria isolated from marine environments. A mixture of bacteria was chosen because they exert influence on one another and create conditions that differ from those obtained using isolated same bacteria. The investigation was carried out by electrochemical, as well as surface and elemental analysis methods.

Although the austenitic grades are generally considered to be very weldable, they are subjected to a number of weldability problems, if proper precautions are not taken. Weld solidification and liquation cracking may occur depending on the composition of the base and filler metal and the level of impurities, particularly S and P [19]. Solid-state cracking, including ductility dip, reheat for stress relief and Cu contamination, have also been encountered in these alloys.

The primary fabrication method for components made of stainless steel is fusion welding (TIG Welding). Austenitic stainless steels are fully austenitic in their wrought condition. In welded condition austenitic stainless steel have duplex microstructure consisting of an austenitic matrix with 3 - 8% delta ferrite. In the case of low temperature aging (<550°C) of weld, the five metallurgical processes namely, spinodal decomposition, precipitation of alpha prime, secondary
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austenite, G-phase and metal carbides are identified as responsible to aging embrittlement of the ferrite phase. Acetobacter suboxydans are used in the present corrosion study. No literature is available with respect to this bacterial corrosion. Hence its role in marine corrosion is found out and corrosion rates are measured.

2. Experimental Procedure

2.1. Welding and Aging and Specimen Preparation

UNS31603 samples were welded by Tungsten Inert Gas Welding without using filler material, but introducing chromium slo as to reduce solidification cracks. Using muffle furnace, aging was done at temperature of 450°C for 10000 hours. Samples from weld region and base metal dimension of 1.5 x 1.5 was cut at height of 1cm. it was then mounted on epoxy resin such that exposed area for corrosion tests was 2.25cm².

The metal surface area was wet ground up to 600 grit finish (SIC grinding paper), degreased with acetone and dried. Test specimens were soaked in distilled water for 3 hrs and sterilized by immersion in 70% ethanol for 1h before assembling and exposure. The composition of the Base Metal (BM) and Weld Metal (WM) are given in Table 1. Corrosion tests were conducted for the samples.

2.2. Bacteria Culturing:

The bacteria G. Rose was obtained from the national collection of industrial microorganisms (NCIM), Pune and cultured in the biotech laboratory of the Chemical Engineering Department, NITK. The details of growing these microorganisms are given below:

Initially the medium for acetobacter suboxydans bacteria was prepared in a conical flask. Composition of the medium is given in Table 2. Then this flask was kept in autoclave at 15psi and 120°C for 15 mins. Then it is kept in shaker for 24 hours for allowing the dormant microbe to grow. After 24 hours of shaking it was then transferred to the media where the sample was kept. Acetobacter suboxydans (G.Rose) was active at temperatures 10°C, 25ºC, and 40°C. These are gram positive rod shaped.

### Table 1. Typical chemical composition of AISI 316L stainless steel.

| Zones | Compositional elements (%) |
|-------|-----------------------------|
| WM    | C  | Si  | Mn  | P  | S  | Cr  | Ni  | Mo  | Al  | Cu  | Co  |
| WM    | 0.052 | 0.7 | 1.97 | 0.015 | 0.0011 | 16.38 | 13.29 | 1.88 | 0.014 | 0.098 | 0.3 |
| Ti    | 0.0036 | 0.018 | 0.032 | 0.014 | <0.003 | 0.0024 | 0.0074 | 0.0027 | 0.0015 | 0.0004 | 65.2 |
| BM    | C  | Si  | Mn  | P  | S  | Cr  | Ni  | Mo  | Al  | Cu  | Co  |
| BM    | 0.057 | 0.69 | 1.94 | 0.012 | <0.001 | 16.37 | 12.89 | 1.85 | 0.012 | 0.102 | 0.301 |
| Ti    | 0.0038 | 0.018 | 0.031 | <0.010 | <0.003 | 0.0022 | 0.0062 | 0.0019 | <0.001 | 0.0009 | 65.7 |

### Table 2. Medium for culturing Acetobacter Suboxydans (G. Rose)

| Sorbitol | 5.0 gms |
| Yeast Extract | 0.5 gms |
| Distilled Water | 100ml |
| Agar | 2.0gms |

Adjust pH to 6.2
2.3. Tafel Method

Tafel test was performed using potentiostat (CHI604A) to investigate the corrosion properties of the samples. The exposed surface area of the sample, which is shown in Fig 1, was 2.25 cm². The test set up is shown in the Fig 2. Here working electrode is the specimen being tested, the calomel electrode is the reference electrode and platinum electrode the counter electrode. These three electrodes are kept in the media for corrosion test in the corrosion cell. The test was performed at a scan rate of 0.01V/s, from -0.2V to +0.8V with respect to the corrosion potential \(E_{\text{corr}}\). Open circuit potential was noted for each experiment.

Corrosion rate (CR) was calculated using the formula:

\[
CR \text{ (mils/yr)} = \frac{0.129 \times (I_{\text{corr}} / A \times EW)}{D}
\]

where,
- \(I_{\text{corr}}\) = Current in Amps.
- \(D\) = density of the specimen material in g/cm³.
- \(A\) = area of the specimen in cm².
- \(EW\) = gram equivalent weight.

3. Results and Discussion

As welding studies have shown the formation of primary ferrite during solidification reduces the vulnerability of these steels to hot cracking. Therefore, the composition of weld filler metals and castings are adjusted to promote primary ferrite solidification. During cooling, most of the primary ferrite transforms to austenite but small residual amount of ferrite is retained at room temperature. The presence of residual ferrite has additional benefits: the strength is increased and the alloy’s resistance to stress corrosion cracking is improved. With the presence of ferrite as a second phase in the welded steel, the stability of these alloys during elevated temperature applications is of concern. Various studies said that delta ferrite transforms into various phases like carbides, sigma, chi and secondary phases etc. When temperature is greater than about 550°C, these transformed products influence the mechanical and corrosion properties [20-22]. From Fig 3(a-b) SEM images shows the presence of biofilm on the metal surface which forms pits on the surface as passivity is not uniform and breakdown of the film. Also in Fig 3(c-f) without microbial film adhesion there is corrosion still rate of corrosion is less when compared it with biofilm adhesion samples. Fig 5 shows the plots if corrosion rates at different temperatures in presence and absence of biofilm. It clearly represents that aged sample at 450°C for 10000 hours are severe corroded when it is compared with as received samples. The biofilm consists of galactose and other compounds. L-galactose reacts with the enzyme L-galactose dehydrogenase where the lactone ring opens and forms again but with between the carbonyl on C1 and hydroxyl group on the C4 resulting in L-galactonolactone. L-galactonolactone then reacts with the mitochondrial flavoenzyme L-galactonolactone dehydrogenase to produce ascorbic acid. An interesting fact about L-ascorbic acid is that it has shown to have a negative feedback on 316L as it oxidizes the surface after exposure to marine water, absorbs salts and forms clusters which break the passive layer formed on the surface. Hence corrosion is more severe.

As a mild reducing agent, ascorbic acid degrades upon exposure to air, converting the oxygen to water. The redox reaction is accelerated by the presence of metal ions and light. It can be oxidized by one electron to a radical state or doubly oxidized to the stable form called dehydroascorbic acid. From Fig 4, 1380 cm⁻¹ suggests the presence of carboxyl or amide groups and the one at 1500 cm⁻¹ can be reasonably described to adsorbed amine groups of proteins. After 24 h immersion, the most intense and broad feature appearing at 1050-1160 cm is characteristic of C-O and C-O-C bonds belonging to carbohydrate groups from polysaccharides. With an increase of the O-H stretch at 3400 cm⁻¹, this simply suggests that the concentrations of both amines and polysaccharides at the surface increase with time. There was increasing surface concentration of compounds containing C-N bonds on the one hand, and C=O and C-O bonds on the other. From Fig 4, 1380 cm⁻¹ suggests the presence of
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**Figure 3.** SEM images of G. Rose on the surface of the metal (a) Cluster of G.Rose on the metal surface at lower magnification. (b) Surface defects at higher magnification in presence of G.Rose. (c) SEM image of as-received BM with microbial adhesion (d) SEM image of as-received WM with microbial adhesion (e) SEM image of BM aged at 450°C for 10000 hours without microbial adhesion (f) SEM image of WM aged at 450°C for 10000 hours without microbial adhesion
Figure 4. FTIR plot of the deposit on the samples 316L BM & WM in the aged and as-received condition in natural sea water.

Figure 5. Plots of corrosion rates Vs exposure time at (a) 40°C, (b) 25°C & (c) 10°C in the presence and absence of bacteria.
### Table 3. Corrosion rates (mpy) of AISI 316L samples in aged and as received condition in static sea water by EC method without G.Rose.

| Months | As received BM | As received WM | Aged BM at 450°C | Aged WM at 450°C |
|--------|----------------|----------------|------------------|------------------|
| 1      | 4.89           | 5.01           | 6.34             | 6.68             |
| 2      | 6.88           | 7.06           | 9.3              | 8.98             |
| 3      | 11.69          | 12.67          | 15.07            | 16.83            |
| 4      | 15.78          | 17.99          | 21.55            | 23.56            |
| 5      | 21.95          | 24.58          | 26.38            | 29.11            |
| 6      | 26.46          | 31.5           | 34.80            | 36.7             |
| 7      | 32.78          | 38.28          | 42.29            | 43.22            |
| 8      | 36.08          | 44.67          | 48.33            | 51.07            |
| 9      | 41.89          | 51.85          | 56.78            | 59.77            |
| 10     | 48.32          | 56.28          | 62.78            | 66.25            |
| 11     | 53.98          | 63.70          | 69.84            | 72.91            |
| 12     | 59.11          | 70.08          | 76.89            | 76.03            |

### Table 4. Corrosion rates of 316L BM & WM in as received condition and aged at 450°C for 10000 hours in presence of bacteria G.Rose

| Months | As received BM | As received WM | Aged BM at 450°C for 10000 hrs | Aged WM at 450°C for 10000 hrs |
|--------|----------------|----------------|---------------------------------|--------------------------------|
| 1      | 5.6            | 6.31           | 8.41                            | 8.76                           |
| 2      | 8.9            | 8.91           | 11.36                           | 11.55                          |
| 3      | 13.66          | 13.81          | 20.14                           | 21.2                           |
| 4      | 18.96          | 19.21          | 29.38                           | 30.14                          |
| 5      | 26.2           | 27.04          | 39.11                           | 40.26                          |
| 6      | 34.5           | 35.33          | 50.04                           | 51.3                           |
| 7      | 40.31          | 41.4           | 59.8                            | 60.23                          |
| 8      | 47.98          | 48.26          | 67.7                            | 68.5                           |
| 9      | 56.88          | 57.29          | 80.14                           | 80.96                          |
| 10     | 63.57          | 64.14          | 92.35                           | 92.56                          |
| 11     | 72.9           | 73.46          | 111.43                          | 114.56                         |
| 12     | 84.07          | 85.1           | 126.51                          | 128.44                         |

### Table 5. Corrosion rates (mpy) of AISI 316L samples in aged and as received condition in static sea water by EC method without G.Rose.

| Months | As received BM | As received WM | Aged BM at 25°C | Aged WM at 25°C |
|--------|----------------|----------------|-----------------|-----------------|
| 1      | 3.56           | 3.38           | 5.98            | 6.12            |
| 2      | 5.32           | 5.81           | 7.64            | 8.28            |
| 3      | 8.5            | 8.79           | 10.91           | 12.9            |
| 4      | 11.58          | 11.9           | 15.77           | 16.70           |
| 5      | 14.39          | 15.4           | 23.58           | 21.67           |
| 6      | 17.99          | 19.6           | 30.48           | 29.67           |
| 7      | 21.65          | 23.78          | 36.99           | 37.23           |
| 8      | 25.45          | 26.94          | 43.08           | 45.77           |
| 9      | 28.37          | 30.04          | 49.7            | 51.38           |
| 10     | 32.03          | 33.35          | 55.82           | 59.36           |
| 11     | 36.11          | 38.69          | 61.11           | 65.08           |
| 12     | 40.09          | 42.79          | 69.87           | 71.50           |
### Table 6. Corrosion rates of 316L BM & WM in as received condition and aged at 450°C for 10000 hours in presence of bacteria G.Rose.

| Months | Corrosion rates (mpy) at 25°C | As received BM | As received WM | Aged BM at 450°C for 10000 hrs | Aged WM at 450°C for 10000 hrs |
|--------|-----------------------------|----------------|----------------|-------------------------------|-------------------------------|
| 1      |                             | 6.21           | 6.74           | 10.11                         | 10.56                         |
| 2      |                             | 8.81           | 8.93           | 12.7                          | 12.9                          |
| 3      |                             | 11.06          | 11.84          | 16.91                         | 17.32                         |
| 4      |                             | 14.8           | 15.11          | 21.44                         | 21.8                          |
| 5      |                             | 20.7           | 21.45          | 33.75                         | 34.21                         |
| 6      |                             | 29.31          | 30.64          | 41.81                         | 41.9                          |
| 7      |                             | 31.44          | 32.49          | 50.64                         | 51.7                          |
| 8      |                             | 40.36          | 41.07          | 61.35                         | 61.5                          |
| 9      |                             | 51.58          | 51.78          | 72.5                          | 72.8                          |
| 10     |                             | 64.95          | 66.05          | 84.64                         | 84.89                         |
| 11     |                             | 72.81          | 73.51          | 97.5                          | 97.8                          |
| 12     |                             | 86.36          | 86.99          | 101.5                         | 101.74                        |

### Table 7. Corrosion rates (mpy) of AISI 316L samples in aged and as received condition in static sea water by EC method without G.Rose.

| Months | Corrosion rates in static sea water at 10°C |
|--------|-------------------------------------------|
| As received BM | As received WM | Aged BM at 450°C | Aged WM at 450°C |
| 1      | 1.31 | 1.67 | 1.89 | 1.87 |
| 2      | 3.56 | 3.58 | 4.32 | 4.69 |
| 3      | 4.93 | 5.21 | 7.11 | 7.45 |
| 4      | 6.72 | 6.88 | 10.08| 10.32|
| 5      | 7.77 | 8.31 | 13.56| 13.67|
| 6      | 8.96 | 10.03| 17.89| 18.83|
| 7      | 10.43| 12.08| 20.8 | 21.79|
| 8      | 12.23| 14.76| 23.65| 25.8 |
| 9      | 14.87| 17.77| 28.66| 29.09|
| 10     | 16.06| 19.54| 32.08| 32.57|
| 11     | 17.99| 22.68| 36.8 | 38.37|
| 12     | 20.1 | 27.9 | 43.2 | 44.76|

### Table 8. Corrosion rates of 316L BM & WM in as received condition and aged at 450°C for 10000 hours in presence of bacteria G.Rose.

| Months | Corrosion rates (mpy) at 10°C |
|--------|-----------------------------|
| As received BM | As received WM | Aged BM at 450°C for 10000 hrs | Aged WM at 450°C for 10000 hrs |
| 1      | 4.66 | 4.84 | 6.81 | 6.96 |
| 2      | 6.31 | 6.5 | 9.65 | 9.88 |
| 3      | 9.44 | 9.5 | 12.64| 12.98|
| 4      | 11.26| 11.51| 15.44| 15.65|
| 5      | 13.8 | 13.86| 18.14| 18.8 |
| 6      | 14.9 | 15.2 | 21.6 | 21.9 |
| 7      | 16.21| 16.5 | 29.8 | 29.04|
| 8      | 18.51| 18.7 | 32.5 | 32.73|
| 9      | 19.44| 19.9 | 35.14| 35.24|
| 10     | 23.5 | 23.86| 39.4 | 39.56|
| 11     | 26.4 | 27.54| 41.73| 42.34|
| 12     | 30.11| 30.55| 45.9 | 45.99|
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Table 9. Values of $I_{corr}$, $R_p$, $\beta_c$, $\beta_a$ from EC methods.

| Condition (Sea Water) | Tafel Extrapolation |
|-----------------------|----------------------|
| Temp ($^\circ$C)      | Zones                |
|                       | $I_{corr}$ (µA)      | $R_p$ (Ω)   | $\beta_c$ (v/dec) | $\beta_a$ (v/dec) |
|                       | Absence of G.Rose    | Presence of G.Rose | Absence of G.Rose | Presence of G.Rose | Absence of G.Rose | Presence of G.Rose |
| As received BM         | 99.16                | 254.6        | 455.2             | 153.0             | 5.52              | 6.357                 | 4.16             | 4.843 |
| 10°C                  |                      |              |                   |                   |                   |                       |                 |
| As received WM         | 135.4                | 299.0        | 329.1             | 117.0             | 5.84              | 6.21                 | 3.94             | 6.19  |
| Aged BM at 450°C for 10000 hrs | 193.0       | 349.0        | 173.6             | 125.2             | 7.06              | 5.69                 | 5.90             | 4.28  |
| Aged WM at 450°C for 10000 hrs | 227.2       | 438.8        | 201.8             | 84.2              | 5.75              | 6.08                 | 3.76             | 5.69  |
| 25°C                  |                      |              |                   |                   |                   |                       |                 |
| As received BM         | 107.6                | 156.2        | 416.2             | 280.3             | 5.99              | 6.04                 | 4.10             | 3.98  |
| As received WM         | 118.8                | 159.8        | 379.2             | 235.7             | 5.99              | 6.55                 | 4.11             | 5.08  |
| Aged BM at 450°C for 10000 hrs | 348.6       | 345.4        | 100.2             | 87.1              | 6.24              | 6.42                 | 6.27             | 8.04  |
| Aged WM at 450°C for 10000 hrs | 431.0       | 438.8        | 78.1              | 84.2              | 6.96              | 6.80                 | 6.77             | 5.69  |
| 40°C                  |                      |              |                   |                   |                   |                       |                 |
| As received BM         | 118.8                | 159.8        | 235.7             | 379.2             | 5.59              | 6.525                | 4.11             | 5.018 |
| As received WM         | 199.4                | 277.8        | 148.7             | 180.4             | 5.79              | 6.55                 | 4.54             | 5.56  |
| Aged BM at 450°C for 10000 hrs | 534.8       | 708.0        | 55.2              | 60.0              | 5.78              | 6.35                 | 4.53             | 8.53  |
| Aged WM at 450°C for 10000 hrs | 299.0       | 345.4        | 87.1              | 117.0             | 6.20              | 6.42                 | 6.97             | 8.34  |

From Fig 5(a-c) corrosion rates in presence of G.Rose is severe at all temperatures of 10°C, 25°C and 40°C. WM at aged condition show severe response to corrosion as ageing for long time causes decomposition of the delta ferrite into second phase particles. Aging for long duration causes recrystallisation and grain growth. This increases grain boundary attack as ferrite content in weld metal (WM) decomposes to form carbides and second phase particles which is confirmed in [22]. Hence corrosion is more in aged WM and BM. Also the EC results obtained in Table 3 – 9 shows the severity of the corrosion in aged samples. Table 3-8 shows the corrosion rates and this clearly indicates that presence of G.Rose increase corrosion also in aged samples at 450°C for 10000 hrs. Table 9 shows the increase in $I_{corr}$ values when G.Rose is present. Hence $I_{corr}$ is directly proportional to corrosion rates. Polarisation resistance ($R_p$) is high in absence of G.Rose and its value decreases when G.Rose is induced.

4. Conclusions

Acetobacter suboxydans G.Rose cause severe corrosion to 316L in sea water. Hence corrosion in sea water is more due to the chemical reactions and organic and inorganic mixtures depositing on the metal surface. Along with these bacteria metallurgical factors like grain growth due to recrystallisation also play role in supporting the corrosion. Carboxyl groups support corrosion and increase corrosion rate in presence of G.Rose. Pitting was severe in presence of G.Rose.

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