Department of Chemistry

Microbiologically Influenced Corrosion of Common Alloys Used For Subsea Applications

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To my lovely husband and soul mate, Andrés; my wonderful parents, Lilia and Antonio; my beloved sister, Fabiola and my dear sweet niece, Valentina.

For their endless love, support and encouragement.

The role of the infinitely small in nature is infinitely large.

LOUIS PASTEUR (1822-1895)

DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Laura Lizeth Machuca Suarez
Perth, October 12th 2012

ABSTRACT

Microbiologically Influenced Corrosion (MIC) is an electrochemical process in which microorganisms initiate, facilitate, or accelerate metal corrosion reactions. MIC has been reported to account for 50 per cent of the total cost of corrosion. In particular, MIC of oil and gas production equipment and pipelines has significant costs and potentially serious environmental repercussions. The complex interactions between chemical, electrochemical, and biological factors in the environment near the material surface have made identification of MIC mechanisms difficult. In particular, the preservation of subsea equipment is a significant knowledge gap in the current understanding and control of MIC. To date, there is limited information on the interactions of high corrosion-resistance alloys with microorganisms in natural seawater, particularly those associated with hydrostatic testing of pressure equipment, ballast and the preservation of sunken subsea equipment. Furthermore, the susceptibility of high-resistance alloys to MIC in seawater at typical temperatures for offshore assets is yet to be quantified and poses a significant dilemma for materials engineers. Incorrect materials selection can be costly and increases the risk of serious environmental damage from premature equipment failure.

In this research, MIC was investigated for several offshore construction alloys including UNS S31603, UNS S31803, UNS S32750, UNS 31254 stainless steels and UNS N08825 and UNS N06625 nickel-based alloys in seawater. Carbon steel was also included in certain studies since it has been for years the dominant offshore material. These materials represent a wide range of categories and grades with a distinctive set of general properties, including corrosion resistance, chemical composition and microstructure. Experiments were designed to elucidate the effects of biofilms, oxygen, temperature and exposure time on corrosion resistance. Research aims were achieved through a series of short and long-term corrosion and microbiological studies in natural seawater using closed and open experimental systems. Natural seawater for laboratory experiments was obtained from the same geographical location at 20 metres depth off Rottnest Island at the Indian Ocean (Western Australia). Results from

this research are presented in the form of a series of scientific publications which form the individual chapters of this thesis. In **Chapter 2** the corrosion properties of the alloys in seawater at temperatures from 5 to 40 °C are reported. Pitting and crevice corrosion were investigated by potentiostatic and potentiodynamic electrochemical analysis. Artificial crevices were prepared based on the spring loaded assembly developed by the project Crevcorr of the European Commission. This study allowed critical potentials and temperatures for pitting and crevice corrosion of the selected alloys in seawater to be defined. A unified comparative analysis of this type had hitherto not been reported. Subsequently, short-term laboratory studies were conducted to investigate the effect of biofilms on corrosion performance in seawater (**Chapters 3-7**). The basic experimental design involved the exposure of bare and artificially creviced materials to raw and treated seawater for different exposure times, modifying experimental variables such as temperature and oxygen content. These studies were conducted under closed experimental conditions, without seawater replenishment, in order to simulate the stagnant conditions at the interior of subsea equipment filled with treated seawater. Experimental methods included immersion and accelerated corrosion tests along with surface and microbiological analyses. The phenomenon of ennoblement was investigated routinely by monitoring the corrosion potential over time. Molecular microbiology studies by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and DNA sequencing of bacterial 16S rRNA gene fragments were used to characterize microbial composition and structure in biofilms developed on the alloys. Microbial adhesion was investigated by fluorescence microscopy and scanning electrode microscopy (SEM). Surface features were analysed by 3D optical microscopy and surface profile measurements. The long-term corrosion performance of the alloys in seawater is reported in **Chapter 8**. Pitting and crevice corrosion studies were conducted in alloys exposed to raw and treated seawater over a period of 1.5 years. To complement the results from closed experimental studies and to provide a more comprehensive and accurate analysis of the risk of MIC of offshore materials, further experiments were conducted in an open system where seawater was replenished constantly over the period of exposure. **Chapter 9** presents a study

conducted in a field laboratory where corrosion performance and biofilm community composition were evaluated in natural and treated costal seawater in a continuous flow system over 90 days. Finally, **Chapter 10** presents a study of the risk of MIC and localized corrosion associated with seawater ingress into flowlines, filled with treated seawater, during subsea tie-in operations for the installation and commissioning of deep-water pipelines for offshore fields. In particular, the effect of oxygen and troublesome bacteria on corrosion performance was investigated. A study addressing this industrial concern had not been published previously despite its broad significance and practical importance.

Results from this research allowed the identification of the critical factors that influence the performance of offshore construction materials in natural seawater. UNS S32750, UNS S31254 and UNS N06625 exhibited high critical potentials and temperatures for localized corrosion and therefore high resistance to localized corrosion at the typical offshore conditions, regardless of the presence of biofilms and oxygen content. UNS S31603, UNS N08825 and UNS S31803 showed to be susceptible to localized corrosion in seawater at temperatures from 5-10°C, 10-20 and 20-30°C, respectively. For these alloys, the risk of localized corrosion and MIC is increased by prolonged exposure to seawater, the rise in seawater temperature and the presence of biofilms. Localized corrosion was highly influenced by oxygen and biofilms provided a critical temperature was surpassed. Anaerobic conditions favoured crevice corrosion initiation and enhanced the negative effects of microbial adhesion on creviced alloys in seawater.

The effect of biofilms on corrosion performance was subject to the availability of nutrients in the system. In stagnant closed systems, biofilms did not ennoble the corrosion potential of the alloys but reduced localized corrosion resistance via decreasing critical potentials for localized corrosion initiation. Biofilms initiated pitting and crevice corrosion by other mechanisms that were not identified. In the continuous open system, active biofilms quickly developed and ennobled the corrosion potential of all materials about +400-500 mV. This ennoblement triggered localized corrosion on UNS S31603, UNS N08825 and UNS S31803. Long-term exposure studies indicated that seawater filtration, in accordance

with hydrostatic testing procedures, reduces corrosion rates but does not prevent localized corrosion if exposure conditions required for localized corrosion initiation are attained. However, filtration reduces the likelihood of localized corrosion and MIC by removing sediments or sand so that crevice formation is prevented and microbial activity is restricted. Furthermore, seawater treatments used for hydrotesting waters conferred protection against localized corrosion provided oxygen is restricted to minimal levels in the system.

The application of PCR-DGGE and DNA sequencing as a molecular tool to examine biofilm community composition proved to be a powerful method to investigate the effects of environmental conditions and substratum surface on the microbial community structure in biofilms. This method showed that biofilm populations on corrosion resistant alloys are highly specific in their preference for substratum surface and oxygen conditions. Microbial colonization was shown to be more copious and diverse on nickel based-alloys than on stainless steels. Analysis of biofilm communities on high-resistance alloys in seawater has not been reported previously. These data extend our knowledge on microbial ecology associated with corrosion resistant materials in seawater and highlight the importance of conducting future mechanistic studies on the precise interactions of biofilms with the different passivating oxide films formed on these offshore alloys. This will allow elucidation of the main factors that influence microbial adhesion on these alloys and will assist in developing new strategies and methods to prevent MIC in subsea equipment. This research has contributed to filling previous knowledge gaps and has formed the foundations of essential guidelines for design criteria, risk assessment and asset integrity management based on materials selection and water treatments in the application to sunken subsea equipment in the oil and gas industry.

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LIST OF PUBLICATIONS

This thesis is assembled by publications, either published, accepted or submitted, which form the individual chapters of the thesis. Publications are refereed and available in refereed scholarly media.

Publications are listed as follows:

Chapter 2

L.L. Machuca, S. I. Bailey, R. Gubner, Systematic study of the corrosion properties of high-resistance alloys in natural seawater, Corrosion Science, 64 (2012) 8-16.

Excellence Research Australia (ERA): A* rated publication; Impact factor: 3.734.

Chapter 3

L.L. Machuca, S.I. Bailey, R. Gubner, E. Watkin, A. Kaksonen, Microbiologically influenced corrosion of high-resistance alloys in seawater, in: Corrosion 11, Paper N. 11230, NACE International. Houston, Texas.

Excellence Research Australia (ERA): A rated conference.

Chapter 4

L.L. Machuca, S.I. Bailey, R. Gubner, E. Watkin, M.P. Ginige, A. Kaksonen, Bacterial community structure in natural marine biofilms and the corrosion of carbon steel in seawater, in: 18th International Corrosion Congress, Paper 371, Perth, Australia, 2011.

Excellence Research Australia (ERA): A rated conference.

Chapter 5

L.L. Machuca, S.I. Bailey, R. Gubner, E. Watkin, M.P. Ginige, A. Kaksonen, Crevice corrosion of duplex stainless steels in the presence of natural marine biofilms, in: Corrosion 12, Paper N. C2012-0001486, NACE International. Salt Lake City, Utah.

Excellence Research Australia (ERA): A rated conference.

Chapter 6

L.L. Machuca, S.I. Bailey, R. Gubner, Microbial corrosion resistance of stainless steels for marine energy installations, Advanced Materials Research, 347-353 (2012) 3591-3596.

Excellence Research Australia (ERA): B rated publication.

Chapter 7

L.L. Machuca, S.I. Bailey, R. Gubner, E. Watkin, M. P. Ginige, A. Kaksonen, K. Heidersbach. Effect of oxygen and biofilms on crevice corrosion of UNS S31803 and UNS N08825 in natural seawater. Corrosion Science (2012). Submitted and under review.

Excellence Research Australia (ERA): A* rated publication; Impact factor: 3.734.

Chapter 8

L.L. Machuca, S.I. Bailey, R. Gubner. Crevice corrosion studies on corrosion resistant alloys in stagnant natural seawater. Advanced Materials Research (2013). Accepted for publication.

Excellence Research Australia (ERA): B rated publication.

Chapter 9

L.L. Machuca, R. Jeffrey, S.I. Bailey, R. Gubner, E. Watkin, M. P. Ginige, A. Kaksonen. K. Heidersbach. Corrosion performance of offshore construction materials in the presence of active biofilms in seawater. Corrosion Science (2012). Submitted.

Excellence Research Australia (ERA): A* rated publication; Impact factor: 3.734.

Chapter 10

L.L. Machuca, L. Murray, R. Gubner, S.I. Bailey. Evaluation of the effect of seawater ingress into 316L lined pipes on corrosion performance**.** Materials and Corrosion (2012) Submitted.

Excellence Research Australia (ERA): B rated publication; Impact factor 1.173.

STATEMENT OF CONTRIBUTIONS BY OTHERS

I hereby declare that the work presented in this thesis was primarily designed, experimentally executed, interpreted, and written by the first author of the individual manuscripts (Laura L. Machuca). Contributions by colleagues are described in the following. The signed statements by co-authors are in the Appendix 8.

Chapter 2

Stuart Bailey and Rolf Gubner provided significant contributions to the conception and design of this study. They also provided intellectual input on the interpretation of the results and critical revision of the manuscript.

Chapter 3

Stuart Bailey, Elizabeth Watkin, Rolf Gubner provided significant contributions to the conception and design of this study. Stuart Bailey provided critical input on the electrochemical analysis. Anna kaksonen provided technical support for PCR-DGGE analysis. Elizabeth Watkin and Anna Kaksonen provided intellectual input on the interpretation of the molecular microbiology results. All co-authors participated in the preparation of the manuscript.

Chapters 4-5

Stuart Bailey and Rolf Gubner contributed to data interpretation. Elizabeth Watkin, Maneesha Ginige and Anna Kaksonen provided support to the analysis of microbiological data. All co-authors provided critical revision of the manuscript.

Chapter 6

Stuart Bailey provided critical input on the electrochemical analysis. Stuart Bailey and Rolf Gubner contributed to data interpretation and preparation of manuscript.

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Chapter 8

Stuart Bailey and Rolf Gubner contributed to the conception and design of this study and helped in data interpretation and preparation of manuscript.

Chapter 9

Robert Jeffrey, Stuart Bailey, Elizabeth Watkin, Rolf Gubner and Krista Heidersbach provided significant contributions to the conception and design of this study. Robert Jeffrey provided the facilities to conduct the experiments and monitored the experiments. Australia. Elizabeth Watkin, Maneesha Ginige and Anna Kaksonen provided support to the analysis of microbiological data. Kayley Usher provided technical and scientific support with SEM facilities. All co-authors provided intellectual input on the data interpretation and critical revision of the manuscript. Experiments were conducted at the NSW Department of Primary Industry Fisheries Research Centre at Taylors Beach, New South Wales, Australia.

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Lynn Murray and Rolf Gubner provided significant contributions to the conception and design of this study. Lynn Murray provided the industry background to develop and plan this study. All co-authors provided intellectual input on the data interpretation and critical revision of the manuscript.

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CONFERENCE PRESENTATIONS

- **L. L. Machuca**, S.I. Bailey, R. Gubner, E. Watkin, A. Kaksonen., Microbiologically influenced corrosion of high-resistance alloys in seawater. Corrosion 2011, NACE International. Paper N. 11230. Houston, Texas. March 13-17, 2011. Oral presentation, symposium EG 187X (Microbiologically Influenced Corrosion).

- **L. L. Machuca**, S.I. Bailey, R. Gubner, E. Watkin, M.P. Ginige, A. Kaksonen, Bacterial community structure in natural marine biofilms and the corrosion of carbon steel in seawater. 18th International Corrosion Congress, Paper 371. Perth, Western Australia. November 20-24, 2011. Oral presentation, MIC symposium.

- **L.L. Machuca,** S.I. Bailey, R. Gubner, E. Watkin, M.P. Ginige, A. Kaksonen, Crevice corrosion of duplex stainless steels in the presence of natural marine biofilms. Corrosion 12, NACE International. Paper N. C2012-0001486, Salt Lake City, Utah. March 11-15, 2012**.** Oral presentation, symposium EG 187X (Microbiologically Influenced Corrosion).

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Chapter 1

Introduction and Overview

1.1 Literature Review

Microbiologically Influenced Corrosion (MIC)

Metallic corrosion is an interfacial process leading to surface degradation of a metal [1, 2]. It involves electrochemical reactions between the metal and its environment. In aqueous media these reactions are governed by physical and chemical parameters including pH, redox potential, conductivity, material composition and temperature among others [2, 3]. Microorganisms may also

profoundly influence these processes, facilitating, initiating or accelerating them, especially when the microorganisms are in close contact with the metal surfaces [4-6]. Both, microbial and electrochemical processes simultaneously influence the metal-solution interface [7, 8]. The resulting changes at the near-surface environment influence, in their turn, the dynamic of biofilm processes [9, 10], the composition, diversity and interactions of biofilm communities developing on surfaces [11-13] and the electrochemical processes at the metal-solution interface [14, 15]. Further complexity is introduced to the system by the structural and physiological heterogeneities of biofilms [16, 17] as well as the complex genetic control systems in microorganisms [18]. The resulting modification, acceleration or inhibition of the corrosion reactions at the metal surface is known as microbiologically influenced corrosion (MIC).

MIC has been reported for metals [19], alloys [20-22], asbestos cement [23], concrete [24, 25] and composite materials [26-29] exposed to different environments including freshwater [30], seawater [31, 32], drinking water systems [33], foodstuffs [34], soils [35-37], fuels [38], physiological fluids [39], and sewage treatment systems [40, 41]. A particular system can become more susceptible or resistant to MIC depending upon environmental factors, water composition and material composition [42, 43].

Quantifying the cost of corrosion generally, and more specifically the cost associated with MIC, in the oil and gas industry is not easily done. Estimates on the cost of corrosion worldwide indicate a dollar figure in excess of AU$2.2 trillion [44]. In the U.S, the total cost of corrosion was estimated to equal $276 Billion in 2001, approximately 3.1 % of the nation's gross domestic product (GDP) [45]. It was estimated that the annual cost of all forms of corrosion to the oil and gas industry was $13.4 billion, of which MIC accounted for about $2 billion. However, closer examination of the National Association of Corrosion Engineers (NACE) data indicates that corrosion costs in the U.S. currently exceed $1 trillion dollars, approximately 6.2% GPD [46] but data are needed to confirm this estimation. In Australia it has been estimated that corrosion may be costing the Australian economy more than $30 billion each year [47]. It has

been estimated that 40% of all internal pipeline corrosion in the gas industry can be attributed to microbial corrosion [48] and that 70% of the corrosion in gas transmission is due to problems caused by microorganisms, with the American refinery industry losing $1.4 billion a year from microbial corrosion [49]. Costs associated with repairs, replacements and down time are huge and include costs in cooling water systems, power generation plants, oil and gas production, transportation and storage, water distribution and heat exchangers tubing in nuclear power generating plants among others [50]. MIC has been reported to account for as much as 50 per cent of the total cost of corrosion [50]. It must be emphasised that these cost estimations do not include the cost of pollution, environmental repercussions and loss of life due to equipment failure. In addition, costs are generally underestimated due to misdiagnosis of corrosion causes. Particularly, it is very intricate to establish a direct association between biological effects and corrosion failure as MIC does not produce a unique type of corrosion [51]. The mere isolation or identification of MIC-related microorganisms from a particular environment is not sufficient to relate these microbes with a corrosion failure.

Biofilms

Owing to its economic and environmental importance, MIC has been the subject of extensive studies for the past decades [52-55]. Regardless of the particular reactions and processes governing MIC in different environments, there is agreement in the literature that microbial adhesion and the consequent biofilm formation is the determining step in MIC [56-58]. Biofilms are sessile communities of microbial cells and extracellular products associated with a substratum [59-61]. Virtually, any industrial system where water contacts a surface is susceptible to biofilm formation. Biofilms seem to be a preferential mode of life for microorganisms as it offers a more sustainable environment for their survival, growth and reproduction [62]. In addition, biofilm development

allows mutualistic and synergistic interactions between microorganisms and affords protection from external harsh conditions [18, 63].

The formation of biofilms involves a sequence of events strongly influenced by hydrodynamic, physical, physico-chemical and biological factors [64-67]. Initial adhesion of microorganisms to a surface seems to be highly controlled by surface properties [68, 69]. Initial bacterial cell-surface interactions are mainly governed by physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, the effect of surface electrostatic charge and hydrophobic interactions [69-72]. Hydrophobic interactions are defined as the attraction between apolar or slightly polar molecules, particles or cells, when immersed in water. It seems that microbial cells with hydrophobic properties prefer hydrophobic material surfaces whilst the ones with hydrophilic characteristics prefer hydrophilic surfaces [73]. In terms of surface charge, in most aqueous media and at neutral pH the microbial cells and solid substrata are almost always negatively charged, due to the ionization of their surface molecules. This means that electrostatic forces are repulsive and do not facilitate adhesion processes. This is, however, affected by factors such as growth medium, pH and ionic strength [67]. It appears that microorganisms change their behaviour and adhesive properties after association with a surface and express phenotypic traits that are often distinct from those that are expressed during planktonic growth [74]. Thus, initial repulsion barriers between microbial cell and substratum surfaces seem to be lessened by the presence of microbial surface polymers such as lipopolysaccharides, extracellular polymers, proteins, capsules, pili, fimbriae, flagella and appendages which provide binding and adsorption sites [75-77]. In addition, many investigators have demonstrated that materials immersed in aquatic environments are rapidly and spontaneously covered by a conditioning film of adsorbed dissolved organic and/or inorganic matter already present in the environment or produced by microorganisms before any bacterial attachment takes place [78]. Investigations of the composition and the growth kinetics of this adsorbed layer on steel surfaces in natural seawater have indicated that nitrogen-containing species and carbohydrates are the primary adsorbed

compounds [78]. This conditioning film has been shown to influence the substratum surface properties including surface charge and hydrophobicity, which play an important role in subsequent colonization by microorganisms [72, 79]. Likewise, this film provides a source of nutrients for pioneer colonizing microorganisms thus favouring microbial attachment [80].

Biofilm development is a complex process affected by the bulk fluid, the substratum surface, the metabolism and growth of attached cells and the synthesis and accumulation of exopolymers. The bulk fluid serves as a source of nutrients, ions and new microorganisms whilst the substratum surface provides a medium for nutrient adsorption and concentration. The transport of nutrients from the bulk phase to the interface is one of the most critical rate-limiting factors. In this case, the attached cells may be at an advantage simply by remaining on a stationary surface in a moving aqueous phase because of the constant replenishment of nutrients and removal of waste products. Fluid flow influences not only the transport of dissolved solutes into and out of the biofilm but also the forces to the move and detach the biofilm [64, 81]. The synthesis of exopolymers has been widely recognized as a crucial step in biofilm development. Common to virtually all biofilm matrices are extracellular polymeric substances (EPS). EPS have been shown to be responsible for either or both of microbial attachment and biofilm growth [77, 82, 83]. In addition, the presence of EPS helps maintain the integrity and architecture of the biofilm allowing large numbers of microorganisms to coexist. The extent and nature of the EPS production seem to be dependent on the physiological state of the microbial cells and nutrient availability [84]. EPS are mainly composed of water and microbial macromolecules and provides a complex array of dynamic microenvironments surrounding the attached cells. The complexity of this biofilm matrix is increased by the microbial diversity and community that develops within biofilms [85]. Within biofilms, different polymers are likely to be produced by individual species and blend together to generate a very heterogeneous EPS. In addition, the metabolic activities of these mixed communities, together with diffusional processes, result in concentration gradients of nutrients, signalling compounds and microbial waste within biofilms that are highly heterogeneous throughout the biofilm [17, 86]. It has

been observed that at the periphery of the biofilms, rapid utilization of oxygen and nutrients will result in depletion of these constituents in the underlying region. As the bacteria respond to these gradients, they adapt to the local chemical conditions, which can change over time as biofilms develop. These adaptation processes are mainly governed by genetic control systems [74]. Intercellular communications within a biofilm rapidly stimulate the up and down regulation of gene expression enabling temporal adaptation such as phenotypic variation and the ability to survive in nutrient deficient conditions [18, 74]. This communication amongst microorganisms through specialized molecules has collectively been termed *quorum sensing* [87, 88]. This has been of particular interest to many researchers whose studies have generated important contributions to the understanding of the processes involving microorganisms and their metabolic activities under biofilms.

General Mechanisms of MIC

Several mechanisms have been hitherto proposed to describe how microorganisms themselves or their metabolic activities can influence the corrosion reactions. However, the diverse physiological capacities of microorganisms, the complexity and heterogeneity of the biofilm matrix and the variable nature of the MIC phenomenon have made detailed mechanisms elusive. Microorganisms do not produce a unique type of corrosion but rather they influence or shift the mechanisms for corrosion. Most studies report MIC as a mode of localized corrosion [89-91].

The physical presence of microbial cells on the surface, in addition to their metabolic activities, modifies the electrochemical reactions at the metal surface-solution interface. Electrochemical processes involve the release of metal ions to the environment and the movement of electrons within the material and to a site where they are consumed by species in contact with the surface [92]. This involves the establishment of two half-cell reactions; an oxidation reaction at the anode that results in metal dissolution and a reduction reaction of chemical

species at the cathode. The role of the microorganisms is either to assist in the establishment of this electrolytic cell (indirect) or to stimulate the anodic or cathodic reactions (direct) [93]. Both, the corrosion mechanism and the causative microorganisms for MIC are affected by the nature of the substratum material and environmental conditions [42, 94, 95]. Mechanisms that rule MIC in freshwater environments seem to be quite different to those observed in seawater and other natural water bodies. Similarly, physicochemical variables such as ion concentration, temperature and oxygen content seem to favour the growth of particular species and restrain the growth and metabolism of others on a particular substratum [96].

The mere presence of biofilms introduces physical anomalies on a metal surface that enable the formation of differentiation cells and induce the creation of local anodes and cathodes at the surface [97]. Generally, these microfouling deposits present a patchy distribution on the metal surface. The physical presence of a biofilm acts as a diffusion barrier that retards the movement of species from the bulk solution toward the metal surface and from the metal-biofilm interface outwards into the solution. Nonetheless, mechanisms associated with microbial growth, energy generation and reproduction seem to play a more important role in MIC than the simple physical occurrence of microbial life on surfaces. Microorganisms take up nutrients from their environment, transform them, generate energy to grow, and then excrete waste products into the environment. These processes involve oxidation-reduction reactions and enzymes that catalyse them [98]. Electron donors, which can be organic or inorganic depending on the microbial physiology, are the energy source for microbial growth as energy is released when they are oxidized. Electron acceptors are related to microbial respiration processes. Aerobic respiration involves oxygen as electron acceptor whereas in anaerobic respiration, electron acceptors other than oxygen are used to sustain energy generation [99, 100]. These alternative electron acceptors permit microorganisms to respire in environments where oxygen is absent. Many microorganisms are facultative, meaning that they can grow under either oxic or anoxic conditions [101].

In neutral aerated seawater, oxygen reduction is the main cathodic reaction at the metal surface. In the absence of oxygen corrosion reactions are sustained by secondary cathodic reactants such as hydrogen ion [102]. Under aerobic conditions, areas under respiring colonies or patchy biofilms on the surface, limit the access of oxygen and chemical species to the underlying metallic surface. This creates areas at the surface with different electrochemical potentials or differential concentration cells. Areas of low oxygen, or other nutrient concentration beneath such deposits, are the anodic sites where metal dissolution proceeds. If the aerobic respiration rate within the biofilm is greater than the oxygen diffusion rate, the cathodic mechanism changes and the biofilm-surface interface become more suitable for the growth of anaerobic microorganisms [103].

Under anaerobic conditions, a diversity of microorganisms can have an effect on the corrosion of metals. One of the most widely recognized models of corrosion associated with the anaerobic metabolism in microorganisms is related to sulphide production by anaerobic prokaryotes, e.g. sulphate-reducing bacteria (SRB). These microorganisms have long been acknowledged as the chief culprits of anaerobic corrosion and as a result have been the focus of most research in MIC [32, 53, 104, 105]. Corrosion mechanisms attributed to SRB and other sulphide producers include anodic and cathodic depolarization by bacterial enzymes and metabolites, sulphide-induced stress corrosion cracking (SCC) and hydrogen-induced cracking [104-107]. The metabolism of iron and/or manganese reducers has also been associated with the anaerobic corrosion of metals [108]. These microorganisms carry out the anoxic reduction of ferric iron (Fe^{+3}) and manganese (Mn^{4+}) coupled to the oxidation of several electron donors for energy metabolism. These microorganisms are known to promote corrosion of iron and its alloys through reactions leading to the dissolution of protective oxide films on the metal surface, thus exposing the underlying metal to the corrosive medium [109]. However, the involvement of these microbes in the corrosion of steel is controversial and the activity of metal reducers has also been associated with corrosion inhibition [110]. Similarly, denitrifying bacteria, a quite metabolically diverse group of microorganisms, have been

involved in anaerobic corrosion. Biological anaerobic oxidation of Fe^{2+}, Fe^0 and H_2 coupled to nitrate reduction in nitrate reducing bacteria has been observed to enhance iron corrosion [111].

More recently, it has been demonstrated that certain bacteria are able to switch from natural soluble electron acceptors such as oxygen, sulphate or nitrite to solid anodes. These bacteria may adhere to the anode surface and catalyse the oxidation of organic compounds, transferring the electrons produced directly to the anode [112]. On the other hand, certain microbial species have also been shown to reduce nitrate or fumarate with a graphite or stainless steel cathode as electron donor [113]. Mechanisms involved in direct microbial electron transfer include periplasmic and outer-membrane *c*-type cytochromes and the presence of pili that might serve as biological nanowires [114]. This direct microbial electron transfer has been shown to play a role in the anaerobic corrosion of steels [94, 115, 116]. Under these conditions, the cathodic reaction enhanced by bacteria seems to be less sensitive to mass-transfer compared to reactions using oxygen or other soluble species.

Among the proposed mechanisms of MIC, there is one involving the activities of aerobic metal-depositing microorganisms. These microorganisms catalyse the oxidation of soluble iron and manganese species to insoluble oxides. Biologically deposited manganese minerals, e.g. MnO_2, have been shown to provide a powerful cathodic reaction on electrically conducting surfaces [117]. It has been suggested that reduction of MnO_2 is kinetically faster and more favourable than the reduction of oxygen. Moreover, since manganese minerals are formed on the surface and in electrical contact with the surface, the cathodic reduction is not diffusion limited and provides a highly efficient reduction reaction [118]. Under-deposit corrosion due to the oxygen depleted areas under these deposits has also been reported [119]. Similarly, biomineralized MnO_2 deposited on stainless steel cathodes has been shown to increase the corrosion rate of galvanically coupled mild steel anodes [118] and the likelihood of localized corrosion in steels [117].

Other causes of microbial corrosion include the concentration of aggressive chemical species within biofilms in contact with the surface and the metal-binding properties of microbial EPS. Values of pH less than 2 have been reported in localized areas near the metal surface compared to pH values of 8 measured at the outer layers of the biofilm [120]. The presence near the surface of organic or inorganic acids produced by bacteria is known to promote the electrochemical oxidation of metals [107]. Bacterial EPS seem to provide a matrix on the surface with chelating properties that concentrate metal ions from the aqueous phase or from the substratum. This has been shown to provide additional cathodic reactions at the metal surface [121].

Despite increasing recognition and documentation of corrosion failures due to MIC, the mechanisms of MIC remained poorly understood. It is very unlikely that one single predominant mechanism exists in MIC. Biofilms, and their interactions with surfaces, are very heterogeneous and complex from all points of view. Therefore MIC would be expected to be affected by the whole biofilm community and their synergistic effects rather than by individual populations or individual metabolic activities.

MIC of Corrosion Resistant Alloys (CRAs) in Seawater

Seawater is one of the most severe of natural corrosive environments. The aggressiveness of seawater lies in its chloride content which is known to deteriorate the resistance of steels [122]. In the majority of ocean waters, the total proportion of dissolved salts in seawater is about 35 parts per thousand and may vary slightly between different sea locations. The corrosive effects of seawater on metals have always been considered to be greater than the corrosion caused by salt solutions having the same composition. This difference is usually attributed to the presence of biological activity in natural environments and some other organic and inorganic molecules that form metallic complexes which can also catalyse corrosion reactions. The presence of these components in seawater, and the fact that they are poorly defined and

most likely subject to geographical variation, has made it difficult to simulate the natural system for corrosion investigations in the laboratory.

High-resistance alloys have been used increasingly for a wide range of applications in marine environments due to their combination of strength and corrosion resistance. The increased corrosion resistance of these materials is provided by the presence of higher concentrations of alloying elements such as chromium, nickel, molybdenum and nitrogen. The type and quantity of alloying depends on the desired mechanical and corrosion resisting properties. In environments containing oxygen, these steels form a thin (1-10 nm thickness), passivating surface oxide film which renders them passive. This passive film is an amorphous structure of chemisorbed oxygen bonding to the surface with an electrostatic bonding between oxygen anions and metal cations [123]. In this passive state, very slow uniform corrosion takes place. However, under certain circumstances the passive film can break down locally, particularly in the presence of halides and some organic acids. The protective film has self-healing properties when damaged and may repassivate if oxygen is present. Nonetheless, the rupture of the passive film may be permanent and localized corrosion occurs in the underlying metal. Localized corrosion can initiate due to surface irregularities and inclusions, e.g. sulphide inclusions, differential aeration and concentration cells, irregular surface coatings or deposits and non-uniform fluid velocity, among others [1].

Pitting and crevice corrosion are the most widespread forms of localized corrosion in corrosion resistant alloys in seawater. Crevice corrosion occurs in narrow gaps into which an aggressive medium may penetrate such as overlapping surfaces, incompletely sealed gasket/metal interfaces or naturally occurring deposits. Pitting, on the other hand, can initiate at different vulnerable sites on the surface freely exposed to the environment. Therefore, pitting initiation has been described as a more stochastic process [122]. Crevice corrosion has been regarded as a form of pitting where metastable pitting at the occluded environment is stabilized by the resistive barrier of the crevice geometry [124, 125]. Owing to this, crevice corrosion may initiate under less

aggressive conditions and is considered as a more detrimental form of localized corrosion when compared with pitting [126].

Mechanisms of initiation, propagation and repassivation of pitting and crevice corrosion in alloys are influenced by factors such as halide concentration, pH, potential and temperature. Passivity breakdown may naturally occur if the potential is lowered from the passive potential into an active region. This can occur if the cathodic reactant supporting passivity, such as oxygen in the electrolyte, is depleted as occurs in crevices or occluded regions on a metal surface [127]. However, passivity breakdown commonly takes place at high potentials in the region where passivity is expected to dominate. In chloride solutions, a critical pitting potential has been described as the minimum potential required for stable pitting in alloys. Above this potential, pits propagate almost indefinitely until total perforation of the metal results. Below this potential, continuous breakdown/repair events take place on the surface of the alloys [128]. This critical potential is often observed to decrease with increasing halide concentration and temperature [129, 130].

It is widely known that corrosion resistant alloys such as stainless steels are easily covered by biofilms within a few hours of exposure to natural seawater [55]. The effects of biofilms on the surface of passive alloys in seawater have been a topic of debate and discussion during recent decades. The main types of attack that affect corrosion resistant alloys in seawater, such as pitting and crevice corrosion, can be notoriously favoured by the presence of biofilms [131, 132]. For instance, microbial respiration within the crevices formed on a surface may accelerate the depletion of oxygen in the crevice solution and decrease initiation times for localized corrosion. It is well-known that passive alloys immersed in natural seawater undergo a shift of the corrosion potential (E_{corr}) in the noble or anodic direction; a phenomenon collectively termed ennoblement [57, 133]. Ennoblement of passive alloys has been widely studied and numerous reports from geographically diverse sea locations have been made over the years. Since ennoblement does not occur when an alloy is exposed to sterile seawater, the phenomenon has been attributed to

microorganisms [55]. The significance of this phenomenon lies in its influence on the susceptibility to corrosion of anode materials in galvanic couples and the initiation and propagation of localized corrosion [20, 57, 134]. It has also been described that ennoblement decreases the salinity level below which a given alloy should be resistant to localized corrosion attack [134]. This ennoblement effect has been reported when alloys are exposed to both seawater and freshwater and it has been observed on varied materials including austenitic, ferritic and duplex stainless steels, titanium and even platinum [50]. Ennoblement has mostly been associated with aerobic biofilms on steels, whereas only a few studies conducted under anaerobic conditions have reported ennoblement of the corrosion potential [135].

Many studies have focused on investigating the mechanisms of ennoblement. Ennoblement is often associated with an increase of cathodic reaction rate that results from either the catalysis of the preexisting cathodic reaction such as the oxygen reduction in aerated waters or the reduction of an alternative oxidizer produced by the biofilm. Either thermodynamic or kinetic effects can cause an ennoblement of the corrosion potential. Hypotheses have been proposed that involve thermodynamic effects such as localized acidification or an increase of the partial pressure of oxygen increasing the reversible potential of the oxygen electrode, and kinetics including electrochemical reduction mediated by the activity of manganese oxidizing bacteria, oxygen reduction catalysis by organometallic compounds, microbial enzymes, hydrogen peroxide formation and hydrogen sulfide [136-139]. More recently, investigators have demonstrated that ennoblement of several steels in seawater can be the result of a direct electron transfer mechanisms between biofilm and steel in the absence of soluble electron donors or acceptors [94, 115, 116]. Despite much research on MIC of passive alloys in seawater, the mechanism by which biofilms cause ennoblement still remains uncertain and to date unifying mechanisms for global observations have not been identified.

It is known that accumulation of sulphur on the surface of high-resistance alloys retards passivity and enhances dissolution of the metal [2]. Research on the

influence of SRB on corrosion resistant alloys in seawater has indicated that sulphide-rich layers are formed on the steel surface in the presence of SRB and that it leads to loss of passivity and pitting initiation. Results indicated that MIC attack in the presence of SRB was controlled essentially by the anodic processes [140]. Synergistic effects between SRB and iron reducing bacteria have been shown to accelerate corrosion rates on stainless steels and facilitate pitting initiation in the presence of chlorides [141]. In addition, the growth of SRB within biofilms has been shown to enhance crevice corrosion of a duplex stainless steel exposed to a solution containing 3.5% NaCl inoculated with SRB. Results also indicated that the austenite phase of the duplex microstructure appears to be more susceptible to SRB-influenced attack [142]. In addition, the observation of pits with curved-rod shape has suggested a direct role of SRB cells on localized corrosion of stainless steel in enriched artificial seawater inoculated with SRB [143].

Subsea Applications and Preservation of CRAs

Pipeline commissioning in the oil and gas industry typically involves cleaning, flooding with treated seawater, gauging and hydrostatic testing. Hydrostatic testing is a common practice to verify pressure equipment does not leak or have manufacturing flaws. The water used in these practices can come from a variety of sources including potable, river or seawater, generally chosen based on consideration of convenience and economic drivers. Seawater is routinely used in the hydrotesting of subsea pipelines. This practice can contaminate the internal surface with microorganisms, sand and salts, even after the water has been removed, increasing the possibility of MIC [144]. In addition, equipment and pipelines can lay dormant on the seabed for extended periods (days, months or years) as a consequence of unforeseen delays and unfavourable weather conditions. Over these extended periods, the activity of residual microbial life and newly introduced bacteria can increase, as the effectiveness of the preservation chemicals decays and the seepage of seawater mixes with the volumes of treated fluids. This stagnant water may permit debris, sand and

marine life to settle and form biofilms. These biofilms may present a serious threat once the pipelines become operational, because fluids transported in pipelines may contain sufficient nutrients for bacteria to flourish [145]. This process can and has been reported to result in significant damage to sunken subsea equipment from MIC. Therefore, seawater used for hydrotesting, ballast and preservation of sunken subsea equipment, must be properly treated in order to reduce the possibility of contamination and severe corrosion damage, to prolong pipeline and equipment service life and prevent failures [146, 147].

The selection of an appropriate treatment method for hydrotest and preservation fluids that will prevent corrosion of equipment prior to start-up is becoming an issue of increasing importance to the oil and gas industry. The general rule is that if water is left stagnant for more than 7 days, the water should be treated to prevent problems. The potential for corrosion can be minimized using macro filtration to reduce the volume of solids entering the system, an oxygen scavenger to remove oxygen in the water, biocide doses to control bacterial growth and if necessary, treatment of the water with a corrosion inhibitor. Biocides are chemicals that are added to the water to kill bacteria. If biocides are not used in contained environments (like pipelines during commissioning) populations of organisms will certainly thrive in the stagnant water and MIC is likely to occur. Biocides should be thoroughly mixed in the water to effectively kill bacteria in the entire pipeline or vessel. To date, research studies addressing the industrial concerns regarding the preservation of offshore equipment are very limited.

1.2 Aims of this research

The main objective of this research is to assess the susceptibility of offshore construction materials to MIC, elucidating the effects of oxygen, exposure time, temperature and biofilms on corrosion resistance.

Specific aims and objectives are to:

- Characterize the corrosion properties of CRAs in natural seawater.
- Evaluate the effect of oxygen, temperature and biofilms on the corrosion performance of CRAs in natural seawater.
- Evaluate of the effects of short and long-term exposure to stagnant natural seawater on the performance of CRAs.
- Characterize the microbial diversity and biofilm communities developed on offshore construction materials in natural seawater.
- Assess the degree to which environmental conditions and substratum surface may affect the microbial diversity and structure in biofilms.
- Identify the critical factors that influence the performance of offshore construction materials in natural seawater.
- Assess the risk of MIC and localized corrosion of CRAs in natural seawater treated in accordance with hydrotesting procedures.

Research aims will be achieved through a combination of corrosion and microbiological research conducted in collaboration between the Corrosion Centre (Corr-CERT) at Curtin University and the CSIRO Land and Water at Floreat.

1.3 Significance and Contribution of this research

MIC has been associated with significant corrosion related failures with devastating repercussions. Consequently, this is a topical research area, although there is currently only limited research on the long term preservation of subsea equipment [148] and the effects of subsea temperatures on the microbial activity and ultimately MIC of common offshore construction alloys. The susceptibility of various alloys to MIC at subsea temperatures is yet to be quantified and poses a significant dilemma for materials engineers. In one reported instance, MIC damage was so severe that the equipment failed during commissioning, resulting in a costly overhaul (> AUD$1-2 million for work over

and replacement) and environmental damage from the uncontrolled release of hydrocarbon [149]. These consequences could have been avoided had there been a better understanding of the preservation of subsea equipment and the susceptibility of common alloys to MIC. Experiences like this are drivers that have compelled Chevron, the partner organisation, to support research in this field. The results of this research will fill current knowledge gaps and will allow developing essential guidelines for risk assessment and asset integrity management based on materials selection and water treatments in the application to subsea equipment. Incorrect materials selection can be costly and increases the risk of serious environmental damage from premature equipment failure.

Several different methods have been used to identify the pitting and crevice corrosion properties of certain passive alloys in different seawater simulating solutions [129, 150-155]. The differing laboratory conditions, testing procedures and varying media used to represent seawater, have led to inaccuracies in the integrated analysis of the localized resistance of passive alloys in natural seawater. Consequently, this study aims to develop a comparative analysis of both pitting and crevice corrosion properties of the various alloys in natural seawater using a consistent test methodology across all tests. A unified analysis of this type has not been conducted previously and will be of significant contribution to the oil and gas industry.

Moreover, most research studies on MIC have typically involved short-term exposures; applied specific bacterial groups and usually addressed only a single stress. In addition, most research findings on MIC have been obtained from the use of traditional microbial culture techniques. These culture methods have always been of major importance to advance our understanding of corrosion processes. However, these methods are restricted to cultivable microorganisms and do not reveal the complexity of microbial communities in different natural environments. It is known that 99% of microorganisms existing in nature are unable to be cultured by selective enrichment cultures and they will, therefore, be excluded when enumerated with growth media [156]. In addition, it has been

recognised that the complex mechanisms of MIC are not limited to particular groups of microorganisms. Microbial life in the environment is mostly characterized by multiplicity (many species together), nutrient limitation, changing environments, and a structured distribution of the biomass. It is therefore not too surprising that those traditional investigations of bacteria grown in the laboratory as pure cell lines with excess nutrients under constant and controlled conditions in liquid suspensions do not really contribute directly to an understanding of the ecology of microorganisms. Investigating the effects of environmental conditions and substratum surface on microbial community structure and composition in biofilms using culture-independent techniques, e.g. molecular microbiology methods, and ultimately their relationship with MIC will provide a better insight into the mechanisms of MIC of passive alloys in seawater and much more information for detailed studies of the metabolic pathways involved. Research studies on microbial populations developed on different passive alloys in seawater have, to our knowledge, never been reported.

Failure as a result of MIC damage during hydrotesting has been reported in the oil and gas industry. However, despite the broad significance and practical importance of evaluating the risk of MIC in subsea equipment filled with hydrotest water, research studies addressing this industrial concern are very limited. Long-term experimentation and the investigation of the effects of seawater treatments on corrosion resistance will provide very useful information to develop a more accurate risk assessment for offshore assets in relation to the requirements for long-term preservation.

1.4 Overview of the Research design

Common construction materials of subsea equipment selected for this research include carbon steel ASTM A572-50, austenitic stainless steels 316L (UNS S31603), super austenitic stainless steel 254SMO (UNS S31254), 13Cr

martensitic stainless steel, duplex stainless steel 2205 (UNS S31803) and super duplex stainless steel 2507 (UNS S32750) as well as nickel alloys Inconnel 825 (UNS N08825) and 625 (UNS N06625). Natural seawater, from a consistent geographical location, will be used to perform corrosion tests and to develop natural biofilms under laboratory controlled conditions. The basic experimental design will consist of exposing bare and artificially creviced alloys to raw and treated seawater for different exposure times, modifying experimental variables such as temperature and oxygen content. Artificial crevices were prepared based on the spring loaded assembly developed by the project Crevcorr of the European Commission.

Research studies will be conducted under both closed experimental conditions, in order to simulate the stagnant conditions at the interior of subsea equipment filled with treated seawater, and continuous flowing conditions. Abiotic control experiments will be conducted. To achieve aerobic conditions for aerobic assays, a continuous flow of filtered air will be injected. For anaerobic assays, nitrogen gas will be used as the inert gas to purge the test solution. Incubators and hot plates will be used to obtain test temperatures ranging from 5 and 40°C. This range of temperatures was reported by Chevron ETC, the partner industrial organization, as typical for offshore assets. Experimental methods will include immersion and accelerated corrosion (potentiostatic and potentiodynamic electrochemical analysis) along with surface and microbiological analyses. The phenomenon of ennoblement will be investigated routinely by monitoring the corrosion potential over time.

Molecular microbiology methods including polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and DNA sequencing of bacterial 16S rRNA gene fragments will be used to characterize microbial composition and structure in biofilms developed on the alloys. Sequences can be compared with data available in sequence databases which will, therefore, allow the construction of microbial community profiles. Microbial adhesion will be examined by fluorescence microscopy and scanning electrode microscopy (SEM). Surface features will be analysed by 3D optical microscopy and surface

profile measurements. Long-term localized corrosion studies of alloys in raw and treated seawater will be conducted over a period of 1.5 years, conducting corrosion and microbiological analyses every six months.

1.5 Outline of the thesis

This thesis is assembled as a hybrid of publications and submitted manuscripts as follows:

In **Chapter 2** the corrosion properties of the selected high-resistance alloys in natural seawater at temperatures from 5 to 40 °C are reported. This study defines the critical conditions of potential and temperature for each particular material in natural seawater. In this study, a complete and thorough comparison of the pitting and crevice resistance of the alloys in natural seawater is reported for the first time. This study highlights the superior resistance of the highest alloyed materials compared to low grade alloys and provides a powerful, rapid and reproducible means to evaluate pitting and crevice corrosion. In this study, a preliminary ranking of the alloys in their resistance to localized corrosion in seawater is established.

In the following chapters, the effect of biofilms on the characterized corrosion properties of the selected alloys in natural seawater and the manner in which oxygen and temperature may affect these biofilms and MIC are reported.

In **Chapter 3** the susceptibility to MIC of crevice-free alloys in natural seawater at 30 °C is reported. This temperature represents a typical temperature for offshore assets and a suitable temperature for the growth of the indigenous microorganisms in the seawater. This study illustrates the effects of biofilms and short-term seawater aging on the localized corrosion resistance of alloys in seawater under closed experimental conditions. Furthermore, it reveals the complexity in marine biofilm communities on the different alloys and shows the

degree to which material composition may affect the bacterial community and shift the microbial diversity in biofilms. Moreover, it provides with the appropriate electrochemical settings to evaluate MIC without disturbing the biofilm structure on corrosion specimens. This work discusses the possible relationship between electrochemical activity and biofilm composition.

Chapter 4 presents a study on the corrosion of carbon steel in natural seawater. In particular, these data show the relationship between carbon steel corrosion and biofilm community structure changes associated with the presence and absence of oxygen in seawater. The corrosion of carbon steel in seawater has long been the subject of extensive research. However, the complexity of the corrosion reactions taking place in seawater has generated controversy over the precise mechanism of both aerobic and anaerobic corrosion of carbon steel. This study highlights the crucial role of oxygen in influencing both the corrosion reactions in carbon steel in seawater and the composition and activities of the biofilm community on carbon steel. This work suggests that diversity in biofilm communities does not correlate with the extent of MIC.

On the basis of the characterized potential-temperature dependence on the onset of localized corrosion on the selected alloys in seawater (**Chapter 2**) along with the results from initial MIC studies at 30 °C (**Chapter 3**) follow up research studies were designed to investigate localized corrosion, mainly crevice corrosion, on certain alloys over others. Alloys UNS S31603, UNS S31803 and UNS N08825 were selected to conduct further studies as these materials, in their freshly ground condition, exhibit some degree of susceptibility to crevice corrosion in seawater at temperatures below 40 °C, temperatures that also support the growth of the mesophilic indigenous microbial populations in the seawater.

In **Chapter 5** the effect of biofilms and short-term seawater aging on crevice corrosion of UNS S31803 in seawater at 20 °C is reported. Preliminary results (**Chapter 3**) showed that UNS S31803 exhibits a critical temperature (T_{tr}) in the range from 20-30 °C at which crevice corrosion could initiate provided the

material had initially attained a transpassive potential. This study reports the influence of seawater aging on biofilm structure and its effect on the critical potentials and critical temperature for crevice corrosion in seawater. These results highlight the determining role of temperature on corrosion resistance and describe the likely effects of biofilm formation on decreasing crevice corrosion resistance when minimal conditions of potential and temperature are not reached.

In **Chapter 6** the influence of short-term seawater aging and temperature on corrosion resistance and microbial activity is reported for alloys UNS S31603 and UNS S31803. This study illustrates that the detrimental effects of temperature and biofilms on localized corrosion and MIC are determined by substratum material composition.

Chapter 7 presents a comprehensive study on the effect of oxygen and biofilms on crevice corrosion of UNS S31803 and UNS N08825 in seawater at 30 °C. This work highlights the critical role of oxygen in both the mechanism of crevice corrosion and the structure of the biofilm community developed on a creviced surface. This study also indicates that biofilm community composition, microbial colonization and the detrimental effects of both temperature and biofilms on crevice corrosion are determined by material composition. It is again indicated that microbial diversity in biofilms does not correlate with the extent of MIC.

In **Chapter 8** the long-term corrosion performance of all the selected off-shore materials in seawater is reported. In particular, pitting and crevice corrosion resistance is reported for alloys in both treated and raw untreated seawater under stagnant conditions over a period of 18 months. This study indicates that the pattern of microbial colonization as well as the degree of localized attack is determined by material composition. These effects were not affected by exposure time and seawater treatment.

Chapter 9 presents a study conducted at a test rig in a field laboratory in New South Wales, Australia. This study illustrates the corrosion performance of the alloys in both raw and treated seawater in a continuous flow system over 90 days. This study highlights the differences found in biofilm activity and corrosion performance when materials are exposed to open conditions, where seawater is replenished continuously, compared to stagnant conditions. Again, this study highlights the influence of alloying composition on microbial attachment and biofilm structure. The effects of biofilms on corrosion resistance are explained on the basis of the potential-temperature dependence of passive alloys on the onset of localized corrosion.

Finally, **Chapter 10** presents a study of the risk of MIC and localized corrosion associated with seawater ingress into offshore flowlines, filled with seawater treated in accordance with hydrostatic testing procedures. In particular, this study illustrates the effect of oxygen and microorganisms on localized corrosion of alloys exposed to various mixtures of treated seawater. The risk of MIC associated with subsea tie-in operations for the installation and commissioning of deep-water pipelines for offshore fields is an increasing concern for the oil and gas industry. However, a study addressing this matter had not been published previously despite its broad significance and practical importance.

1.6 Review and Discussion

Research studies on the evaluation of the risk of MIC of high-resistance alloys associated with offshore operations require a multidisciplinary approach to accurately address industrial concerns while contributing to the knowledge and understanding of the research field. Today, there is scattered data from inconsistent test methodologies and limited information in the open literature regarding the effect of typical ocean temperatures and exposure conditions for subsea assets on MIC. This lack of information has prompted this investigation into the practices and procedures of treating fluids for the extended

preservation of subsea equipment constructed from standard CRA materials. The purpose was to investigate the relationship between corrosion resistance and microbiological activity at several ocean temperatures for the preservation of subsea equipment. The selected CRAs were first characterized in their resistance to localized corrosion in seawater. This initial investigation allowed defining the critical conditions of potential and temperature on the onset of pitting and crevice corrosion for each particular material in natural seawater. UNS S32750 and UNS S31254 displayed excellent resistance to localized corrosion at temperatures below 40 °C. UNS N08825 and UNS S31803 showed good resistance to pitting corrosion at temperatures below 40 °C but displayed poor resistance to crevice corrosion at temperatures above 5 and 30 °C, respectively. UNS S31603 suffered pitting and crevice corrosion in seawater at temperatures from 10 °C and 5 °C, respectively. According to this preliminary evaluation, alloys were ranked in their resistance to localized corrosion in seawater. To assess the effect of seawater aging and biofilms on localized corrosion resistance, short and long-term pitting and crevice corrosion studies were conducted using natural seawater allowing indigenous microorganisms in the seawater to develop biofilms on the CRA surfaces. The effect of biofilms on the corrosion performance of the alloys in seawater was shown to be highly influenced by temperature, oxygen and nutrient availability/flowing conditions. In stagnant closed systems, biofilms did not ennoble the corrosion potential of either crevice-free or creviced alloys in seawater. Biofilms, however, reduced pitting and crevice corrosion resistance via decreasing critical potentials for localized corrosion initiation and repassivation. The mechanism by which biofilms achieved this was not identified. Under closed experimental conditions, an external anodic potential was necessary to trigger localized corrosion on alloys. All alloys remained protected against pitting under open-circuit conditions during short-term exposures. However, data obtained from CRAs exposed to filtered and UV irradiated seawater, indicated that minimal conditions for bacterial growth can activate microbial metabolism and support the formation of active biofilms able to modify the electrochemical properties of the alloys. Moreover, it has been shown that the absence of nutrients in a system, e.g. during stagnation periods, can force some specialized

microorganisms to search for a supplementary electron source and acceptors such as solid stainless steel electrodes which can modify the electrode potential [94, 113, 116].

On the other hand, in continuously flowing seawater, active biofilms quickly ennobled the corrosion potential of all materials by +400-500 mV. This ennoblement triggered localized corrosion under open-circuit conditions on UNS S31603, UNS N08825 and UNS S31803 but did not initiate localized corrosion on the higher alloyed materials UNS S32750, UNS S31254 and UNS N06625. These results are explained on the basis of the potential-temperature dependence of passive alloys on the onset of localized corrosion. Studies on the effect of temperature on MIC of low alloyed materials indicated that MIC of alloys is favoured by the increase in seawater temperature. However, this conclusion is drawn on the basis of data obtained from experiments using seawater up to a maximum temperature of 30 °C. It is believed that at higher temperatures, the growth and activity of mesophilic indigenous microorganisms in seawater is diminished and therefore also will be the risk of MIC. This is probably why higher alloyed materials, which display critical temperatures for crevice corrosion above 40 °C, did not initiate localized corrosion in the presence of biofilms.

Biofilms were shown to shift the mechanisms of carbon steel corrosion in seawater, i.e. from uniform corrosion under aerobic conditions to localized corrosion in the absence of oxygen. This study highlighted the crucial role of oxygen in influencing both the corrosion reactions of carbon steel in seawater and the composition and activities of the biofilm community on carbon steel. The corrosion of carbon steel in seawater has long been the subject of extensive research. Particular attention has been given to the predominant role of SRB in the accelerated corrosion of this steel due to their metabolic production of sulphide ions. Interestingly, PCR-DGGE analysis indicated that the mechanism of pitting under anaerobic conditions did not involve the presence of SRB within the biofilm community. These results highlight the importance of focusing MIC

research studies on the interaction between metallic materials and complex biofilm matrixes rather than individual microbial populations.

Biofilms were also shown to shift the mechanisms of corrosion in highly alloyed steels. Results from this research showed that biofilms were able to trigger crevice corrosion in UNS S31803 at conditions of temperature and potential that would otherwise induce only transpassive dissolution of the alloy. These findings had never been reported before. The mechanisms by which microorganisms triggered crevice corrosion under these conditions are uncertain. However, PCR-DGGE analysis indicated a correlation between changes in microbial community structure and the decreased crevice corrosion resistance of the alloy with exposure time.

Crevice corrosion was shown to be highly influenced by oxygen and biofilms. Crevice corrosion was more severe under totally anaerobic conditions compared to exposure in constantly aerated seawater. In the absence of oxygen, the alloy exhibited higher propagation current densities and the repassivation process was delayed. Moreover, anaerobic conditions favoured the formation of biofilms with more aggressive properties towards localized corrosion resistance of the alloys. The mechanisms of crevice corrosion under anaerobic conditions have been poorly defined in the literature. It is commonly agreed that oxygen removal from the environment will prevent localized corrosion since the lack of a cathodic reactant will prevent the formation of aeration cells and anodic dissolution. The conclusions from this investigation are drawn based on data obtained from experiments applying external polarization to the materials. Under open-circuit conditions, crevice corrosion was not initiated. This data may suggest, however, that in the absence of oxygen, and if passivity is broken by other mechanisms, e.g. catalytic effects of anaerobic biofilms, repassivation processes are less likely to take place and crevice corrosion may be triggered. This is also supported by the fact that microbial colonization of creviced surfaces was shown to be preferential at the cathodic region (exposed area) of the surface.

Long-term exposure studies indicated that seawater filtration, in accordance with hydrostatic testing procedures, reduces corrosion rates of carbon steel and CRAs in seawater. However, it does not prevent localized corrosion under open-circuit conditions if the physicochemical conditions required to initiate localized corrosion are attained during exposure. The mechanisms that triggered localized corrosion under these conditions were, however, not investigated. Nevertheless, filtration may reduce the likelihood of localized corrosion and MIC by removing sediments or sand, thus preventing under-deposit corrosion or the formation of crevices on the surface. Filtration also reduces the source of nutrients for microbial growth. Chemical treatments used for hydrotesting waters were shown to confer protection against localized corrosion and MIC provided oxygen is restricted to minimal levels in the system. In the presence of high oxygen content, the efficiency of the chemical treatments was reduced. Below 20 ppb dissolved oxygen (DO) in the system, the alloys remained protected against pitting at the recommended dosages of chemical treatments. Similarly, in seawater mixtures containing 80% of the recommended dosages of chemical treatment (60 ppb DO), the alloy still exhibits resistance to pitting corrosion. A DO concentration of 2 ppm induced pitting from the first week of exposure even at the recommended dosage of chemical treatments.

The application of PCR-DGGE and DNA sequencing as a molecular tool to examine biofilm community composition proved to be a powerful method to investigate the effects of environmental conditions and substratum surface on the microbial community structure in biofilms. This method showed that biofilm populations on corrosion resistant alloys are highly specific in their preference for substratum surface and oxygen conditions. It was shown that microbial diversity in biofilms formed in seawater is favoured in the presence of oxygen. However, microbial diversity does not correlate with the extent of MIC attack. Microbial colonization was shown to be more copious and diverse on nickel based-alloys than on stainless steels. Analysis of biofilm communities on high-resistance alloys in seawater has not been reported previously. These data extend our knowledge on microbial ecology associated with corrosion resistant materials in seawater.

This research has allowed the identification of the critical factors that influence the performance of offshore construction materials in natural seawater. UNS S32750, UNS S31254 and UNS N06625 render good resistance to pitting and crevice corrosion in seawater at temperatures below 40°C regardless of the presence of biofilms and oxygen. Alloys UNS S31603, UNS N08825 and UNS S31803 are susceptible to localized corrosion in natural seawater at temperatures above 5-10°C, 10-20 and 20-30°C, respectively. The risk of localized corrosion is increased by prolonged exposure to seawater, by the increase in temperature and by the presence of biofilms. However, the lack of mechanistic studies for MIC as part of this research did not allow identification of detailed mechanisms on the selected alloy materials. However, findings from this research will constitute the foundations for future mechanistic studies on the precise interactions of biofilms with the different passivating oxide films formed on these offshore alloys. This will help elucidate the main factors that influence microbial adhesion on these alloys and will assist in developing new strategies and methods to prevent MIC in subsea equipment. As discussed, there are, however, many questions that remain to be answered before a full understanding of the subject is achieved.

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Chapter 3

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Chapter 7

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Effect of oxygen and biofilms on crevice corrosion of UNS S31803 and UNS N08825 in natural seawater

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Abstract

The effect of oxygen and biofilms on crevice corrosion of UNS S31803 and UNS N08825 in seawater was studied. Passivity breakdown occurred through crevice corrosion in UNS N08825 and through transpassive dissolution in UNS S31803 although both alloys displayed crevice corrosion under potentiodynamic conditions. The most severe crevice corrosion occurred in the absence of oxygen and the presence of a biofilm. Microbial adhesion as investigated by fluorescence microscopy occurred mainly outside the crevice and DNA sequencing revealed a shift in biofilm composition as a function of substratum surface and oxygen pressure.

Keywords: stainless steel, alloy, polarization, crevice corrosion, microbiological corrosion.

1. Introduction

In marine environments it is well known that corrosion resistant alloys may still suffer from localized corrosion. In natural seawater there is an increased risk for localized corrosion due to the presence of microorganisms that form biofilms [1-3]. To ensure the appropriate corrosion resistance required for marine applications, a new generation of alloys such as duplex stainless steels (DSS) and nickel-base alloys have been developed. The increased corrosion resistance of these alloys is provided by the presence of higher concentrations of alloying elements such as chromium, nickel and molybdenum. In oxygen containing environments, these alloys form a passivating film on the surface that protects the alloy from aggressive species such as halides. This protective film has self-healing properties when damaged if oxygen is present. However, under certain conditions aggressive ions such as chlorides may compromise the stability of this passivating film, which sometimes, in combination with increased temperatures, may induce permanent rupture of the protective film and promote localized corrosion in the underlying metal [4]. The exact mechanism of passivity breakdown in chloride environments is not known with certainty, but it seems to implicate a potential-controlled adsorption reaction involving chloride ions and specific sites on the metal surface such as irregularities, discontinuities and uneven deposits (e.g. biofilms, debris) [5].

One of the main concerns with high-resistance alloys in marine environments is crevice corrosion (CC). CC is a form of localized attack that occurs within occluded regions or crevices such as overlapping surfaces, incompletely sealed gasket/metal interfaces, threaded joints, irregular penetrating welds or naturally occurring deposits. In the presence of crevices, localized corrosion initiates more easily owing to the presence of a crevice gap where the electrochemical conditions become different to those on the boldly exposed surface [6]. Hitherto, different models for crevice corrosion initiation have been postulated. Existing theories include the

development of a critical crevice solution (CCS) [7], which refers to the formation of a differential aeration cell between the crevice gap and the outer solution followed by a gradual build-up of aggressive species within the crevice electrolyte. An alternative theory advocates the development of a current resistance (IR) drop [8, 9] which refers to the electrode-potential variation between the crevice and the outer surface. This theory stipulates that when the IR drop inside the crevice becomes large enough to force the crevice to move to an active potential, passivity is destroyed and active dissolution of the metal occurs. Crevice corrosion initiation has also been linked to metastable pitting that is stabilized at the occluded environment [10, 11]. This theory suggests that metastable pits formed within a crevice are more likely to become stabilized due to the resistive barrier of the crevice geometry. Sulphide inclusions in the passive film were also proposed to play a role in CC initiation since chemical analysis of the crevice electrolyte during the induction period of CC showed that sulphur species were the dominant compounds [12].

The involvement of microorganisms in marine corrosion has long been recognized [13-16]. Microbial adhesion and the consequent biofilm formation on structural materials in seawater have shown to increase the likelihood of localized corrosion by different means. For instance, ennoblement of passive alloys - a shift of the open circuit potential in the anodic direction due to bacterial presence and/or activity - may reduce the initiation time for pitting and crevice corrosion [17, 18]. Mechanisms of biofilm-enhanced corrosion also include cathodic depolarization [19], production of aggressive metabolites [20] and catalytic enzymes [21, 22] and biodeposit formation leading to crevice type of attack [23]. Biofilms have also been shown to decrease initiation times for localized corrosion by lowering the critical pitting and repassivation potential of alloys [2, 24]. The role of biofilms in crevice corrosion propagation has also been examined [25]. It was suggested that the

increased propagation rate was caused by catalysis of the cathodic reaction by biofilms.

The investigation of biofilm communities on corroding surfaces has become essential to advance the understanding of the complex mechanisms involved in the biofilm-enhanced corrosion processes. The study of biofilm community structure may provide information on the interactions of biofilm populations with different steel grades and physicochemical variables by investigating the shift in biofilm communities in response to environmental conditions. Hitherto, only a few studies have considered analyzing the microbial community structure of biofilms on corroding surfaces [2, 26-28] and only one recent study has been conducted to investigate microbial communities in biofilms developed on creviced surfaces [29].

In marine environments, it is of particular importance to investigate the role of oxygen in the kinetics of metal dissolution as it is a critical element not only in the formation and maintenance of passive films in active-passive alloys, but also it is a determining factor in the structure and physiological activities of biofilms on surfaces [30]. In addition, the role of oxygen in crevice corrosion has been previously reported and oxygen depletion appears to be a prerequisite for the initiation and stabilization of crevice corrosion [7]. Therefore, it appears of vital importance to investigate the interactions between crevice corrosion of high-resistance alloys and biofilm formation in natural seawater, and in particular to study the role that oxygen plays on those interactions. Nonetheless, a comprehensive study with focus on these aspects is lacking.

In this study, experiments were designed to sustain biofilm growth on artificially creviced alloys in natural seawater in the presence and absence of oxygen for up to 30 days. Experiments were maintained under closed experimental conditions, without water replenishment, in order to simulate the interior of off-shore

pressure equipment in which seawater from hydrotesting, ballast or preservation can remain inside the equipment for extended shut-down periods. We investigated crevice corrosion on UNS S31803 DSS and UNS N08825 nickel-base alloy in seawater by conducting corrosion potential (E_{corr}) monitoring, potentiodynamic polarization tests and surface analysis by optical microscopy. In particular we examined the influence of oxygen and marine biofilms on the crevice corrosion initiation and repassivation of the alloys in seawater at 30°C, a typical temperature for offshore assets, and a suitable temperature for the growth of biofilms composed of mesophilic microorganisms. Microbial adhesion was studied using 4,6-diamidino-z-phenylidole (DAPI) fluorescent dye and the biofilm community structure was examined using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S rRNA genes and DNA sequencing to help establish a relationship between biofilm community structure and crevice corrosion associated with the presence or absence of oxygen in seawater.

2. Experimental procedure

2.1. Specimen preparation

Commercial UNS S31803 stainless steel and UNS N08825 nickel-base alloy were used in this study. The chemical composition of these alloys in weight percent is described in Table 1. Samples and spring loaded crevice assemblies were prepared as described previously [31]. The arrangement for crevice evaluation is shown in Figure 1. Prior to each experiment, square coupons of approximately 50 cm^2 surface area and a thickness of 5-7 mm were drilled in the centre with a 7 mm diameter hole and were wet ground up to 600 grit finish (SIC grinding paper), degreased with acetone and dried with nitrogen gas. The crevice formers (outside diameter 20 mm, inside diameter 7 mm, height 15 mm) were made of PVDF (polyvinylidene diflouride), ground to 1200 grit finish, cleaned and dried before

use and four disc springs (nylon coated steel) were used to maintain a constant load corresponding to the 3 N.m applied torque. Test specimens and all pieces of the crevice assembly were soaked in Decon® 90 (Decon laboratories Limited) for 3 hours and sterilized by immersion in 70% ethanol for 1 hour before assembling and exposure.

Table 1.

Alloying composition and PREN value of materials used in the experiments

Alloy	Type	C wt %	Mn wt %	Fe wt %	Cr wt %	Ni wt %	Mo wt %	N wt %	S Wt %	PREN
UNS S31803	Duplex SS	0.015	1.53	bal	22.35	5.72	3.16	0.18	0.001	35.65
UNS N08825	Nickel base alloy	0.05	0.85	22	22.50	bal	3	-	0.03	27

[a]**PREN=** %Cr + 3.3 %Mo + 16 %N (stainless steel) %Cr + 1.5 (% Mo + % W + %Nb) (nickel base alloy)

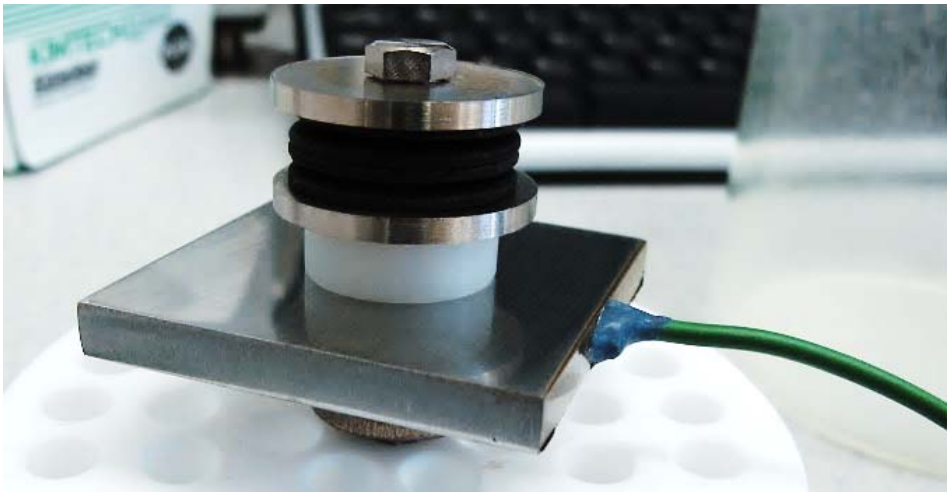

Figure 1. Spring loaded crevice assembly used to evaluate crevice corrosion of alloys.

2.2. Test conditions

Artificially creviced coupons were formed and immersed in natural seawater. Creviced coupons were maintained under open-circuit closed experimental conditions (without seawater replenishment) for 30 days to allow a biofilm to develop on the specimens. Seawater was collected from 20 metres depth in the Indian Ocean off Rottnest Island (Western Australia). The chemical composition of the seawater is shown in Table 2.

Table 2.

Analysis of the natural seawater used in these experiments

Analysis	**Composition**
Salinity [PSU]	35.58
DO [mg/L]	5.06
Conductivity [mS/cm]	48.79
pH	8.2
Chloride [mg/L]	18500
Magnesium [mg/L]	1340
Sodium [mg/L]	11100
Sulphate [mg/L]	2700

To evaluate the effect of oxygen and biofilm formation on crevice corrosion, four reaction vessels were set up for each alloy (Table 3). A schematic of the experimental set-up is shown in Figure 2. A continuous filter-sterilized (0.22 µm nylon syringe filters) gas (either air or N_2) inflow was maintained in all vessels to ensure consistent levels of gas saturation for the duration of the experiment. Dissolved oxygen was monitored using an Orbisphere 3655 oxygen analyser (Hach

Company). Test temperature was maintained at 30°C using a circulating water bath. Coupons were immersed in the electrolyte solution using a Teflon holder placed at the bottom of the cells. Five sample replicates were immersed in each cell which was filled with 7 L of seawater. Control experiments consisted of artificially creviced coupons immersed in filter-sterilized seawater (0.22 μm polycarbonate filters). At the completion of exposure, potentiodynamic polarization tests, surface analyses and biofilm collection from specimens were conducted.

Table 3.

Experiments conducted to evaluate crevice corrosion of alloys in natural seawater

Material	Reaction vessel	Electrolyte solution
UNS S31803	SS[a]-Aerobic test	natural seawater containing bacteria; solution saturated with air
	SS-Aerobic control	sterilized seawater without bacteria; solution saturated with air
	SS-Anaerobic test	natural seawater containing bacteria; solution purged with nitrogen
	SS-Anaerobic control	sterilized seawater without bacteria; solution purged with nitrogen
UNS N08825	N[b]-Aerobic test	natural seawater containing bacteria; solution saturated with air
	N-Aerobic control	sterilized seawater without bacteria; solution saturated with air
	N-Anaerobic test	natural seawater containing bacteria; solution purged with nitrogen
	N-Anaerobic control	sterilized seawater without bacteria; solution purged with nitrogen

[a]SS: stainless steel [b]N: Nickel-based alloy

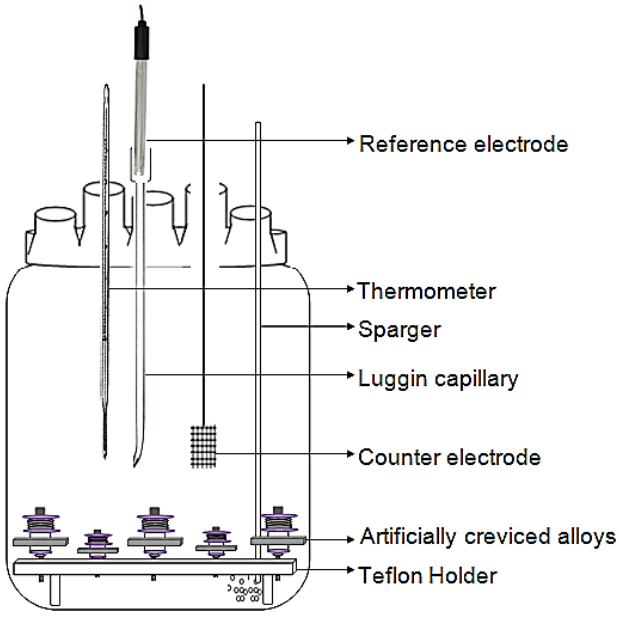

Figure 2. Schematic illustration of the reaction vessel used to perform the experiments.

2.3. Electrochemical measurements

All experiments were conducted using a conventional three electrode cell assembly [32]. The corrosion potential, E_{corr}, of specimens was measured against a double junction Ag/AgCl reference electrode (RE) and monitored daily throughout the immersion period. Cyclic polarization scans were conducted in separate vessels using a three-electrode set up where the creviced alloys served as working electrodes, a platinum coated mesh was used as counter electrode and the Ag/AgCl electrode as RE. Cyclic polarization tests were conducted in fresh natural seawater at 30°C sparged with the same gas used during exposure (either N_2 or air) to identify breakdown potential (E_b) and repassivation potential (E_r) of the aged creviced samples. Before conducting the scans, samples were allowed to stabilize in the fresh seawater at open-circuit for 1 hour. The scans were carried out using a forward and reverse scan rate of 0.167 mV/sec. The sweep direction was reversed when either a current density of 1.5 mA/cm^2 or a potential of 1.5 V vs. RE was

reached. The initial and final point of the scan was set at a potential of -0.1 V vs. E_{corr}. The E_b was identified as the potential where an increase in current density indicated the onset of transpassivity or crevice corrosion (current increase at the passive region). The E_r was identified as the potential for which the forward and reverse scans intersect, which is where repassivation of pits is considered to have taken place.

Some creviced coupons were not potentiodynamically polarized at the end of exposure in order to evaluate crevice corrosion under open-circuit conditions.

2.4. Surface analysis of crevice corrosion

Optical measurements and inspection to evaluate crevice corrosion of the tested alloys were conducted using an infinite focus microscope (IFM G4g system, Alicona Imaging). Prior to surface inspection, specimens were cleaned using the standard procedure [33].

2.5. Evaluation of bacterial adhesion

To evaluate bacterial attachment, alloy surfaces were stained with 4,6-diamidino-z-phenylidole (DAPI), a fluorescent dye that binds strongly to DNA. Specimens were removed from the reaction vessel, separated from their crevice assembly, rinsed with sterile water, stained with DAPI (2µg/mL; Sigma) and incubated in the dark at room temperature for 15 min. DAPI-stained samples were examined with an epifluorescence microscope (Axio Imager.A1; Carl Zeiss, Germany) equipped with a Plan-Neofluar objective.

2.6. Characterization of biofilm community structure by PCR-DGGE analysis of 16S rRNA gene fragments and DNA sequencing

The diversity of the bacterial community in biofilms developed on creviced alloys under aerobic and anaerobic conditions was examined by PCR-DGGE analysis. Biofilm detachment from coupons, DNA extraction from biofilm cells and PCR-DGGE analysis were performed as previously described [34]. Coupons were dismounted and suspended in seawater containing filter-sterilized Tween 20 (0.1% w/v final concentration) and microbial cells were detached by 60 second-sonication steps (solution was refreshed between sonication steps). Biofilm microorganisms in the sonicated suspensions were harvested by centrifuging at 10,000 x *g* for 20 min and resuspended in phosphate buffer solution (PBS). DNA was extracted using a commercial soil kit (PowerSoil™ DNA Isolation Kit, MO BIO Laboratories Inc) and used as template to amplify a specific region of the 16S rRNA gene using nested PCR approach. The outer primer pair was 27F and 1492R and the inner primer pair was 357F-GC and 907R (Table 4) [35, 36]. The PCR-amplified 16S rRNA gene fragments were separated using a DCode Universal Mutation Detection System (BIORAD) as per the manufacturer's instructions. The PCR products were mixed with DNA loading buffer (BIOLINE) and loaded onto 7% (w/v) polyacrylamide gel (40% acrylamide/bis solution, 37.5:1) with a denaturing gradient of 30-60% urea-formamide (100% denaturant: 7M urea, 40% deionised formamide) in 1x TAE buffer (2 M Tris base, 1 M glacial acetic acid, 50 mM, EDTA, pH 8.0). The DGGE was run at 150 V (60°C) for at least 5 hours. DNA bands were visualized using a gel illuminator upon staining with SYBR® Gold nucleic acid gel stain (Invitrogen™) and excised from each lane using sterile scalpel blades. The bands were resuspended in 40 µL of sterile RNase-free water overnight (4°C). Eluted DNA from individual bands was re-amplified by PCR using primers 357F (no GC clamp) and 907R (Table 4). Finally PCR products were visualised by electrophoresis and sequenced with primer 357F at Macrogen, South Korea. DNA sequences were compared against reference sequences in GenBank database using the Basic Local Alignment Search Tool (BLAST; http:/www.ncbi.nlm.nih.gov/blast/) to obtain phylogenetic information [37].

Table 4.

Sequencing primers used in this study for the amplification and sequencing of the full-length and variable region of the 16S rRNA gene.

Primer[a]	Amplification target	Sequence (5' to 3')	Reference
27F[b]	Bacteria	GAGTTTGATCCTGGCTCAG	[35]
357F	Bacteria	CCTACGGGAGGCAGCAG	[36]
357F-GC[c]	Bacteria	CCTACGGGAGGCAGCAG	[36]
1492R[b,d]	Prokaryotes	ACGGdITACCTTGTTACGACTT	[35]
907R[b]	Universal	CCG TCA ATT CMT TTG AGT T	[35]

[a]The number relates to the *E.coli* position to which the 3' end of the primers anneals. F and R correspond to forward and reverse primers, respectively.
[b]Modified from the original paper.
[c]GC is a 40 nucleotide GC-rich sequences attached to the 5' end of the primer. The GC sequence is 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG.
[d]dI denotes a deoxyinosine modification.

3. Results

3.1. Electrochemical measurements

Experiments were conducted to investigate the effect of microorganisms in natural seawater (Test) by comparison with sterile experiments where microorganisms were removed from the seawater by 0.2 µm filtration (Control). Figure 3 shows average E_{corr} versus time data of creviced UNS S31803 (Figure 3 (a)) and UNS N08825 (Figure 3 (b)) exposed to seawater at 30°C under aerobic and anaerobic conditions over 30 days. Under anaerobic conditions UNS S31803 showed a gradual shift of the E_{corr} towards negative values from the first days of exposure

until the completion of the immersion period. The final E_{corr} was approximately 300 mV more negative than the initial $E_{corr.}$ Similar results were found in the anaerobic test and control which indicates that the presence of bacteria in the seawater did not trigger the negative polarization of UNS S31803 with exposure time. On the other hand, UNS S31803 exposed to aerated seawater exhibited a very stable E_{corr} throughout the period of exposure and showed no polarization at any time of the exposure. Again, no differences were observed between the aerobic test and control experiments. Similarly, UNS N08825 showed polarization of the E_{corr} towards negative values with increasing exposure time under anaerobic conditions. The anaerobic test exhibited slightly more active E_{corr} values, on average 100 mV lower, than the anaerobic control throughout the exposure time. Likewise, UNS N08825 in seawater under aerobic conditions displayed an E_{corr} that moved towards more active values with increased immersion time in both the presence and absence of bacteria. A similar extent of E_{corr} polarization was observed in both the aerobic test and control for this alloy.

Potentiodynamic polarization studies were carried out to evaluate potential versus current density relationship of the creviced alloys pre-exposed to the seawater under four different experimental conditions for 30 days. The cyclic polarization behaviour of UNS S31803 and UNS N08825 pre-exposed to seawater is represented by curves in Figures 4 and 5, respectively. Important parameters defining the shape of the active-passive type of polarization curve can be used to compare the localized corrosion resistance of the two different alloys under different experimental conditions [38]. These electrochemical parameters were identified for the tested alloys at all the experimental conditions and results are summarized in Table 5. However, samples exposed to seawater containing bacteria (aerobic test) showed a slightly lower E_b than the E_b of the control (Table 5).

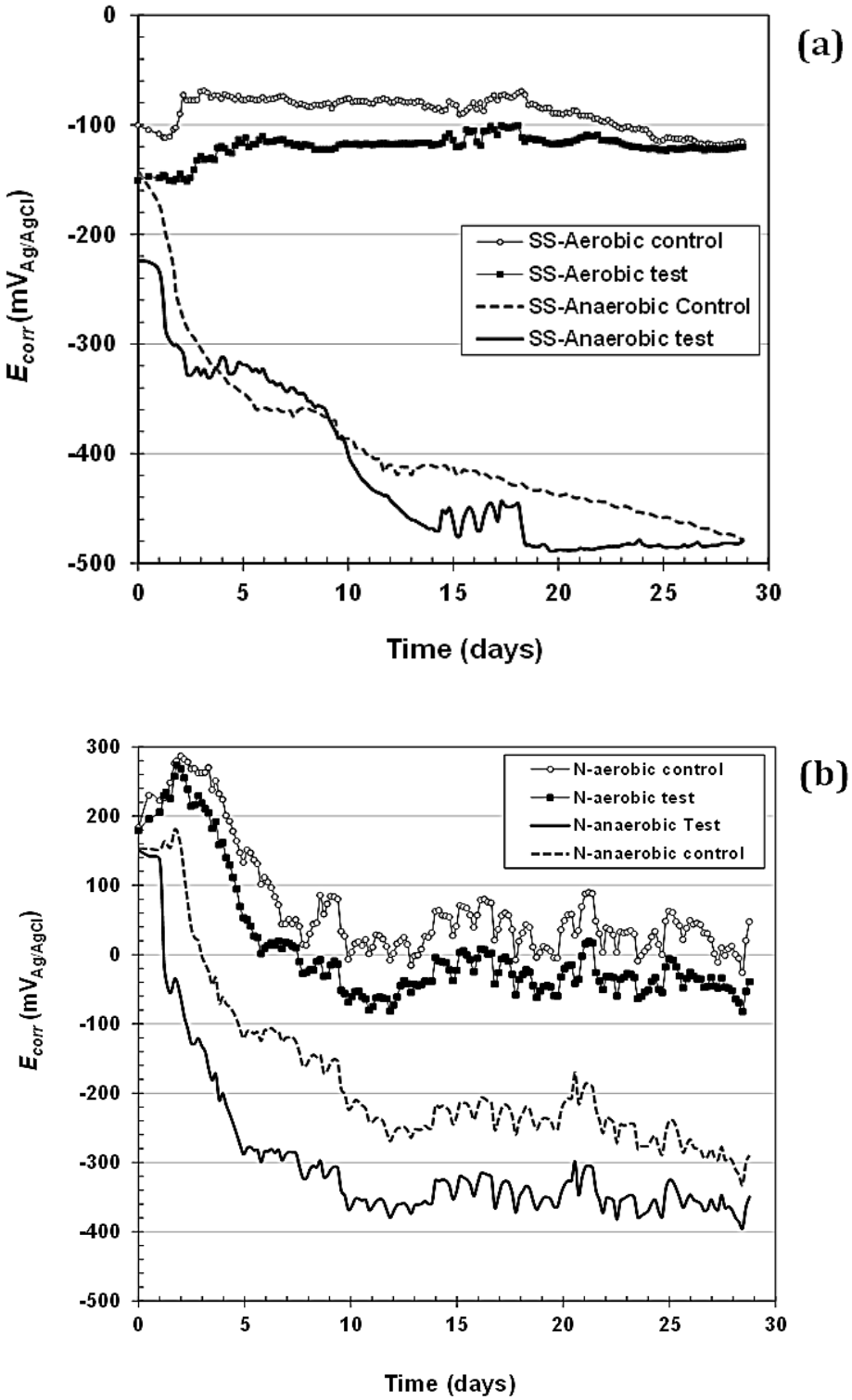

Figure 3. Average corrosion potential, $E_{corr,}$ versus time data of duplicate specimens of (a) UNS S31803 and (b) UNS N08825 exposed to control and test seawater at 30 °C under aerobic and anaerobic conditions for 30 days.

Upon reversal of the scan, the potential versus current transient tracked back along the forward scan for some hundreds of mV until the current density established a steady value as the potential continued decreasing. Then, the current density showed a slight gradual increased during the reverse sweep until the E_r was reached. This resulted in the formation of a hysteresis loop and a subsequent negative (active) repassivation potential which indicated that active dissolution processes had taken place. For UNS S31803 under aerobic conditions, the maximum current density reached during the reverse scan was higher for the test than the control and the E_r was found to be more active in the test than in the control as well (Table 5).

From the potentiodynamic polarization scan it can be seen that UNS S31803 exposed to anaerobic conditions exhibited a very active E_{corr} from which a nearly linear increase in current with potential was observed upon the anodic scan (Figure 4 (b)). Passivity breakdown also occurred at very noble potentials in both test and control under these exposure conditions. In the anaerobic control, a passivation region was formed at a potential near 400 mV (Ag/AgCl). This passivation state was not observed in the anaerobic test. In addition, the anodic polarization curve of UNS S31803 under anaerobic conditions revealed that the E_b was reached at a lower potential in the test than in the control (Table 5). During the reverse scan, UNS S31803 exposed to seawater under anaerobic conditions also displayed a current increase upon reversing of the scan in both test and control. However the maximum current density upon reverse of the scan (i_{max}) as well as the E_r of the alloy remained the same regardless of the presence of biofilm.

In the case of UNS N08825, similar polarization curves were observed for specimens exposed to seawater under aerobic and anaerobic conditions. Under aerobic conditions (Figure 5 (a)), no major differences were observed between test and control. However, slightly higher current densities were reached upon the

reverse scan for the aerobic test than the control. Interestingly, a passivation region is also formed in UNS N08825 test and control experiments under aerobic conditions at a potential near 400 mV (Ag/AgCl) during the anodic scan as observed for UNS S31803. Then, a sudden increase in the current density with increasing potential was observed. The formation of a hysteresis loop between the forward and reverse scans and the very active E_r indicated that UNS N08825 displayed poor resistance to crevice corrosion regardless of the presence of bacteria in the seawater under aerobic conditions. UNS N08825 in seawater under anaerobic conditions exhibited similar polarization behaviour in both test and control (Figure 5 (a)). However, UNS N08825 exposed to seawater with bacteria displayed slightly more active values of $E_{corr,}$ E_b and E_r as compared with the control, which reveals an exacerbating effect of bacterial presence and/or activities on the crevice corrosion of UNS N08825 under anaerobic conditions. As for UNS N08825 exposed to aerobic conditions, a passivation region is formed on UNS N08825 anaerobic control near 400 mV (Ag/AgCl) upon the anodic scan until a second activation state is reached at higher potentials. This passivation region was not observed in UNS N08825 anaerobic test (exposed to seawater containing bacteria) where the current density increased steeply from E_b to the beginning of the reverse sweep.

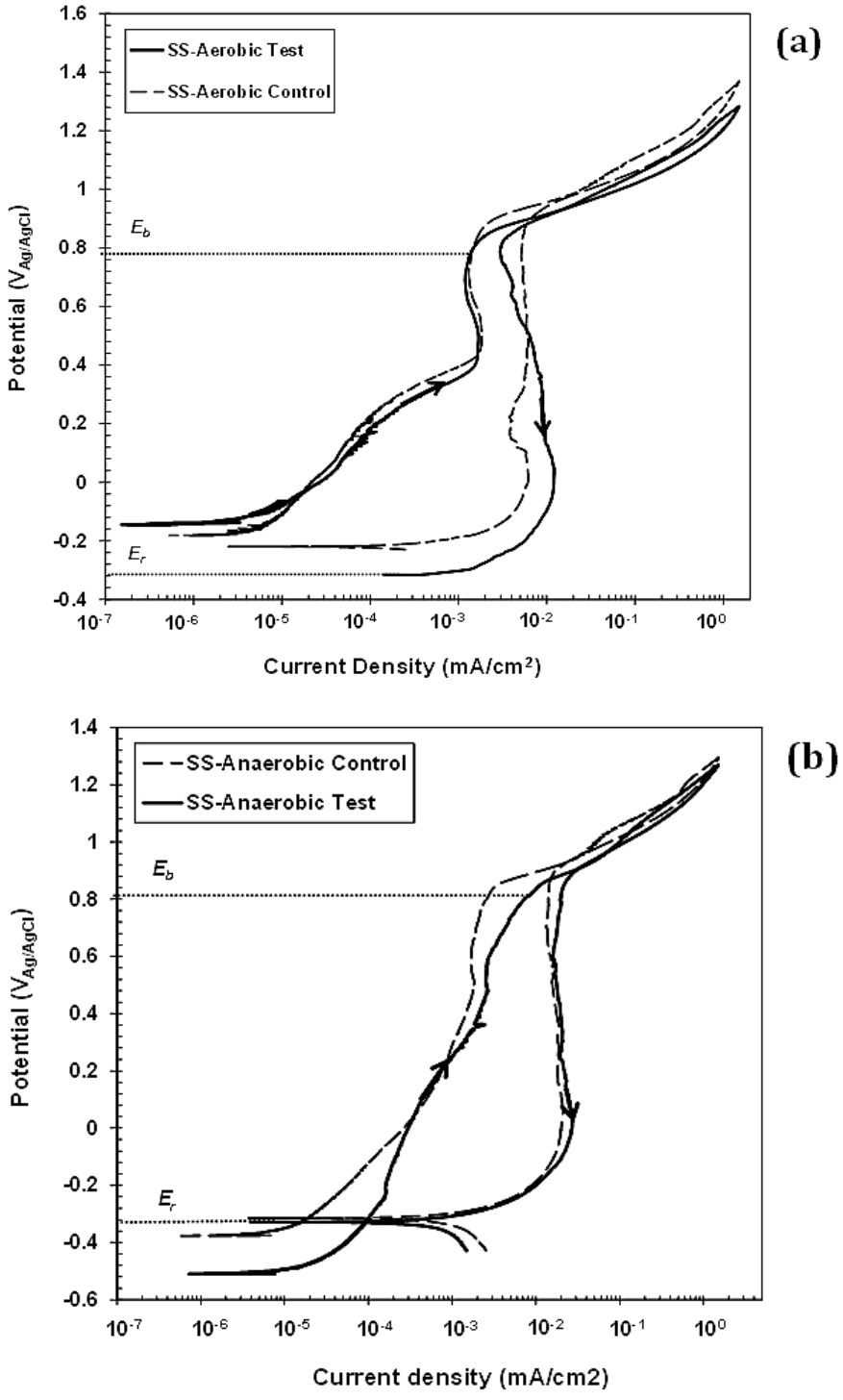

Figure 4. Cyclic potentiodynamic polarization curves showing critical potentials for UNS S31803 pre-exposed to control (sterile) and test (containing bacteria) seawater at 30 °C for 30 days under: a) aerobic conditions and b) anaerobic conditions. E_b: Breakdown potential; E_r: Repassivation potential.

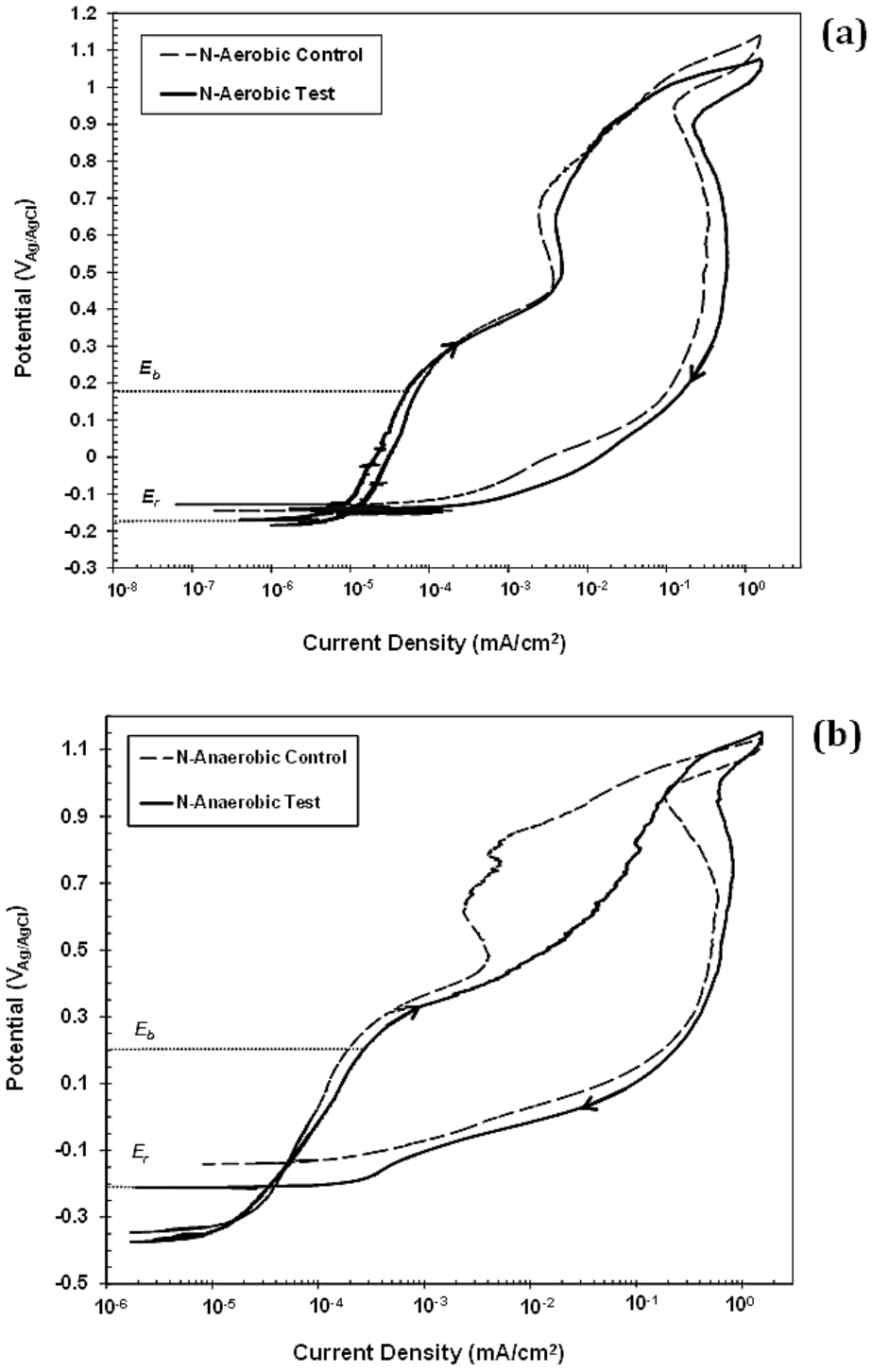

Figure 5. Cyclic potentiodynamic polarization curves showing critical potentials for UNS N08825 pre-exposed to control (sterile) and test (containing bacteria) seawater at 30ºC for 30 days under: a) aerobic conditions and b) anaerobic conditions. E_b*:* Breakdown potential; E_r*:* Repassivation potential.

Table 5.

Electrochemical parameters to evaluate crevice corrosion obtained from cyclic potentiodynamic polarization scans

Material	Experiment	E_{corr}[1] (mV)	E_b[2](mV); Reaction	E_r[3] (mV)	i_{max}[4] (mA/cm^2)
UNS S31803	aerobic test	-155	780; Trans[5]	-310	0.012
	aerobic control	-199	875; Trans	-218	0.0067
	anaerobic test	-526	803; Trans	-326.00	0.0267
	anaerobic control	-394	815; Trans	-326.00	0.021
UNS N08825	aerobic test	-175	187; CC[6]	-138	0.58
	aerobic control	-175	189; CC	-138	0.32
	anaerobic test	-396	220; CC	-200	0.731
	anaerobic control	-386	242; CC	-136	0.668

[1]Corrosion potential.
[2]Breakdown potential.
[3]Repassivation potential.
[4]Maximum current density reached upon the reverse scan.
[5]Transpassive dissolution; [6]CC: crevice corrosion.

3.2. Surface Inspection by optical microscopy

Specimens were examined by optical microscopy after exposure to seawater to identify crevice corrosion and to validate the electrochemical findings. Surface images of electrochemically tested UNS S31803 and UNS N08825 pre-exposed to natural seawater at 30ºC for 30 days under anaerobic conditions are shown in Figure 6. Regardless of the experimental conditions and the type of alloy, the crevice attack was found to be restricted to the border area of the crevice former and did not develop towards either the inner area of the shielded surface or the exposed surface outside the crevice. All UNS S31803 and UNS N08825 coupons exhibited crevice corrosion after electrochemical testing regardless of the presence or absence of microorganisms in the seawater.

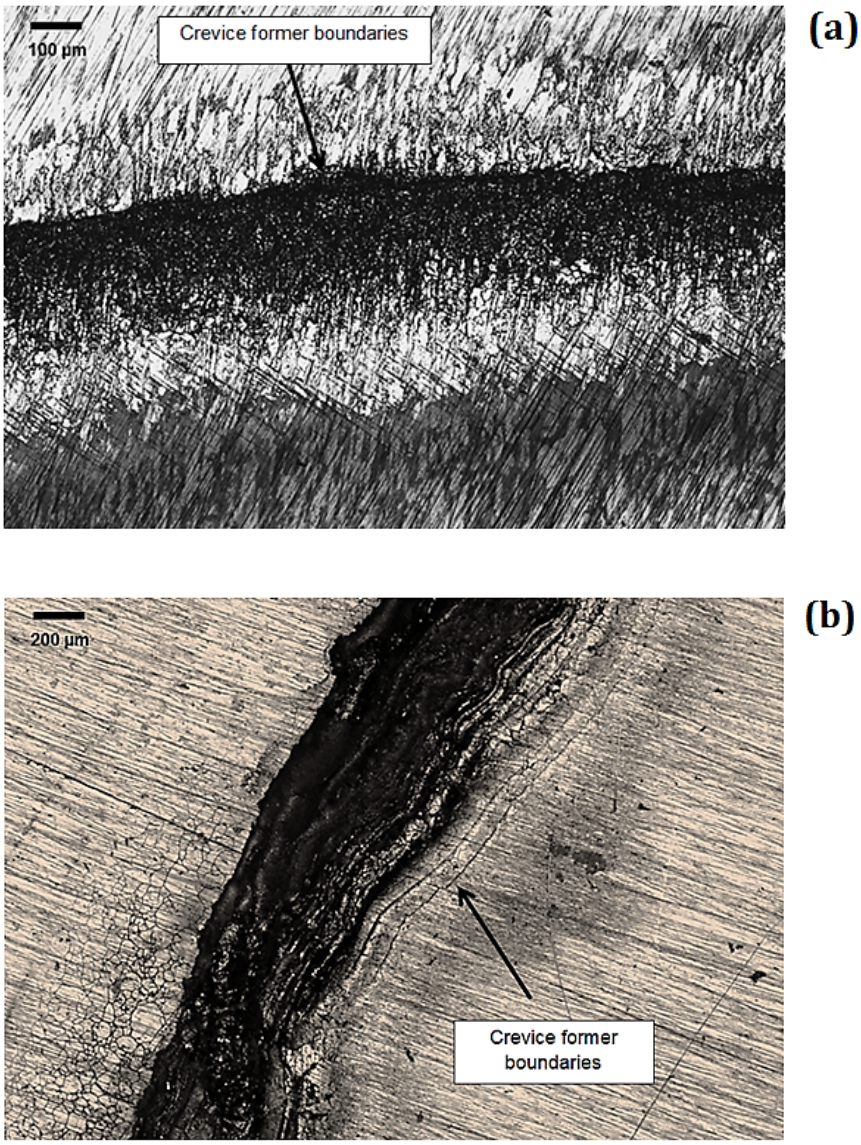

Figure 6. Optical surface images of alloys exposed to natural seawater at 30°C under anaerobic conditions in the presence of bacteria after potentiodynamic polarization test. (a) UNS S31803; (b) UNS N08825. Crevice corrosion was concentrated at the crevice former boundaries. The same pattern of attack was observed for alloys exposed to aerobic conditions (data not shown).

The extent of the crevice attack however depended on the experimental conditions and the type of alloy. Average maximum depths of crevice corrosion found on surfaces of electrochemically polarized specimens are shown in Table 6. Crevice

depths were found to be higher in UNS S31803 and UNS N08825 when exposed to seawater containing microorganisms than in the sterile seawater under either aerobic or anaerobic conditions. Optical measurements of crevice depths and surface inspection correlate well with the results from electrochemical testing. Overall, anaerobic conditions were found to be more detrimental than aerobic conditions for the two tested alloys exposed to natural seawater, and the presence of microorganisms was found to exacerbate the crevice corrosion of the two alloys, regardless of the oxygen pressure.

Table 6.

Average maximum depth of crevice corrosion found on surfaces of electrochemically polarized specimens

Material	**Experiment**	**Max crevice depth (μm)**
UNS S31803	aerobic test	25-32
	aerobic control	18-20
	anaerobic test	55-60
	anaerobic control	45-52
UNS N08825	aerobic test	88-95
	aerobic control	70-75
	anaerobic test	180-196
	anaerobic control	150-160

Figure 7 shows optical microscope images of coupons exposed to seawater containing microorganisms and maintained under open-circuit conditions (potentiodynamic polarization was not conducted at the end of the exposure). Numerous cell-like structures were observed on the surface of UNS S31803 and UNS N08825 (Figure 7 (a)) under both aerobic and anaerobic conditions. These

were not observed on control specimens exposed to sterile seawater. These structures, about 10-20 μm in length with elongated boat-shaped morphologies, were observed forming clusters mainly at the outer region of the crevice former and spreading towards the exposed metal. The size and morphology of these structures are suggestive of diatom cells [39]. Likewise, rod-shaped structures suggestive of bacteria cells were observed all over the surface outside of the crevice area on UNS S31803 and UNS N08825 under both aerobic and anaerobic conditions (Figure 7(b)). These bacteria-like structures were found alone or associated with patchy deposits on the surface. Diatom-like structures and bacteria-like cells were not found inside the crevice. Crevice corrosion was not detected on either UNS S31803 or UNS N08825 maintained under open-circuit conditions regardless of the presence of microorganisms in the seawater.

3.3. Bacterial attachment to surfaces

Microbial adhesion to alloy surfaces exposed to seawater at 30ºC under aerobic and anaerobic conditions for 30 days was confirmed by DAPI-staining technique using epifluorescence microscopy (Figure 8). Microbial attachment was only observed in test and not on sterile control coupons. DAPI-stained surfaces revealed the attachment of biological material including bacteria cells, diatom-like cells and other colonizing structures (Figure 8 (a)) that were most often aggregated into irregular clumps and distributed from the outer circumference of the crevice former towards the exposed surface on both UNS S31803 and UNS N08825. Biological structures were rarely detected inside the crevice area. Interestingly, long branching filaments and net-like structures were always found surrounding cells forming large mats of fused overlapping material on the surface of the two alloys (Figure 8 (b)). Some of these filamentous structures were highly suggestive of fungi. Although microbial attachment was evident in all test coupons, microbial colonization was found to be patchy and uneven and never covered the entire

surface of either UNS S31803 or UNS N08825 coupons under any of the exposure conditions.

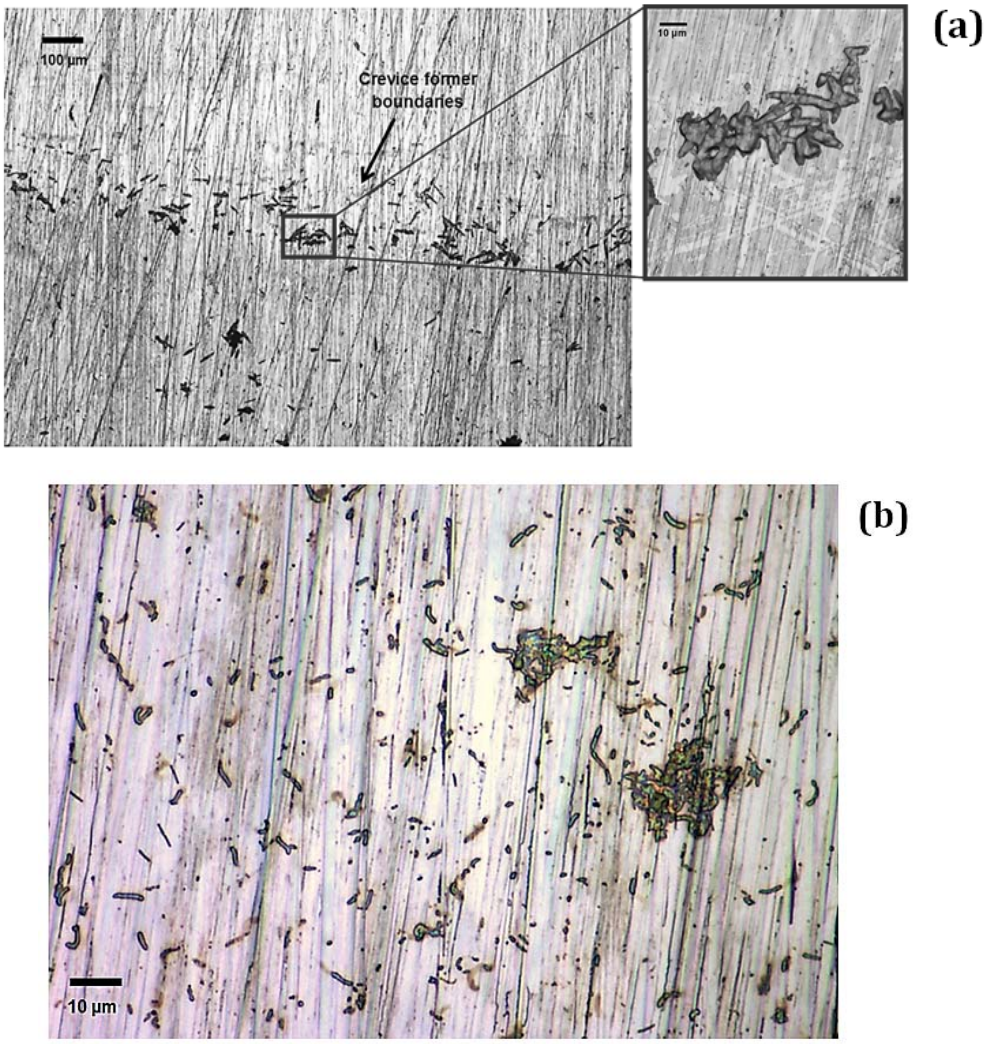

Figure 7. Optical surface images of UNS S31803 and UNS N08825 exposed to seawater under open-circuit conditions. These specimens were not electrochemically polarized. (a) UNS N08825 showing the presence of diatoms-like cells on the crevice former boundaries. An expanded cross section image of the same surface at the crevice boundaries is highlighted to show details of the attachment; (b) Structures very suggestive of bacteria found on the exposed surface (outside the crevice) of UNS S31803. These structures were found in both UNS S31803 and UNS N08825 exposed to seawater under aerobic and anaerobic conditions.

Some differences in the pattern of colonization were observed on surfaces of UNS S31803 and UNS N08825. Surface colonization and diversity was found to be higher on UNS N08825 than on UNS S31803. Long chain-forming bacteria were regularly and uniquely detected on UNS N08825 surfaces (Figure 8 (c)) and the predominant diatom-like cells differed according to the type of alloy (Figure 8 (d-e). No important differences were observed on the overall colonization of specimens of the same alloy exposed to aerobic versus anaerobic conditions.

3.4. Biofilm community structure by PCR-DGGE and DNA sequencing

Bacterial 16S rRNA gene fragments obtained by PCR amplification of DNA extracted from free-living bacteria in the seawater used as electrolyte for corrosion experiments and from biofilm samples developed on artificially creviced alloys were examined by DGGE. DGGE profiles are presented in Figure 9. The number of individual bands obtained in each lane of the DGGE is related to the number of the major bacterial populations present in the sample and indicates the diversity in the bacterial community. Each band was excised from the gel to conduct DNA sequencing and identify individual bacterial populations. The sequence information is given in Table 7. The DGGE fingerprinting reflected the shift in bacterial populations in biofilms in response to environmental conditions. It is clearly seen that biofilm composition was strongly affected by the presence of oxygen in the seawater and by substratum composition and/or microstructure exhibited by the two different alloys. Only one population was commonly encountered (*Marinovum algicola,* bands N14 and S18) in biofilms on both UNS S31803 and UNS N08825. All other bacterial populations identified in biofilms were highly specific in their preference of substratum/growth conditions.

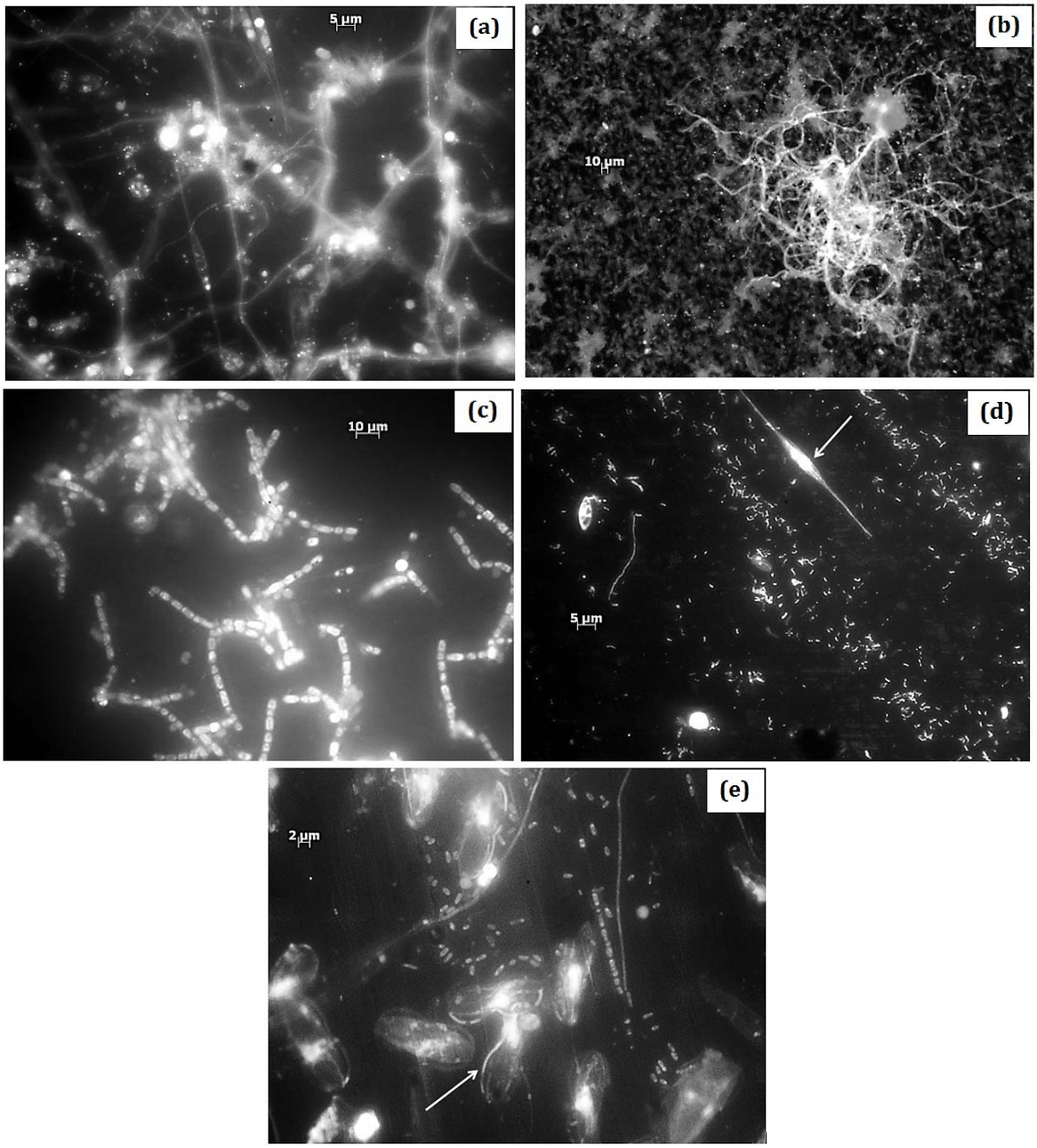

Figure 8. Microbial colonization on the surface of the creviced alloys exposed to seawater for 30 days under open-circuit conditions and then stained with 4,6-diamidino-2-phenylindole (DAPI) and examined by epifluorescence microscopy. (a) Colonizing structures on creviced UNS N08825; (b) long branching filaments and mats of fused overlapping material on UNS S31803; (c) long chain-forming microorganisms on UNS N08825. (d) and (e) show the predominant diatom-like cells on UNS N08825 and UNS S31803 respectively.

Bacterial diversity in biofilms on both UNS S31803 and UNS N08825 was shown to be higher when the biofilm developed under aerobic conditions compared with anaerobic conditions. Under anaerobic conditions, bacterial diversity was higher on UNS N08825 than on UNS S31803. Conversely, bacterial diversity was found to be higher on UNS S31803 than on UNS N08825 when exposed to seawater saturated with air.

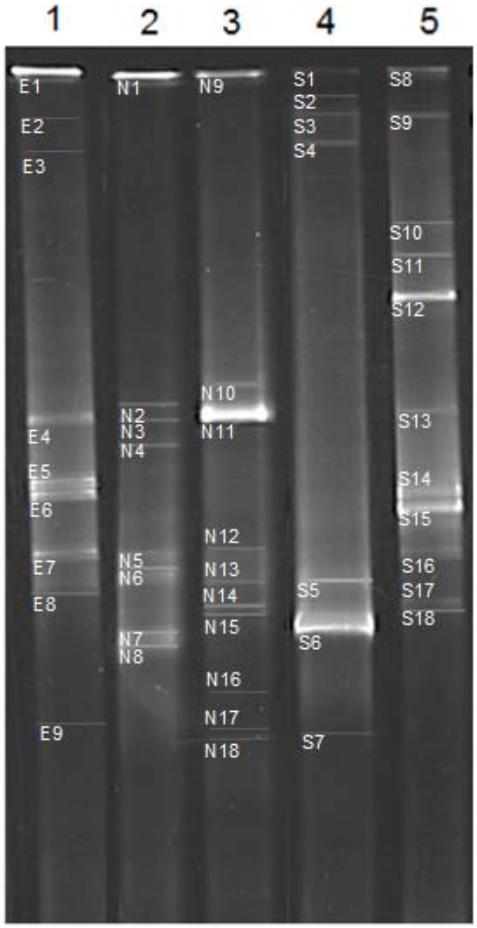

Figure 9. DGGE profiles of 16S rRNA bacterial gene fragments amplified from DNA samples from seawater and biofilms developed on artificially creviced alloys. Lane 1 (E1-E9): populations in seawater sample; Lane 2 (N1-N8): biofilm populations on UNS N08825 anaerobic test; Lane 3 (N9-N18): biofilm populations on UNS N08825 aerobic test; Lane 4 (S1-S7): biofilm populations on UNS S31803 anaerobic test; Lane 5 (S8-S18): biofilm populations on UNS S31803 aerobic test. Each numbered band was excised and DNA sequence information is given in Table 7.

Table 7.

Microorganisms characterized from excised DNA fragments with the highest similarity to the reference sequences in the GenBank database.

DGGE band	Closest relative	% Similarity / Accession number
E1	*Flavobacteriaceaea bacterium*	97%/FM162938
E2	*Methylophaga* sp.	98%/DQ486477
E3	*Methylophaga* sp.	97%/DQ486478
E4	*Alteromonas macleoidii*	98%/CP001103
E5	*Alteromonas genovensis*	98%/HM031989
E6	*Alteromonas* sp	97%/CP002339
E7	*Pseudoalteromonas sp.*	97%/JN681218
E8	*Pseudomonas putida*	97% JF 749813
E9	*Roseobacter* sp.	96%/EU195946
N1	*Flavobacteriaceaea bacterium*	94%/FM162938
N2	*Thalassobius aesturii*	96%/HM032803
N3	*Thalassobius aesturii*	96%/HM032804
N4	*Pseudoruegeria aquimaris*	90%/NR 043932
N5	*Thalassobius aesturii*	94%/HM032803
N6	*Thalassobius aesturii*	90%/HM032804
N7	*Thalasobacter arenae*	96%/NR044471
N8	*Pseudoruegeria aquimaris*	92%/NR 043932
N9	*Methylophaga* sp.	95%/DQ486477
N10	*Lewinella cohaerens*	98%/AM295254
N11	*Lewinella cohaerens*	98%/AM295255
N12	*Loktanella vestfoldensis*	93%/DQ915611
N13	*Loktanella vestfoldensis*	93%/DQ915612
N14	*Marinovum algicola*	95%/FJ752526
N15	*Croceitalea eckloniae*	98%/NR043628
N16	*Loktanella vestfoldensis*	93%/DQ915611
N17	*Roseobacter pelophilus*	96%/AJ968651
N18	*Roseobacter pelophilus*	93%/AJ968651
S1	*Marinobacter excellens*	90%/NR025690
S2	*Marinobacter excellens*	96%/NR025691
S3	*Marinobacter excellens*	90%/NR025691
S4	*Marinobacter excellens*	96%/NR025692
S5	*Marinobacter alcaliphilus*	96%/EU440994
S6	*Marinobacter alcaliphilus*	96%/EU440995
S7	*Marinobacter alcaliphilus*	96%/EU440996
S8	*Idiomarina* sp.	96%/EF554908
S9	*Pseudomonas pseudoalcaligenes*	97%/JF911373
S10	*Pseudomonas xinjiangensis*	96%/HQ696439
S11	*Pseudomonas xinjiangensis*	96%/HQ696440
S12	*Alteromonadales bacterium*	95%/EU180986
S13	*Pseudomonas pelagia*	98%/FJ687951
S14	*Pseudomonas putida*	97% JF 749813
S15	*Halomonas* sp.	98% AB304910
S16	*Halomonas* sp.	98% AB304911
S17	*Alteromonas genovensis*	98%/NR 042667
S18	*Marinovum algicola*	95%/FJ752526

4. Discussion

The effect of oxygen and biofilms on crevice corrosion initiation and repassivation of UNS S31803 DSS and UNS N08825 nickel-base alloy in seawater at 30ºC was studied using a combination of electrochemical measurements and surface analysis. Despite the microbial attachment to the alloys demonstrated by DAPI-staining technique, the biological components of the seawater did not induce ennoblement of the E_{corr} of the alloys at any of the experimental conditions tested. The corrosion potential, E_{corr}, of the two alloys exhibited active (negative) polarization with increased exposure regardless of the presence of oxygen and biofilm. This shift of the E_{corr} to negative values can be related to depolarization effects of chloride ions absorbed on the surface thereby inducing the open circuit (currentless) dissolution of the oxide films and active uniform dissolution of the surface [40]. Although it was shown that biofilm mass was not substantial and did not cover the entire surface, previous studies have shown that a biofilm consisting of cell aggregates colonizing only discrete areas of the surface is able to ennoble the potential of stainless steel exposed to flowing seawater [41]. Likewise, microbiologically influenced corrosion (MIC) studies where ennoblement has been reported were conducted in open systems where seawater was regularly replenished or in culture based experiments where bacteria were maintained in nutrient-rich media [26]. Therefore, our results highlight that while microbial adhesion and biofilm formation are sufficient to induce a change in the E_{corr}, the physiological state that the microorganisms can attain during exposure may be the most important factor. These results suggest that where bacterial growth and activity can be slowed or arrested by limiting nutrients, the biofilms are less likely to accelerate corrosion processes.

Accelerated corrosion tests indicated that passivity breakdown occurred through crevice corrosion, in the passive potential range, only in UNS N08825 under all the experimental conditions. Conversely, in UNS S31803, passivity breakdown

occurred through transpassive dissolution [42] or due to oxidation of H_2O to O_2 in both test and control. It was also observed that in UNS S31803 a transpassive potential was necessary to initiate crevice corrosion regardless of the experimental conditions. This phenomenon has been observed previously in creviced 316 stainless steel [43]. The initiation of crevice corrosion was reported to occur below a critical temperature only when the alloy reached a transpassive potential. It was suggested that below a critical temperature for crevice initiation, crevice growth is likely to take place if a gradual acidification of the crevice solution is attained due to the formation of soluble corrosion product species resulting from transpassive dissolution reactions. Similar results were observed for UNS S31803 and UNS N08825 in natural seawater in a recent study [31]. In that study, critical crevice temperatures (CCT) for UNS S31803 and UNS N08825 in seawater were found to be 33ºC and 47ºC, respectively as measured by a potentiostatic technique. Below the CCT, a repassivation transition temperature (T_{tr}) was identified where crevice corrosion took place only after the alloys reached a transpassive potential.

Results from the present study indicate that the resistance to crevice corrosion was higher in UNS S31803 than in UNS N08825 regardless of the presence of biofilm or oxygen. However, optical surface imaging of specimens maintained under open circuit conditions revealed that both UNS S31803 and UNS N08825 remained free from crevice corrosion regardless of the oxygen pressure and biofilm growth. These results are in agreement with previous investigations concerning the potential and temperature dependence of localized corrosion [44]. It is generally accepted that in aggressive environments where crevice corrosion is likely to take place, crevice corrosion will occur only if E_{corr} is equal to or greater than a critical potential [45] that is temperature dependent. The critical potential is usually considered to be the crevice corrosion repassivation potential (E_r). In environments unable to promote crevice corrosion, the transpassive dissolution potential seems to better represent this critical potential [46]. According to this criterion, E_r values obtained by cyclic polarization scans in this study indicate that

under open circuit conditions crevice corrosion can initiate in UNS N08825 exposed to seawater at 30ºC if a slight ennoblement of E_{corr} is achieved during exposure as indicated by the small E_{corr} - E_r gap. On the contrary, UNS S31803 will remain protected against crevice corrosion under these particular conditions until a transpassive potential is reached.

Electrochemical data also indicated that crevice corrosion was highly influenced by the presence of oxygen in the electrolyte solution. Crevice corrosion was more severe under anaerobic conditions as compared to experiments in constantly aerated seawater. Reinforcing this finding, surface analysis showed that alloys exposed to anaerobic conditions exhibited deeper and wider areas of crevice attack than alloys exposed to aerobic conditions. It is well known that in aerated electrolyte solutions oxygen is actively consumed as the main cathodic reactant. When the electrolyte is continuously oxygenated throughout the exposure, dissolved oxygen would be expected to be easily replenished in the shielded surface. Hence, the continuous supply of oxygen to the electrolyte would ensure the preservation of the passive film both inside and outside of the crevice and would prevent the formation of anodic and cathodic regions in the surface. Conversely, in the absence of oxygen, the oxygen reduction reactions are not the main cathodic reactions in the corrosion cell and hydrogen evolution primarily takes place instead, although other cathodic reactions may also have an effect. Under anaerobic conditions, oxidizing species are rapidly consumed and if not replenished mass transport becomes restricted within the crevice. In addition, very narrow crevice gaps may constrict transport of ionic species inside and outside of the crevice [47]. This will form a differentiation cell on the surface with the occluded area becoming anodic with respect to the exposed surface outside the crevice, which may in turn result in active dissolution within the crevice. Similarly, the repassivation of metastable events may become ineffective in the occluded area as compared with the exposed surface by the same mechanism of mass transport limitation, hence favouring stabilization of nucleation events within the crevice that

would otherwise repassivate if in contact with oxidizing species. These results are in agreement with results from a previous study on the role of oxidants in the crevice corrosion process [48]. This work showed that the replacement of the de-aerated crevice electrolyte, during the initiation of crevice corrosion, with O_2-saturated electrolyte suddenly terminated the crevice corrosion process in a chloride environment. Re-activation of crevice corrosion was observed when the crevice electrolyte was again replaced by de-aerated electrolyte. It was shown that high oxygen content in the electrolyte was sufficient to support cathodic reactions inside the crevice to reduce the IR voltage necessary for the onset of crevice corrosion. These results supported the IR theory described by Pickering [8] that stipulates that an IR-drop or the electrode potential variation between the cavity and the outer surface is the key parameter for metal dissolution in the crevice.

In this investigation, crevice corrosion was only evident when accelerated corrosion methods were conducted, i.e. when external polarization was applied. However, crevice corrosion was greater in the presence of microorganisms. It is well known that physicochemical variables and biological species will have a major effect on the form of the potential versus current density relationship [38]. In the present study, the different electrochemical behaviour displayed by test and sterile controls, as well as imaging of corroded specimens, demonstrated that crevice corrosion was exacerbated by the presence of biofilms for both UNS S31803 and UNS N08825. Similar results have been previously reported for UNS S31803 in seawater [29] although in htat study crevice corrosion did not occur with external polarization unless a biofilm was present. In the present study, the effect of biofilm on creviced UNS S31803 and UNS N08825 in seawater included a decrease in the E_b and $E_{r,}$ and an increase in the maximum current density reached during the reverse scan (i_{max}), which is a measure of the crevice corrosion propagation and the inability of the material to achieve a passive state.

In addition, the effect of the biofilm on crevice corrosion of UNS S31803 and UNS N08825 was also influenced by oxygen. Overall, the biofilm developed under anaerobic condition was more aggressive to the two creviced alloys than the biofilm developed under aerobic conditions, when compared by accelerated corrosion tests. Moreover, it was observed that the onset of passivity in the two alloys was inhibited by the presence of anaerobic biofilms.

These results indicate that the most detrimental effects on active-passive alloys emerge from the interactions of microorganisms with creviced surfaces when oxygen is limited such that passivation events may not take place in the shielded surface. For instance, if the repassivation process is limited after passivity has been broken, microorganisms can reach the underlying metal and help stabilize localized corrosion events. For UNS S31803 biofilm facilitated the onset of the transpassive dissolution reaction whilst in UNS N08825 biofilms decreased the critical potential for crevice corrosion initiation. It is reasonable to assume that the degree to which biofilm affect the electrochemical reactions occurring on the corroding surface would depend on the aggressiveness of the environment towards the corrosion resistance of alloys. Where minimally aggressive conditions are present, e. g. low temperatures, it seems unlikely that biofilm will promote the initiation of crevice corrosion of these alloys under open circuit conditions. More particularly, the case of high-resistance alloys where high temperatures and corrosion potentials may be required to initiate localized corrosion and where these extreme environments can be detrimental to most microorganisms.

DAPI-stained surfaces revealed the spatial distribution of microorganisms and biofilms on the surface of creviced UNS S31803 and UNS N08825. Biological structures were always detected at the border area of the crevice former and distributed towards the exposed surface outside the crevice. Biological structures were negligible in the inner area of the occluded surface. Since MIC proceeds by electrochemical mechanisms, the role of the microorganisms is either to assist in

the establishment of the electrolytic cell (indirect) or to stimulate the anodic or cathodic reactions (direct). Based on the fundamental mechanisms of crevice corrosion, MIC mechanisms could involve one or more of the following: a) microbial restriction of repassivation process at the crevice wall by consumption of oxidizing species in the electrolyte needed for passive film preservation; b) the stabilization of metastable pits by maintaining a concentrated aggressive pit electrolyte solution at the crevice wall hence lowering the E_b of the alloys; c) the metabolic activities of biofilm microorganisms limiting mass transport towards the crevice, thus facilitating the formation of differentiation cells. The preferential attachment of microorganisms to the areas outside the crevice may also suggest that microorganisms supported the cathodic reactions on the creviced surface via de consumption of oxidizing species.

The relationship between alloy grade, crevice corrosion and the presence or absence of oxygen with biofilm community composition was also explored using PCR-DGGE and DNA sequencing. Results revealed that biofilm bacterial populations were highly specific in their preference for substratum and oxygen conditions. It was interesting to see that even when exposed to the same electrolyte and experimental conditions, i.e. oxygen pressure and temperature, the composition of biofilm communities differed with the substratum surface. Under anaerobic conditions, *Marinobacter* species were the dominant populations in biofilms on UNS S31803 whereas *Thalasobbius* and *Pseudoruegeria* were the major populations in biofilms on UNS N08825. A previous study also reported *Marinobacter* species as one of the major bacterial populations in biofilms developed on duplex stainless steels [2]. Interestingly, under aerobic conditions *Pseudomonas* were identified in biofilms on UNS S31803 DSS but were not detected in biofilms on UNS N08825. The shift in biofilm communities as a function of substratum surface has been reported previously [2].

It is well known that the formation of biofilms is the result of a complex series of events, involving interactions between physical, chemical and biological processes and that different biofilm microbes respond to changes in their environment with dissimilar growth patterns. It can be expected that microbial attachment was influenced by the nature of the passivating film formed on the alloy surfaces and by the microstructure of the alloys. UNS S31803 and UNS N08825 exhibit different chemical composition and microstructures. Duplex stainless steel is an iron-based Cr-Ni-Mo alloy with a two phase microstructure (ferrite and austenitic) in approximately similar percentages and UNS N08825 is an austenitic nickel-based Fe-Cr-Mo alloy. Chemical composition and microstructure has shown to have a strong influence on the composition and thickness of oxide films [49]. Previously, it was reported that the austenite phase of the duplex microstructure was more susceptible to attack by bacteria than the ferrite phase [50]. However, the structure of passivating films is continuously altered with exposure time by the chemical and biological species in the environment and microbial-surface interactions become a more complicated process. The biofilm community structure developed on stainless steel and polycarbonate has been shown to change with exposure time [51]. It was shown that bacterial community structure was very variable during the first days of exposure but became stable and indistinguishable in older biofilms with no differentiation based on surface type. The authors suggested that the accumulation of biofilm biomass may have masked exposed surfaces so that surface effects on biofilm structure should decrease, not increase, over time. The authors, however, did not report any measure of the biomass colonizing the surface with time.

Results from this study also showed that microbial diversity was higher in biofilms developed under aerobic conditions than under anaerobic conditions. It is well known that microorganisms vary in their need for, or tolerance of, oxygen. Aerobes are species capable of growth at full oxygen tensions and utilize oxygen in their

metabolism while strict anaerobes may be inhibited or even killed by oxygen. In the middle of these two extremes a wide variety of species can grow under both oxic and anoxic conditions. However, microbial physiological activities within biofilms are far from uniform and individual metabolic activities are typically juxtaposed. In biofilms, microscale chemical gradients are formed and regulate the metabolic pathways in the biofilm populations. It is known that the diffusion of chemical species declines with increasing biofilm depth because they are actively consumed by biological cells in the upper layers of the biofilm. This explains how strict anaerobes can flourish in biofilms developed under aerobic conditions. Hence, it can be expected that bacterial metabolic diversity in biofilms will be more favoured in the presence of oxygen than under anaerobic conditions. However, higher diversity in biofilms does not seem to indicate more aggressiveness of the biofilm towards localized corrosion [34]. This assumption is also supported by the results from the current study.

The phenomena of microbial adhesion and biofilm formation on corroding surfaces are far from being simple and uniform. This is a very complicated system affected by many factors and the evaluation of the relative contributions of these factors is extremely difficult. Although there is a tremendous insight to be gained from the application of molecular tools to the investigation of MIC, more investigations are still needed to advance our understanding of the mechanisms of bacterial function to attain appropriate methods to prevent MIC.

5. Conclusions

From accelerated corrosion tests, immersion tests and surface analysis on UNS S31803 and UNS N08825 in natural seawater at 30ºC the following conclusions can be drawn:

1. Passivity breakdown occurs through crevice corrosion in UNS N08825 in the presence or absence of oxygen and biofilms. In contrast, a transpassive potential is necessary to initiate crevice corrosion in UNS S31803 regardless of the presence of oxygen or biofilms. This highlights the superior resistance of UNS S31803 compared to UNS N08825. Crevice corrosion is restricted to the border area of the creviced surface.
2. UNS S31803 and UNS N08825 maintained under open-circuit conditions do not initiate crevice corrosion regardless of the presence of oxygen or biofilm. However, crevice corrosion is likely to initiate in UNS N08825 if a slight ennoblement of its E_{corr} is achieved during exposure as indicated by the small E_{corr} - E_r gap. In contrast, UNS S31803 will remain protected against crevice corrosion until a transpassive potential is reached during exposure.
3. Crevice corrosion is highly influenced by the presence of oxygen in the electrolyte. Crevice corrosion is more severe under totally anaerobic conditions compared to exposure in constantly aerated seawater.
4. Crevice corrosion is exacerbated by the presence of biofilm only when an external anodic polarization is applied. Biofilm growth does not induce ennoblement of E_{corr} of the alloys under closed-experimental conditions and does not promote initiation of crevice corrosion under open-circuit conditions.
5. The effect of biofilm on crevice corrosion is influenced by the presence of oxygen. A biofilm developed under anaerobic conditions is more aggressive to the alloys than a biofilm developed under complete aerobic conditions.

Analysis of microbial adhesion by DAPI staining and biofilm community structure by PCR-DGGE and DNA sequencing demonstrated that:
1. Microbial attachment and biofilm formation is distributed from the border area of the crevice former towards the exposed surface outside the crevice. Biological structures are negligible at the inner area of the occluded surface.

2. Biofilm bacterial populations are highly specific in their preference for substratum surface and oxygen conditions. Biofilm community structure is shifted in response to alloy composition and/or microstructure and the oxygen pressure conditions of the surrounding environment.
3. Microbial diversity is higher in biofilms developed under aerobic conditions than under anaerobic conditions. However, high diversity was not associated with more aggressive crevice corrosion.

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Chapter 8

L.L. Machuca, S.I. Bailey, R. Gubner. Crevice corrosion studies on corrosion resistant alloys in stagnant natural seawater. Advanced Materials Research (2013).

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Crevice Corrosion Studies on Corrosion Resistant Alloys in Stagnant Natural Seawater

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Abstract

Crevice corrosion (CC) was investigated in a number of selected corrosion resistant alloys in natural seawater containing microorganisms for up to 18 months under stagnant conditions. Experimental controls consisted of tests in natural seawater filtered in accordance with hydrostatic testing procedures. The corrosion potential of alloys was monitored throughout exposure and corrosion was evaluated by weight loss and 3D optical microscopy. CC was initiated on several alloys and corrosion rates in time indicated a positive effect of seawater filtration on the long-term performance of the alloys. Microbial adhesion as investigated by fluorescence microscopy occurred mainly outside the crevice and differed according to the nature of the substratum surface.

Keywords: Crevice Corrosion, Microbial Corrosion, Corrosion Resistant Alloys, Seawater.

1. Introduction

Corrosion resistant alloys (CRAs) have been considered as important alternatives to conventional low grade steels in seawater environments. The outstanding corrosion resistance of these materials is due to the formation of a stable passive layer on the surface which protects them against uniform corrosion. Nonetheless, these alloys are still susceptible to localized corrosion such as pitting and crevice corrosion[1]. Pipeline commissioning for subsea installations typically involves hydrostatic testing procedures using seawater. In the oil and gas industry it is often the case that hydrotest seawater is left inside the pipeline subsequently for many months before the equipment is commissioned. During this holding period, this stagnant water may permit debris, sand and marine life to settle hence increasing the risk of under-deposit and microbial corrosion[2]. Crevice corrosion (CC) is one of the main concerns with corrosion resistant alloys expose to stagnant hydrotest seawater as it takes place under less aggressive conditions when compared with other forms of corrosion.

In this study we investigated the long term crevice corrosion resistance of several CRAs in both filtered and raw natural seawater containing microorganisms using immersion tests. A set of crevice-free coupons were also exposed to the seawater to evaluate pitting corrosion. The corrosion potential was measured constantly and corrosion was monitored after 6, 12 and 18 months exposure by weight loss and surface analysis. Microbial adhesion was evaluated by fluorescence microscopy to study the effect of seawater aging and substrate material composition on the pattern of microbial colonization of creviced surfaces.

2. Experimental Procedure

Tests specimens (square coupons 50x50x5mm) were drilled in the centre (7 mm) and wet ground to 600 grit finish with SiC grinding paper, degreased with acetone

and dried with nitrogen. Test alloys and their composition (in wt %) are described in Table 1. crevice assemblies were prepared based on the design of the CrevCorr Round Robin test[3] using PVDF crevice formers and an applied torque of 3 N.m. The schematic representation of the crevice assembly is shown in Figure 1.a. Specimens and all pieces for the crevice assembly were soaked in decontaminant solution for 3 hours and sterilized by immersion in 70% ethanol for 1 hour before exposure. Triplicate crevice assemblies were placed in 10 L exposure vessels that were filled with natural seawater (NSW) collected from 20 metres depth in the Indian Ocean off Rottnest Island (Western Australia) (Figure 1.b). Experimental controls consisted of crevice assemblies exposed to 60 µm filtered seawater (FSW) in accordance with hydrostatic testing procedures. This filtration does not remove microorganisms but it reduces solid particles in the seawater so that the risk of microbial corrosion and under-deposit corrosion may be diminished. A set of $1cm^2$ alloy coupons were embedded in epoxi resin, wet ground to 600 grit finish and immersed in the testing solution by hanging using a coated copper wire via a spot weld which also functioned as electrical connection between sample and potentiostat to monitor the corrosion potential. The corrosion potential of alloys was measured against a double junction Ag/AgCl reference electrode weekly. Exposure vessels were sealed with liquid silicone rubber and mantained at 20°C using a refrigerated incubator for up to 18 months. At the completion of 6, 12 and 18 months exposure, coupons were cleaned by following the standard procedure[4] and corrosion rates were calculated from weight loss. Surface analysis to evaluate localized corrosion was conducted using a 3D optical infinite focus microscope (IFM G4g system, Alicona Imaging). To examine microbial adhesion, alloy surfaces were stained with 2µg/mL 4, 6-diamidino-z-phenylidole (DAPI) fluorescent dye and examined with an epifluorescence microscope (Axio Imager.A1; Carl Zeiss, Germany).

Table 1.

Test materials and chemical composition

Alloy/UNS number	Type	C wt %	Mn wt %	Fe wt %	Cr wt %	Ni wt %	Mo wt %	N wt %	S wt %
316L/UNS S31603	Austenitic	0.022	1.76	bal	17.4	10	2.03	0.05	0.001
2205/UNS S31803	Duplex	0.015	1.53	bal	22.35	5.72	3.16	0.18	0.001
2507/UNS S32750	Super duplex	0.019	0.82	bal	24.74	6.61	3.73	0.26	0.0003
254SMO/UNS S31254	super austenitic	0.01	-	bal	20.18	18.15	6.10	0.20	0.010
625/UNS N06625	Nickel base alloy	0.1	0.45	5.00	22.5	bal	9	3.85	0.015
825/UNS N08825	Nickel base alloy	0.05	0.85	22	22.50	bal	3	-	0.03

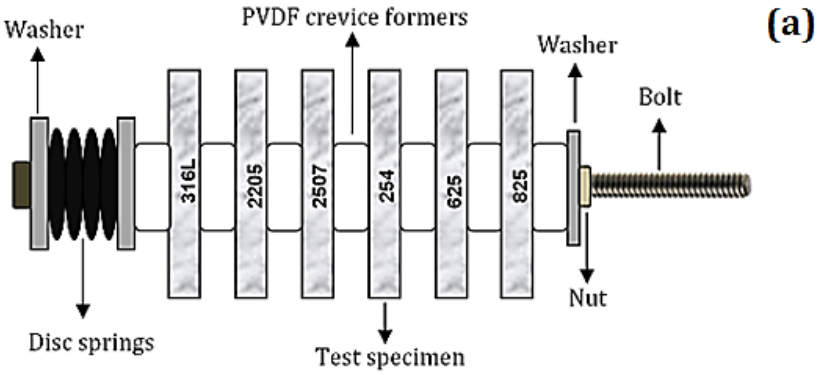

Figure 1. (a) Schematic representation of the crevice assembly used in this study; (b) Experimental vessels used to evaluate long term crevice corrosion.

3. Results and Discussion

Figure 2 shows the corrosion potential vs. time of alloys in NSW and FSW at 20°C measured throughout 18 months exposure. The corrosion potential of alloys in all cases dropped during the first months of exposure and remained fairly stable in the active region with some active–passive stages until the 9-10 months exposure. This drop in the corrosion potential toward negative values can be related to passivity deterioration due to aging in a chloride containing solution. The corrosion potential of alloys was then slightly shifted into positive direction in both NSW and FSW and remained fluctuating until the completion of exposure. The corrosion potential of all alloys remained negative during the 18 months exposure and never reached positive values in either NSW or FSW.

Average corrosion rates calculated from weight loss of triplicate specimens are shown in Figure 3. Corrosion rates were very low in all alloys exposed to both NSW and FSW and showed to decrease with exposure time. The highest corrosion rates were observed in 316L and the two nickel base alloys. Corrosion rates were higher in alloys exposed to NSW than FSW which highlights the positive effect of filtration treatment in the performance of CRAs in seawater. For the duplex stainless steels and the 254SMO alloy corrosion rates were negligible regardless of exposure time and the type of seawater.

3D optical surface images and DAPI stained surfaces of alloys exposed to NSW and FSW at 20ºC for 18 months are shown in Figure 4. Pitting was detected on 316L, 2205 and 825 alloys after 6, 12 and 18 months exposure in both NSW and FSW. Interestingly, grain structures were observed at the crevice former boundaries on 316L exposed to NSW (Figure 4.c). Pit depths were similar in both 316L and 825 alloys and range from 5-20 µm in both NSW and FSW. Maximum pit depth observed in 2205 was 9 µm. Crevice corrosion was not detected on any of the alloys during the first 12 months of exposure but it was detected on 316L and 825 alloys at the 18 months exposures in both FSW and NSW. Crevice depths oscillated between 20-30 µm in 316L and 825 alloys exposed to both FSW and NSW.

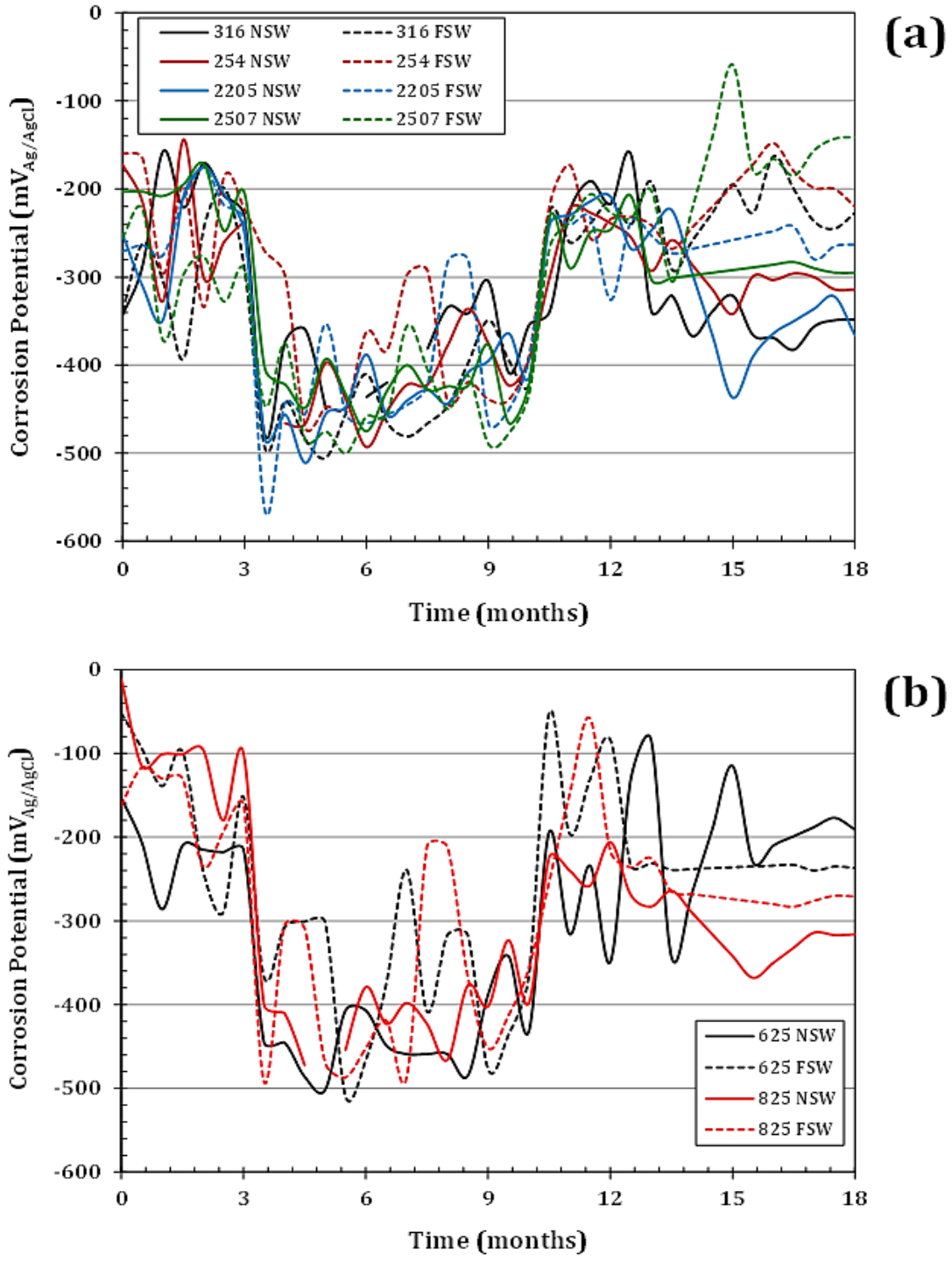

Figure 2. Average corrosion potential vs. time of (a) stainless steels 316L, 254SMO, 2205, 2507 and (b) nickel-based alloys 625 and 825 in natural seawater (NSW) and filtered seawater (FSW) at 20˚C.

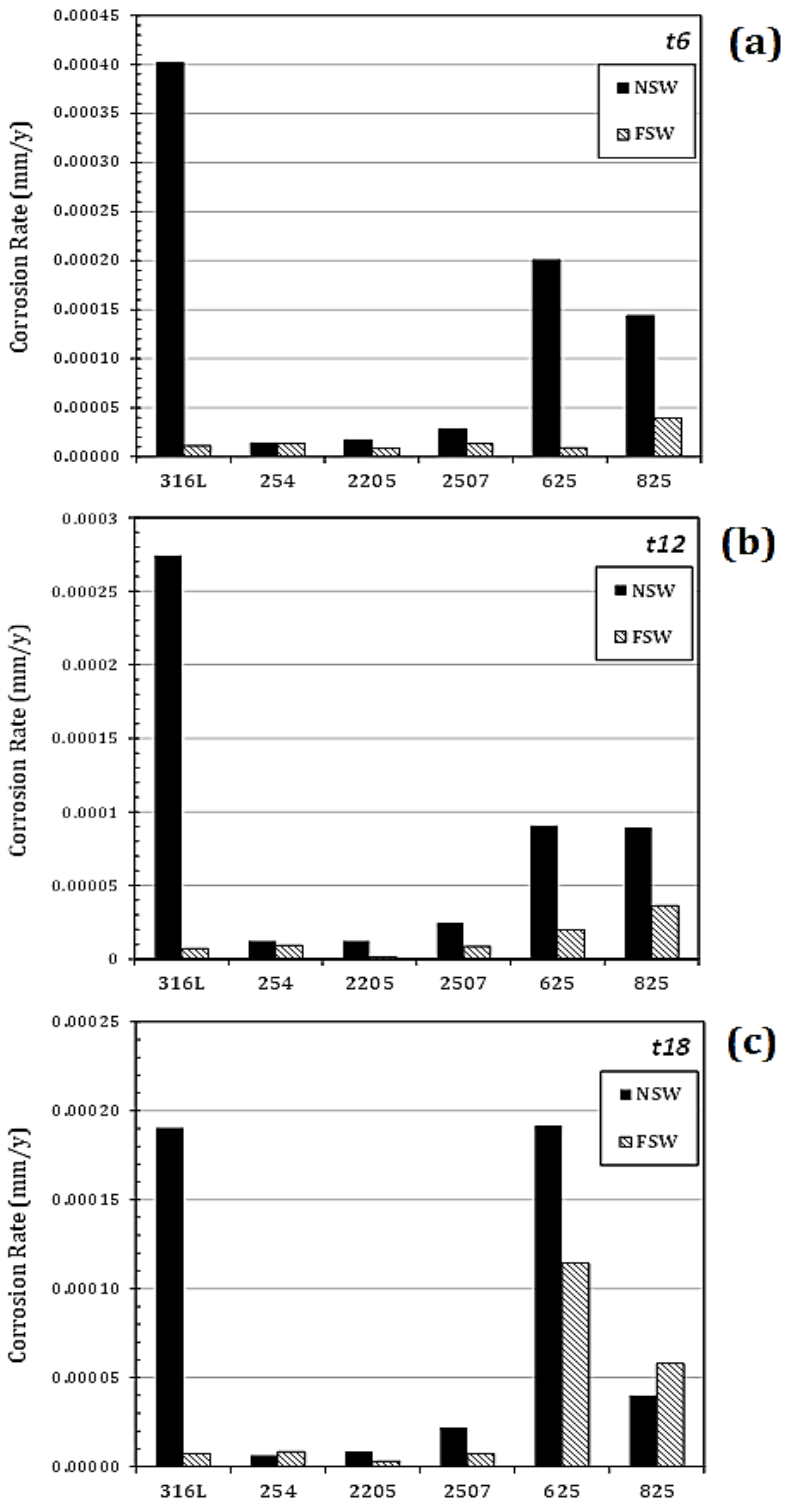

Figure 3. Average corrosion rates from weight loss of CRAs after (a) 6 months, (b) 12 months and (c) 18 months exposure to natural seawater (NSW) and filtered seawater (FSW) at 20˚C under stagnant conditions.

Pitting and crevice depths were conservative regardless of the exposure time. 2507, 254 SMO and 625 alloys exhibited excellent resistance against pitting and crevice corrosion regardless of the exposure time.

Microbial adhesion was confirmed on all alloys exposed to both FSW and NSW. Microbial adhesion was distributed from the outer edges of the crevice former towards the exposed surface of all alloys. Microorganisms were rarely detected inside the crevice area. There were not differences in the pattern of colonization as a function of exposure time. However, microbes colonized the alloys in distinctive patterns according to substratum surface. Microbial colonization on stainless steels (316L, 2205, 2507 and 254SMO) consisted of individual bacterial cells distributed all over the surfaces. In the case of the nickel alloys, microbial colonization was more diverse and consisted of aggregates of diatoms, fungi, bacterial cells and overlapping biological material. Bacterial cells were bigger in size and more abundant on the nickel alloys as compared with the stainless steels. Differences in the pattern of microbial colonization can be associated with the nature of the oxides developed on the ferrous materials compared with the oxides on the nickel alloys which could induce selective adhesion and growth of microorganisms.

The reason why microbial colonization was more copious and diverse on nickel alloys than on stainless steels is uncertain. Interestingly, there seemed to be no effect of seawater filtration on the extent and type of microbial colonization of alloys as indicated by epifluorescence microscopy.

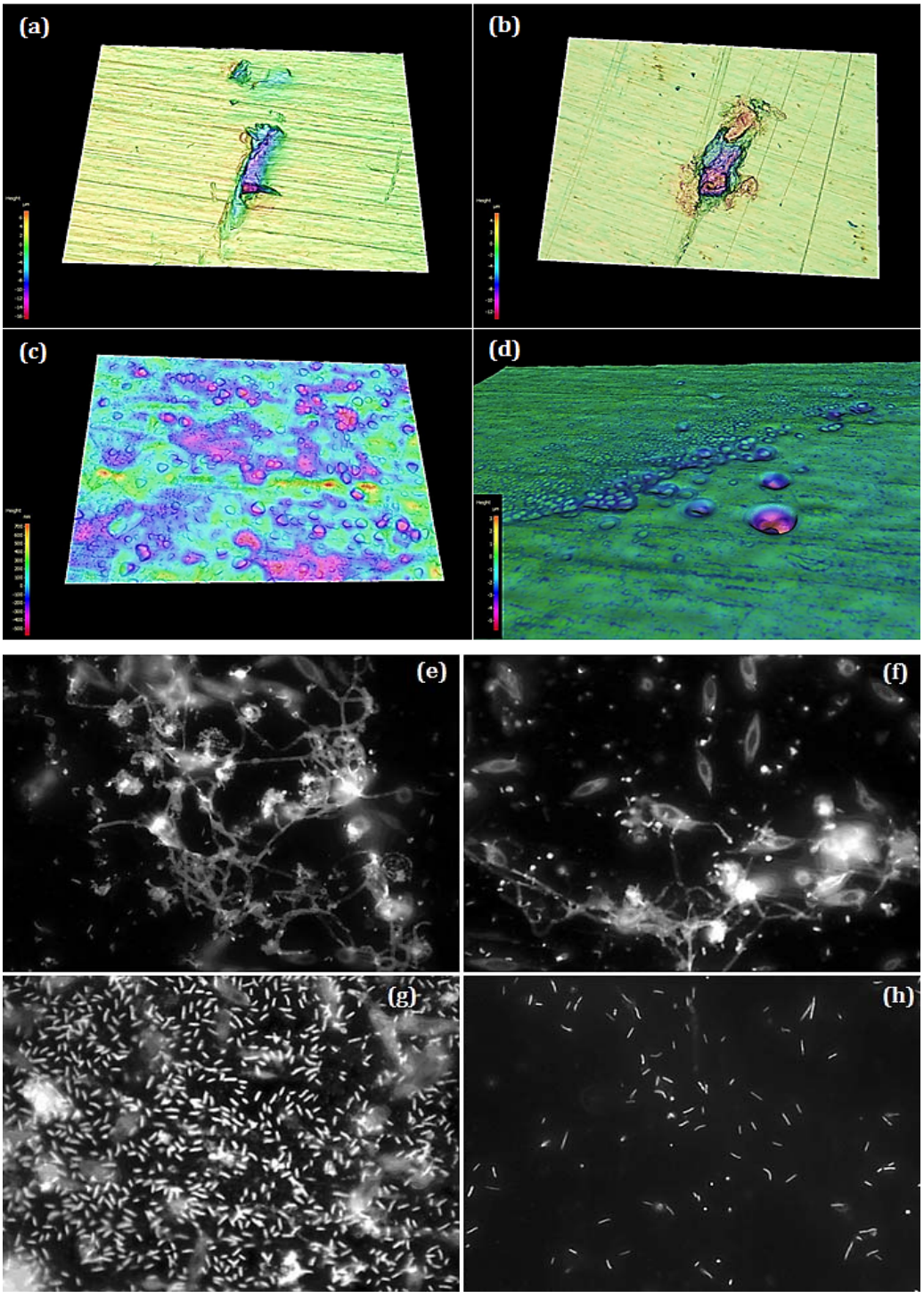

Figure 4. (a-b) crevice attack on (a) 825 alloy and (b) 316L exposed to seawater for 18 months. (c) 316L showing grain structures and localized attack and (c) pitting was concentrated at the outside edges of the crevice former (d). (e-g) DAPI-stained 825 alloy showing extensive microbial adhesion on the surface. (h) DAPI stained 2205 alloy showing some bacteria attached to the surface.

4. Conclusions

- 316L and 825 alloys are prone to pitting and crevice corrosion in both raw and filtered seawater at 20ºC under stagnant conditions. The extent of the localized attack was the same for both alloys in raw and filtered seawater. Pitting and crevice depths were conservative regardless of the exposure time.

- 2205, 2507, 254SMO and 625 alloys possess excellent resistance to pitting and crevice corrosion in both raw and filtered seawater at 20ºC under stagnant conditions.

- Microorganisms colonized the alloys in distinctive patterns according to substratum surface. Microbial colonization was more copious and diverse on nickel alloys (625 and 825) than on stainless steels (316L, 2205, 2507 and 254SMO) and the pattern of colonization was not affected by exposure time and seawater filtration treatment.

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Chapter 9

L.L. Machuca, R. Jeffrey, S.I. Bailey, R. Gubner, E. Watkin, M. P. Ginige, A. Kaksonen. K. Heidersbach. Corrosion performance of offshore construction materials in the presence of active biofilms in seawater. Corrosion Science (2012).

Manuscript submitted

Corrosion performance of offshore construction materials in the presence of active biofilms in seawater

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Abstract

The corrosion potential of several offshore structural steels and nickel alloys was measured in natural coastal seawater in a continuous flow system over 90 days. Biofilms formed and ennobled the electrodes potential by +400-500 mV in both raw and filtered-UV irradiated seawater. Biofilms induced localized corrosion on certain materials by shifting E_{corr} into a critical potential for localized corrosion. Ennobling biofilms were composed of microbial cells, diatoms and extracellular polymeric substances and differed in their structure and bacterial diversity between materials. Results are explained on the basis of the potential-temperature dependence of alloys on the onset of localized corrosion.

Keywords: steel, alloy, SEM, microbiological corrosion, pitting corrosion

1. Introduction

The selection of suitable materials for the construction of offshore oil and gas facilities requires thorough attention to avoid loss of production, costly maintenance and environmental repercussions. Carbon steel has been the dominant offshore construction material since it has tremendous advantages of a large experience base and strong technical background along with moderate cost. However, high-resistance alloys such as stainless steels and nickel-based alloys are important structural materials due to their combination of high strength and resistance to corrosion in aggressive offshore environments, decreasing the total life time system-cost of oil and gas production facilities. High-resistance alloys suffer negligible general corrosion in seawater due to a protective, predominantly chromium oxide, film that forms immediately on the surface with exposure to air [1, 2]. However, these alloys do still suffer localised corrosion, i.e. pitting corrosion, crevice corrosion, stress corrosion cracking (SCC) and microbiologically influenced corrosion (MIC) [3-7]. The susceptibility of these alloys to localized corrosion in seawater has been shown to be strongly dependent upon potential, temperature and chloride concentration [3, 5, 8-10]. A greater understanding of the offshore environment and more detailed knowledge of the conditions under which offshore structures and systems have to operate will assist the selection of the most appropriate materials.

There remain significant knowledge gaps in the preservation of offshore equipment, including the control of microbiologically influenced corrosion. Although many studies have investigated the interactions of biofilms with low grade steels in seawater [11-16], there is only limited information regarding MIC of highly alloyed stainless steels and nickel-based alloys in seawater [6, 17-19]. Previous studies on MIC have postulated several mechanisms through which microorganisms can aggravate localized corrosion on active-passive alloys. It has

long been known that natural biofilms are able to shift the E_{corr} of active-passive alloys in the noble direction, a phenomenon collectively known as ennoblement. Ennoblement has been widely studied and numerous reports from geographically diverse sites have been published over the years [20-24]. Since ennoblement has not been observed in steels exposed to sterile seawater, the phenomenon has been attributed to microorganisms. Although a unified mechanism of potential ennoblement has not been established, several mechanisms have been proposed. These mechanisms include cathodic depolarization [25-27], production of aggressive metabolites [28], catalytic enzymes, metal-organic compounds [23, 29] and manganese deposition [30], among others. The significance of this phenomenon lies in its influence on the susceptibility to corrosion of anode materials in galvanic couples and the initiation and propagation of localized corrosion [14, 31]. Other MIC mechanisms include biodeposit formation leading to a crevice type of attack [7], acceleration of propagation rates for crevice corrosion, decrease of the critical potentials for pitting and crevice corrosion initiation [6, 32] and direct microbial-steel electron transfer [33, 34].

The influence of marine biofilms on localized corrosion of high-resistance alloys in seawater has been investigated previously under closed experimental conditions without seawater replenishment [6, 19, 35]. These alloys proved to be resistant to localized corrosion under open-circuit conditions and biofilms did not induce ennoblement of the corrosion potential. In these studies, a high driving potential was necessary to trigger localized corrosion on the test alloys. From those results, it was suggested that the absence of metabolically active biofilms, which may be necessary to induce ennoblement, was due to depletion of nutrients in the seawater with exposure time. It is known that under conditions of nutrient depletion, biofilm growth is limited. Additionally, the biofilm matrix is altered due to changes in the production of extracellular biopolymers, microbial metabolic activity becomes restricted, the physical biofilm structure community is weakened and desorption of

microbial cells from the substratum is enhanced [36]. This can ultimately lead to the onset of microbial dormancy.

The aim of the current study was to develop metabolically active biofilms with physiological states more likely to accelerate electrochemical reactions at the microbial-substratum interface on several off-shore construction materials, and determine their effects on corrosion performance. This was achieved by exposing the materials to continuously flowing natural seawater, ensuring a constant loading rate of nutrients and promoting the formation of mature and active biofilms.

Results from this study are intended to complement previous reports on the performance of high-resistance alloys in seawater under closed experimental conditions, providing a more comprehensive and accurate analysis of the risk of MIC in seawater for these alloys. The selected test materials were exposed to coastal seawater for 90 days in a test rig within the NSW Department of Primary Industry Fisheries Research Centre at Taylors Beach, New South Wales, Australia [37]. The effect of biofilms on the performance of the test materials was examined by monitoring corrosion potential over time and conducting surface analyses. Microbial diversity and community structure in biofilms on the different materials was investigated by polymerase chain reaction of bacterial 16S rRNA gene fragments flowed by denaturing gradient gel electrophoresis (PCR-DGGE). Molecular characterization of biofilm communities has become crucial to understand the complexity of the interactions of biofilms with substratum surfaces and the surrounding environment [11, 38-41]. The sensitivity of this technique allowed us to assess the degree to which exposure conditions and material composition affect the bacterial community and shift the microbial diversity in biofilms.

2. Experimental procedures

2.1. Specimen preparation

Commercial carbon steel, stainless steels UNS S31603, UNS S31803, UNS S32750, UNS S31254 and the nickel-base alloys UNS N08825 and UNS N06625 were used in this study. The chemical composition of alloys in weight per cent is presented in Table 1. Square coupons (20 mm x 20 mm x 5mm thick) were cut from the supplied plate samples and drilled in one corner with a 2 mm diameter hole. An electrical connection was established via a copper wire soldered to one side of the coupon.

Table 1.

Test materials and chemical composition

Material	Type	C wt %	Mn wt %	Fe wt %	Cr wt %	Ni wt %	Mo wt %	N wt %	Nb wt %	S wt %
UNS S31603	Austenitic SS	0.022	1.76	bal	17.4	10	2.03	0.046	-	0.001
UNS S31803	Duplex SS	0.015	1.53	bal	22.35	5.72	3.16	0.18	-	0.001
UNS S32750	Super Duplex SS	0.019	0.819	bal	24.74	6.61	3.73	0.262	-	0.0003
UNS S31254	Super austenitic SS	0.01	-	bal	20.18	18.15	6.1	0.2	-	0.010
UNS N08825	Nickel base alloy	0.05	0.85	22	22.5	bal	3	-	-	0.03
UNS N06625	Nickel base alloy	0.1	0.45	5	22.5	bal	9	-	3.85	0.015
Carbon steel	ASTM A572-50	C 0.155, Al 0.025, Mn 0.65, P 0.020, S 0.010, Si 0.15.								

To prevent crevice corrosion, samples were electrocoated with a protective epoxy (Powercron® 6000CX, PPG Industrial coatings) at the surface area where the spot weld was made for electrical connection and uncovered weld areas further covered by epoxy resin (Belzona 1391, Belzona polymerics Ltd.). Prior to exposure, coupons were wet ground to a 600 grit finish, soaked in Decon® 90 (Decon laboratories Limited) for 3 hours and sterilized by immersion in 70% ethanol for 1 hour.

Coupons were finally dried with nitrogen, weighed in triplicate and total coupon areas were measured using a digital gauge. Coupons were suspended by nylon strings in the experimental tanks.

2.2. Test conditions

Two lots of triplicate coupons of each material were exposed to streams of continuous low velocity (<1 mm/s) natural coastal seawater in a test rig established in a field laboratory within the Port Stephens Fisheries Centre site at Taylors Beach, New South Wales, Australia. A detailed description of the experimental rig is given elsewhere [37]. One stream was untreated and passed straight into a sealed 200 L experimental tank (referred to as raw seawater). A second stream was pumped into settling tanks followed by a series of filters down to 5 µm, passed over ultra violet lamps before going into another sealed 200 L experimental tank. This tank was continuously irradiated with ultraviolet (UV) light (lamps placed above water) in order to kill microorganisms without changing the chemical properties of the water (referred to as treated seawater). The chemical composition of the seawater is shown in Table 2. Water temperatures in the experimental tanks were recorded daily for the duration of the experiment (Tinytag Aquatic, Gemini Data Loggers, UK).

2.3. Electrochemical studies and determination of corrosion rates

The corrosion potential (E_{corr}) of each electrode was measured against a type CCS1-perm portable Ag/AgCl seawater reference electrode (Silvion Limited) and recorded every four hours using a multichannel data logger (dataTaker DT605, dataTaker Pty Ltd). Coupons were withdrawn after 90 days immersion, cleaned following the standard procedure [42]and weighed in triplicate to calculate weight loss and corrosion rates of each sample [42]using the following formula:

CR = kW/DAT,

Where CR= corrosion rate, mm/y; k= constant, 8.76 x 10^4; W=weight loss, grams; D=density, g/cm3; A= area in cm2; T= time of exposure in hours.

Table 2.

Analysis of the seawater at the experimental rig (Taylors Beach, NSW, Australia).

Analysis	Raw seawater	Treated seawater
Ammonia [mg/L N]	0.0085	0.051
Dissolved Oxygen [%]	86.7	82.1
Nitrate [mg/L N]	0.044	0.086
Nitrite [mg/L N]	0.003	<0.002
Salinity [PSU]	34.2	34.3
Sulphate [mg/L]	3500	3300
Total Phosphorous [mg/L P]	0.015	0.028

2.4. Evaluation of biofilm formation by SEM

Natural marine biofilms formed on the coupons exposed to coastal seawater for 90 days under freely corroding conditions were examined by scanning electron microscopy (SEM). Biofilm-coated coupons were removed from experimental tanks and fixed in glutaraldehyde (2.5% in 0.025 M phosphate buffered saline (PBS) (Sigma), pH 7.4) containing 0.15% w/v Alcian Blue (Sigma Aldrich) and incubated at room temperature for a minimum of 22 h. Coupons were then washed with PBS (0.025 M, pH 7.4) and fixed in sterile 1% osmium tetroxide for 30 min. Coupons were then dehydrated through a graded series of ethanol (15 min each):

50, 75, 90 and 100% dry ethanol successively. Dehydrated coupons were critical-point dried (CPD) in liquid carbon dioxide using an E3000 Critical Point Dryer (Quorum Technologies). After CPD, coupons were sputter-coated with platinum (5 nm thickness) using a SC7640 Sputter Coater, Quorum Technologies. SEM imaging was performed on a Zeiss 1555 VP-FESEM using an in-lens detector with a 30 mm aperture, accelerating voltage of 3 kV and a working distance of 4 to 5 mm.

2.5. Optical surface analysis for corrosion evaluation

Localized corrosion of the alloys was evaluated by surface optical measurements and inspection using an infinite focus microscope (IFM G4g system, Alicona Imaging).

2.6. Analysis of bacterial diversity by PCR-DGGE

The composition of the bacterial community in biofilms on high-resistance alloys and carbon steel was examined by PCR-DGGE analysis. At the completion of the exposure, 2 coupon replicates of each material from each tank were suspended in seawater containing filter-sterilized Tween 20 (0.1% w/v final concentration) separately. Microbial cells were detached by 60 second-sonication steps (solution was refreshed between sonication steps) until no microbial cells were observed in the solution under a phase contrast microscope. For each material, suspensions from all sonication steps were filtered through 0.2 μm pore diameter polycarbonate membrane filters (IsoporeTM, Millipore Corp.) and DNA from microorganisms on the membrane filters was extracted using a DNA extraction kit (PowerSoil™ DNA Isolation Kit, MO BIO Laboratories Inc). DNA was used as template to amplify a specific region of the bacterial 16S rRNA gene using nested PCR approach. The outer primer pair was 27F and 1492R and the inner primer pair was 357F-GC and 907R (Table 3) [43, 44]. The PCR-amplified 16S rRNA gene

fragments from different species were separated by DGGE using a DCode Universal Mutation Detection System (BIORAD) as per the manufacturer's instructions. The PCR products were mixed with DNA loading buffer (BIOLINE) and loaded into 7% (w/v) polyacrylamide gel (40% acrylamide/bis solution, 37.5:1) with a denaturing gradient of 30-60% urea-formamide (100% denaturant: 7M urea, 40% deionised formamide) in 1x TAE buffer (2 M Tris base, 1 M glacial acetic acid, 50 mM, EDTA, pH 8.0). The DGGE was run at 150 V at 60°C for 16 hours. DNA bands were visualized using a gel illuminator following staining with SYBR® Gold nucleic acid gel stain (Invitrogen™).

Table 3.

Primers used in this study for the amplification of the full-length and variable region of the bacterial 16S rRNA gene.

Primer[a]	Amplification target	Sequence (5' to 3')
27F[b]	Bacteria	GAGTTTGATCCTGGCTCAG
357F	Bacteria	CCTACGGGAGGCAGCAG
357F-GC[c]	Bacteria	CCTACGGGAGGCAGCAG
1492R[b,d]	Prokaryotes	ACGGdITACCTTGTTACGACTT
907R[b]	Universal	CCG TCA ATT CMT TTG AGT T

[a]The number relates to the *Escherichia coli* position to which the 3' end of the primers anneals. F and R correspond to forward and reverse primers, respectively.
[b]Modified from the original paper
[c]GC is a 40 nucleotide GC-rich sequences attached to the 5' end of the primer. The GC sequence is 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG.
[d]dI denotes a deoxyinosine modification.

3. Results

3.1. Corrosion Potential

Figure 1 shows average E_{corr} evolution in time of the test materials exposed to raw and treated seawater for 90 days. For all the high-resistance alloys exposed to raw seawater (Figure 1(a)) E_{corr} shifted from negative to positive values during the first 5 days of exposure. For most of the alloys, E_{corr} continued to increase with time and only minor active-passive states were observed over the exposure period. For UNS S31603, large active-passive peaks were more evident during exposure as compared with the other alloys. For most of the alloys, these active peaks were detected after 20-30 days exposure to raw seawater except for UNS N08825 where a drastic shift of E_{corr} towards active values was observed after a maximum ennoblement had been attained. E_{corr} of all alloys exhibited a clear tendency to ennoble with exposure. High-resistance alloys exposed to treated seawater displayed a fairly stable E_{corr} during the first 40 days of exposure with a slight trend towards negative values with time (Figure 1(b)). Interestingly, after 40 days exposure, E_{corr} of all alloys shifted to electropositive values and increased gradually with exposure. For some alloys, large active-passive transition peaks were evident after 50 days exposure. In all alloys, E_{corr} ennobled approximately +400-500 mV relative to the initial $E_{corr.}$ The maximum ennoblement attained during exposure was slightly higher for alloys exposed to raw seawater compared to alloys exposed to treated seawater although values were comparable. E_{corr} of carbon steel exposed to raw seawater slightly decreased during the first 5 days exposure and then displayed a smooth tendency towards more positive values with time (Figure 1(c)). E_{corr} of carbon steel was ennobled about +50 mV relative to the initial E_{corr}. E_{corr} of carbon steel exposed to treated seawater also showed a slight increase over time although the material did not display considerable ennoblement.

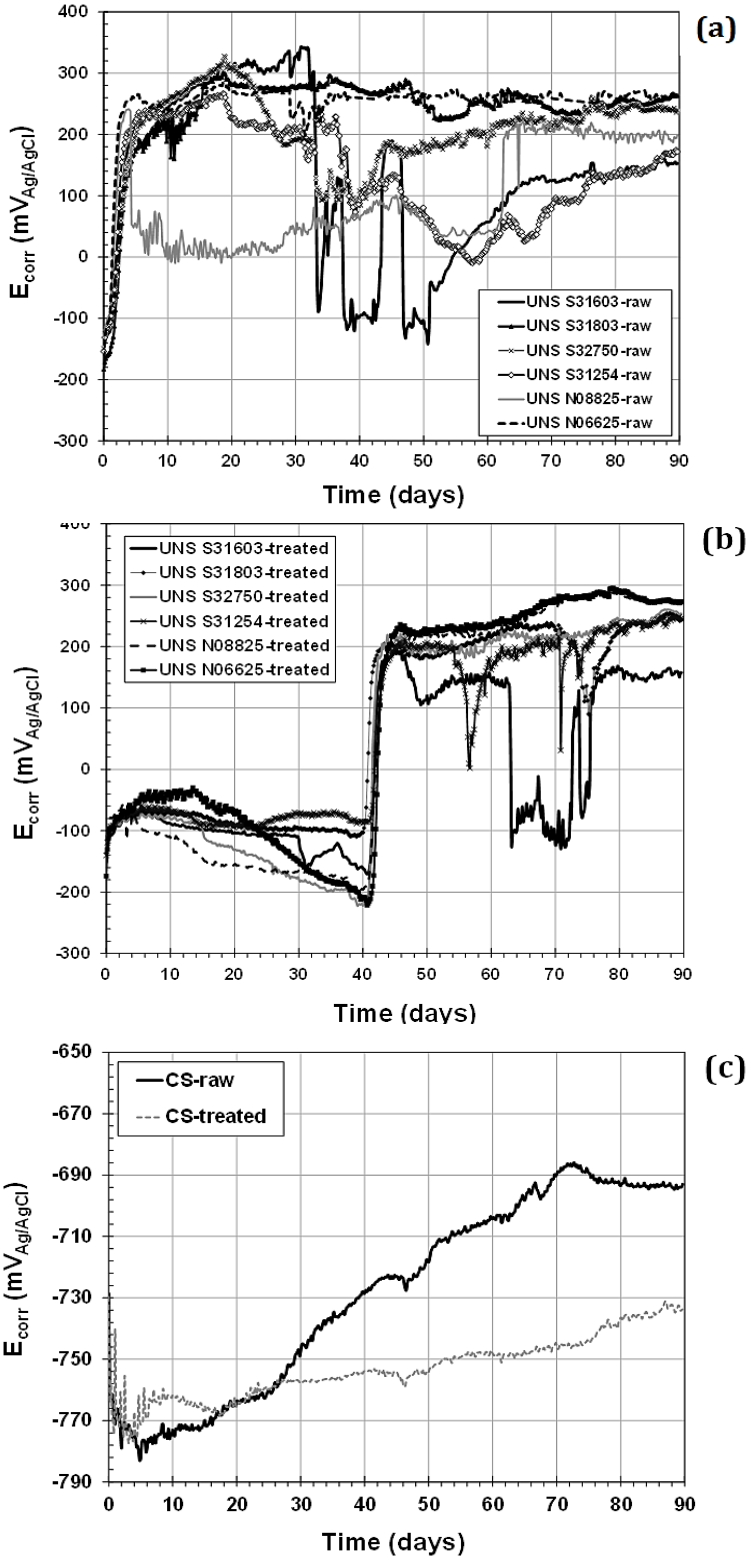


Figure 1. E_{corr} as a function of time for test materials exposed to flowing natural coastal seawater. (a) high-resistance alloys in raw seawater; (b) high-resistance alloys in treated seawater; and (c)carbon steel in raw and treated seawater.

3.2. Temperature

The exposure temperature in the experimental tanks measured over the course of the immersion period is plotted in Figure 2. Initial exposure temperatures were about 18-19°C and reached maximum values of ~28°C after 30 days of exposure. Average temperatures were 23.1°C and 22.7°C for treated and raw seawater tanks, respectively. The slight increase in temperatures in the treated seawater tank is attributed to the effect of ultra-violet lamps.

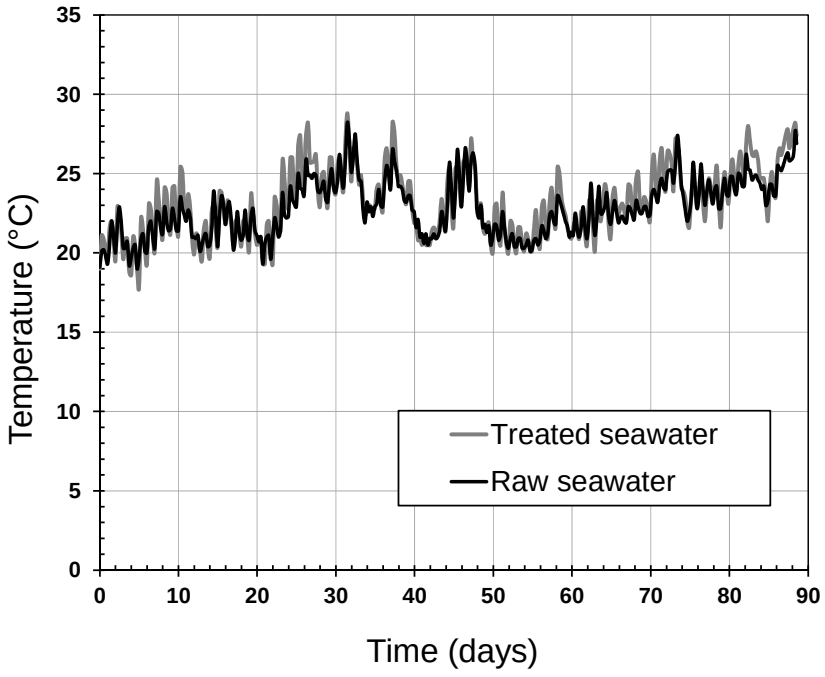


Figure 2. Variation of temperature over time for flowing raw and treated seawater in the experiments.

3.3. Corrosion rates

Average corrosion rates calculated from weight loss of test materials are shown in Figure 3. Corrosion rates of carbon steel and high-resistance alloys in raw and

treated seawater were very low. In all cases, corrosion rates were higher in materials exposed to raw seawater than treated seawater. The highest corrosion rates were observed in carbon steel and UNS N08825.

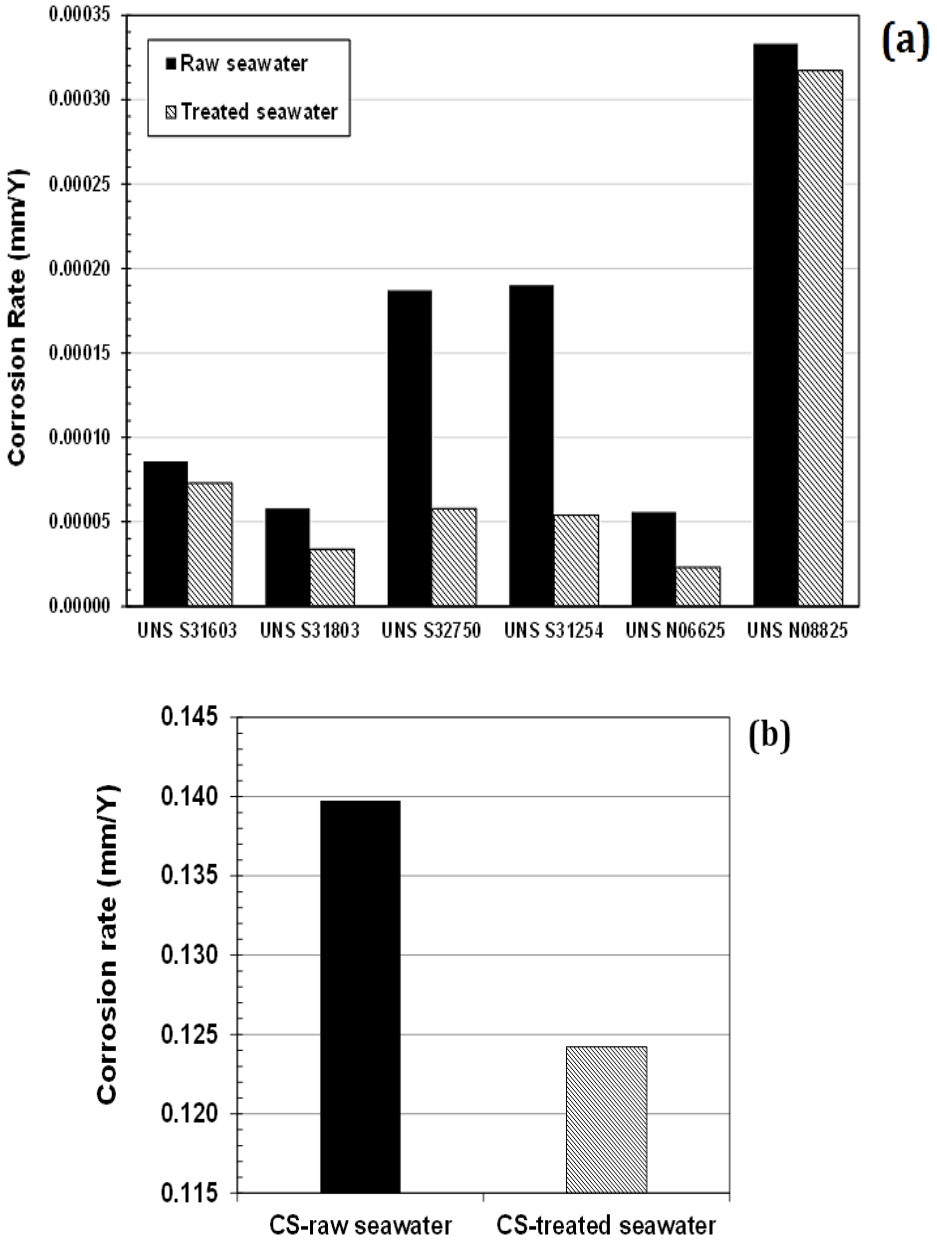


Figure 3. Corrosion rates by weight loss of test materials exposed to flowing natural costal seawater for up to 90 days. (a) high-resistance alloys in raw and treated seawater and (b) carbon steel in raw and treated seawater.

3.4. Surface analysis

Surface analysis by optical microscopy and pit profile measurements were conducted at the completion of the exposure. Figure 4 shows surface images of the test materials. Localized corrosion was observed on carbon steel, UNS S31603, UNS S31803 and UNS N08825 exposed to raw and treated seawater and was not detected on UNS S32750, UNS S31254 and UNS N06625. The extent of localized corrosion was similar for each alloy exposed to either raw or treated seawater but more severe in some alloys than in others. In particular, UNS N08825 exhibited the highest pit densities and pit depths ranged from 10-50 μm in raw and treated seawater (Figure 4(a)). On UNS S31603 numerous pit were also detected and pit depths ranged from 10-20 μm (Figure 4(b)).

In addition, grain structures were evident on a small region of the surface of UNS S31603 and localized corrosion was evident along the grain boundaries (Figure 4(c)). This intergranular attack was detected on UNS S31603 exposed to raw seawater whereas no evidence of intergranular corrosion (IGC) was observed for UNS S31603 coupons exposed to treated seawater. On UNS S31803, several shallow pits were also found with pit depths that ranged from 5-10 μm in both raw and treated seawater. Pits were not detected on UNS S32750, UNS S31254 and UNS N06625.

Carbon steel exhibited severe localized corrosion and the attack was more severe in raw seawater than in treated seawater (Figure 4(d)). For carbon steel exposed to raw seawater, pit depths ranged as much as 20-70 μm (Figure 4(e)) and the surface was more severely attacked than for carbon steel exposed to treated seawater. Pits observed on carbon steel exposed to treated seawater ranged from 10-20 μm.

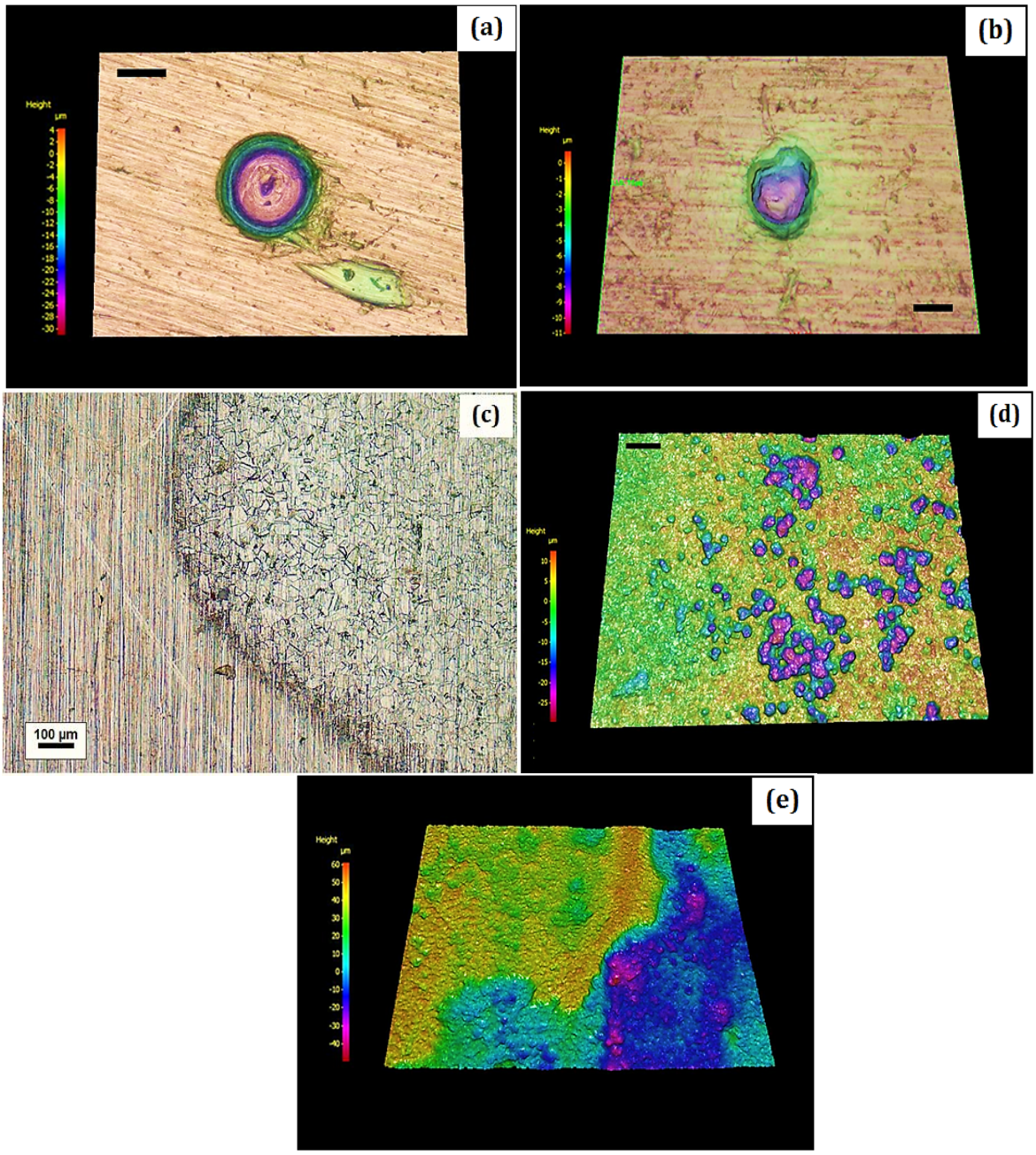
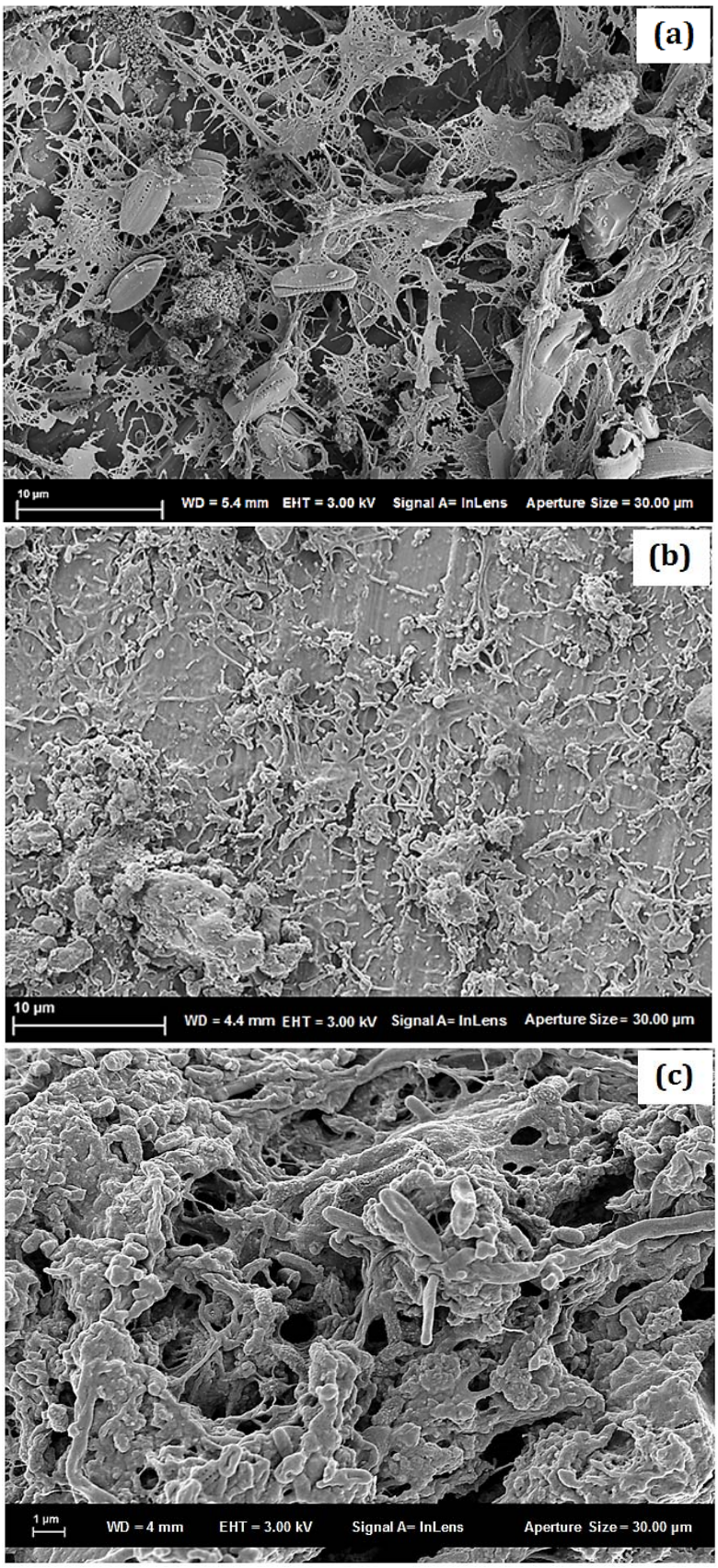


Figure 4. Optical microscope surface images of tests materials exposed to flowing natural coastal seawater under open-circuit conditions after biofilm removal and cleaning. (a) 34 µm pit on UNS N08825 exposed to raw seawater (scale bar correponds to 50 µm); (b) 12 µm pit on UNS S31603 exposed to treated seawater (scale bar correponds to 20 µm); (c) intergranular corrosion (IGC) on UNS S31603 exposed to raw seawater. IGC was not observed on UNS S31603 exposed to treated seawater; (d) severe pitting observed on carbon steel exposed to raw seawater. Pits depths ranged from 20-70 µm; (e) surface edges severely attacked in carbon steel exposed to raw seawater.

3.5. Biofilm imaging

SEM images of biofilms developed on high-resistance alloys and carbon steel in seawater are shown in Figure 5. Biofilms were not only observed on coupons exposed to raw seawater but also on coupons exposed to treated seawater. There were no noticeable differences in physical structure between biofilms developed in raw and treated seawater. The pattern of colonization and biofilm structure differed between materials. Ennobling biofilms on high-resistance alloys consisted of microbial cells, diatoms and extracellular polymeric substance (EPS) (Figure 5[a]). Biofilms on UNS S31603 (high pit densities) were thin and irregular along the surface forming net-like structures and patchy deposits and aggregates (Figure 5[b]). The alloy surface was not fully covered by biofilms. Biofilms formed on UNS S31803 (shallow pits), UNS S32750 and UNS S1254 (not pitted) partially covered the surface and were thicker and more complex than those formed on UNS S31603 (Figure 5[c]).

Biofilms developed on the nickel based alloys UNS N08825 (high pit densities and depths) and UNS N06625 (not pitted) were indistinguishable between the two alloys. These biofilms were very dense with microbial cells, filamentous structures and diatoms embedded in copious amounts of EPS and fine overlapping material on the entire surface (Figure 5[d]). This fusing material appeared as a connecting structure among biofilm components. These biofilms were most often aggregated into high and irregular mushroom-like structures. On carbon steel coupons, individual microbial cells and aggregates were encrusted with corrosion products over the entire surface of coupons exposed to both treated and raw seawater (Figure 5[e]). There was no correlation found between biofilm thickness and the extent of pitting corrosion on the different materials.


(a)
10 µm
WD = 5.4 mm EHT = 3.00 kV Signal A= InLens Aperture Size = 30.00 µm
(b)
10 µm
WD = 4.4 mm EHT = 3.00 kV Signal A= InLens Aperture Size = 30.00 µm
(c)
1 µm
WD = 4 mm EHT = 3.00 kV Signal A= InLens Aperture Size = 30.00 µm

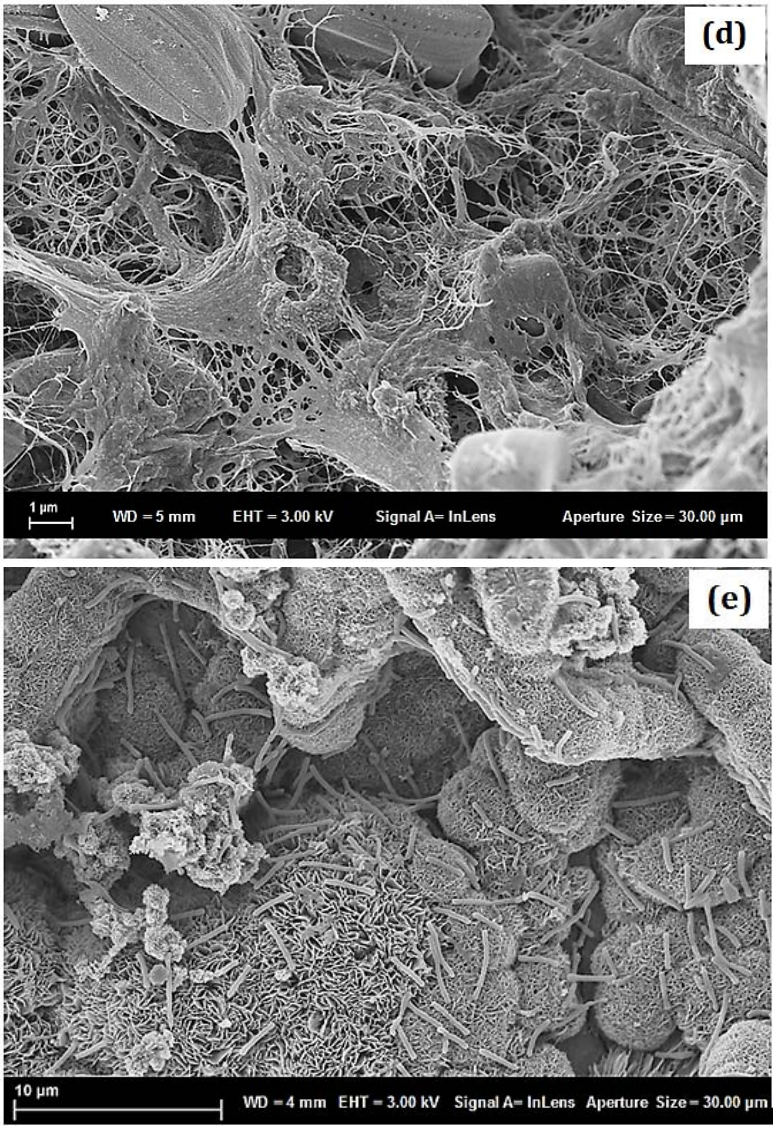


Figure 5. SEM micrographs of biofilms formed on the various test materials exposed to flowing coastal seawater. [a] biofilm containing microbial cells and diatoms (indicated by arrows) embedded in extracellular polymeric substance (EPS) on UNS S31803 exposed to treated seawater; [b] biofilms consisting of mats of fused overlapping material and net-like structures formed on UNS S31603 exposed to treated seawater. [c]mature biofilm showing microbial cells embedded in abundant EPS on UNS S32750 exposed to treated seawater; [d] dense and complex biofilm formed showing diatoms and microbial cells, filamentous structures and diatoms embedded in copious amounts of EPS with connecting structures formed on UNS N08825; [e] individual microbial cells encrusted with corrosion products over the surface of carbon steel coupons exposed to raw seawater.

3.6 PCR-DGGE analysis of bacterial diversity

PCR-DGGE analysis of biofilms revealed a shift in bacterial diversity and community composition in biofilms as a function of substratum material (Figure 6). Bacterial DNA was extracted from all the materials exposed to both treated and raw seawater. The individual bands seen in each lane of the DGGE are related to the predominant bacterial populations present in biofilms formed on the different materials and the number of bands gives an indication of the bacterial diversity in biofilms. It is clear that for each substratum material the number and type of bacterial populations in biofilms grown in raw seawater differed from those formed in treated seawater although some DNA bands were detected in both biofilms (e.g. Fig.6, CS-band 1). Likewise, several bands were commonly encountered in biofilms developed on different materials (e.g. Fig.6, band 13) although some bands were only found in biofilms grown on certain materials but not on others (e.g. Fig.6, band 21). There was no correlation found between the number of bands (bacterial diversity) and pitting corrosion on the different materials.

4. Discussion

The increase of E_{corr} on high-resistance alloys in raw seawater was similar to values reported by researchers in other regions [20, 45, 46]. The rapid ennoblement of E_{corr} appears to be associated with the active microbial colonization of the alloys following exposure. Despite the differences in biofilm morphology, structure and composition on the test alloys, as indicated by microscopy and PCR-DGGE, the extent of ennoblement was comparable between the alloys. The extent of pitting did not correlate with either higher bacterial diversity or biofilm thickness as suggested by SEM.

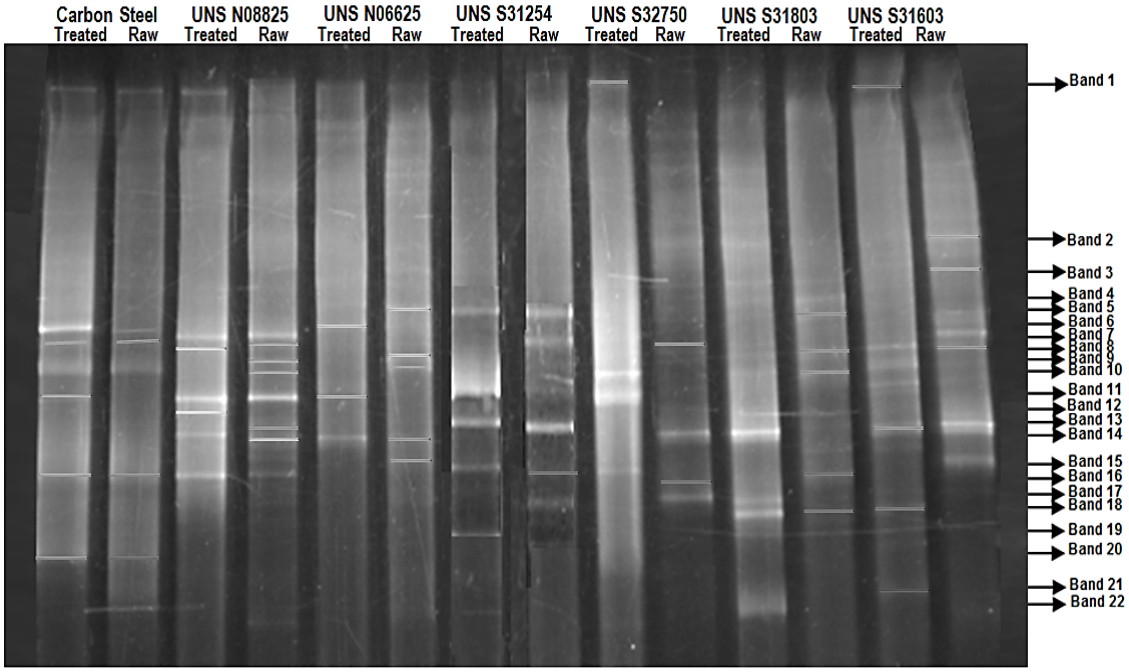


Figure 6. Denaturing Gradient Gel Electrophoresis (DGGE) profiles of dominant bacterial populations in biofilms formed on the different test materials exposed to both raw and treated seawater. Arrows indicate the bands in the gel, each one theoretically corresponding to one bacterial population in each biofilm community.

Today, it is commonly agreed that ennoblement is directly associated with biofilm formation [15]. Several mechanisms have been proposed to explain this phenomenon but unifying mechanisms for global observations have not been identified. Numerous researchers have shown that increased cathodic reduction rates accompany ennoblement of E_{corr} [20, 47]. In marine waters, this has been attributed to organometallic catalysis [48], acidification of the metal surface [49], catalysis by bacterially produced enzymes [23, 24], the combined effects of low pH and production of hydrogen peroxide within biofilms [49] and microbially produced inhibitors [50]. More recently, it has been demonstrated that certain bacteria are able to switch from natural soluble electron acceptors and donors to solid anodes so that there is a direct transfer of electrons between biofilms and steels. Microbial direct electron transfer has also been described as a mechanism of ennoblement of steels in seawater [51-53]. Under these conditions, the enhanced

cathodic reaction by bacteria seems to be less sensitive to mass-transfer compared to reactions using oxygen or other soluble species. Ennoblement has also been related to the settlement of living sea diatoms on stainless steels [20]. It was suggested that ennoblement was caused not by the metabolism but by the metabolite of the sea diatoms contained in a thin organic film adhered to the steel. In the present study, sea diatoms were found embedded in the EPS of ennobling biofilms on the different alloys which may suggest they could have played a role in the observed ennoblement. It is probable that all of these mechanisms, either singly or in combination, may contribute to the ennoblement of the alloys. However, the precise mechanisms of ennoblement by biofilms on high-resistance alloys were not investigated.

The growth of ennobling biofilms on alloy surfaces exposed to filtered- UV irradiated seawater indicates that the disinfection treatment did not sterilise the water. Filtration and settlement reduce the amount of sediments, larger organisms and organic matter. UV radiation damages proteins and membranes and indirectly damages DNA by creating reactive oxygen compounds (e.g., H_2O_2, O_2^- etc.) causing single-strand breaks in DNA [54]. Microbial survival during UV radiation could be due to several mechanisms. It has been known for many years that biofilms may grow in harsh environmental conditions due to the intrinsic or acquired resistance of microorganisms [55]. Bacterial cells can respond to stress by activation and expression of new groups of genes, sporulation and the production of neutralising enzymes that prevent cellular damage [55]. Microbial survival of UV irradiation has been shown to result from protection via particle shielding [56]. Bacteria may be shielded from radiation damage by particles or clumping, allowing even susceptible strains to survive. Results from E_{corr} measurements indicated that biofilms ennobled alloys only after 40 days of exposure to treated seawater. These results may indicate that near 40 days exposure, conditions became more favorable to stimulate microbial activity. Results showed that exposure temperature reached

maximum values at the 30-40 days exposure. Higher exposure temperatures may have increased the growth rates of microbes that were present on coupons. Higher coastal temperatures may also have increased microbial diversity potentially introducing new species into the system. Survival during UV radiation could result from the low susceptibility to UV radiation of some new species [56].

The presence of biofilms on coupons in both seawater tanks explains why alloys ennobled and pitted to the same extent regardless of seawater treatment. The influence of the disinfection treatment on species of bacteria on surfaces is indicated by the DGGE profiles of biofilm bacteria; however, it is interesting that this did not result in differences in the appearance of biofilms or pitting.

Localized corrosion of UNS S31603, UNS S31803 and UNS N08825 covered by biofilms formed in both treated and raw seawater was demonstrated by optical surface analysis. Localized corrosion was not detected on UNS S32750, UNS S31254 and UNS N06625 despite the large ennoblement of E_{corr} of these alloys during exposure and the presence of biofilms as evidenced by SEM. These results can be explained on the basis of the known potential-temperature dependence on the onset of localized corrosion in active-passive alloys. It is known that localized corrosion is considered to occur if the corrosion potential of a steel in a given environment surpasses a critical potential [5, 8, 9]. At lower potentials, pit initiation is followed by rapid repassivation, a stage commonly referred to as metastable pitting. Both a critical potential and temperature are required to stabilize pit growth on the alloys surface. In a previous study, critical potentials and temperatures for pitting and crevice corrosion of high-resistance alloys in seawater were investigated [5]. It was observed that critical potentials for localized corrosion initiation and repassivation decreased with increasing exposure temperature and lowering of temperature similarly ennobled the critical potential. It was also shown that pitting corrosion did not take place below a transition temperature regardless of the applied potential but crevice corrosion occurred

below a transition temperature when the alloys reached a high anodic potential in the transpassive region. In the same study, UNS S32750 and UNS S31254 exhibited excellent resistance to crevice and pitting corrosion in natural seawater at temperatures below 40°C under potentiostatic and potentiodynamic test conditions at potentials as high as 1500 $mV_{Ag/AgCl}$. UNS S31603, UNS N08825, and S31803 were reported to initiate localized corrosion at seawater temperatures below 40°C provided an anodic potential was applied (critical potential). This is in agreement with the results from the present study where UNS S32750 and UNS S31254 remained protected against localized corrosion at the experimental seawater temperatures (17-29 °C) despite the fact that biofilms ennobled E_{corr} to +200-300 $mV_{Ag/AgCl}$ during exposure. For UNS S31603, UNS N08825, and S31803, however, the same conditions of potential and temperature sufficed to cause localized corrosion of the alloys. These results demonstrate that biofilms can trigger localized corrosion in active-passive alloys by shifting E_{corr} into a critical potential range provided the temperature has exceeded a critical value. Similarly, ennoblement of E_{corr} decreases the critical temperature below which a given alloy should be resistant to localized corrosion initiation.

Surface analysis also showed areas of intergranular corrosion (IGC) on UNS S31603 exposed to raw seawater whereas no evidence of IGC was observed for UNS S31603 exposed to treated seawater. IGC is a form of localized corrosion along grain boundaries caused by anodic dissolution of specific regions at the surface such as regions depleted of alloying elements. Non-uniform intergranular attack can be due to factors that locally impair the corrosion process such as the formation of deposits. This may weaken the oxide film at specific locations, allowing halides such as chloride ions greater access to the underlying metal and facilitating localized attack. IGC has been previously reported for 70Cu-30Ni alloy under elliptical deposits of embedded diatoms [57] whereas IGC was not observed on the same alloy exposed to artificial seawater. It was suggested that sulphides

produced by bacteria may have reacted with nickel in grain boundaries causing preferential dissolution of the steel in these regions. It has been shown that selective colonization by bacteria results in depletion of Cr and Fe relative to nickel at the grain boundaries on 316L stainless steel [58]. It was also reported that individual cells were seen along surface grain boundaries prior to biofilm formation suggesting that initial colonization is non-random and highly selective for grain areas. In addition, it was shown that sulphur compounds preferentially accumulate at grain boundaries particularly in the presence of sulphate-reducing bacteria (SRB). On the basis of these findings, it is plausible to consider that microorganisms played a role in the onset of IGC of UNS S31603 exposed to raw seawater. However, since biofilms also formed on UNS S31603 exposed to treated seawater, where IGC was not detected, IGC cannot be attributed to the mere presence of microorganisms. Given that PCR-DGGE revealed differences in the bacterial community in biofilms formed in raw and treated seawater, it is possible that IGC initiation could have been particularly favoured by the distinctive metabolic activities of the biofilm community developed in the raw seawater system.

PCR-DGGE also revealed that bacteria differed in their preference for material type. Different microbial communities on different alloys has been reported previously [6]. It is likely that microbial adhesion and biofilm formation are affected by the nature of the passive films and the microstructure of alloys. Chemical composition and microstructure have been shown to have a strong influence on the composition and thickness of oxide films, which may contribute to the selective attachment of microorganisms.

In addition, SEM revealed variations in physical structure and amount of EPS produced in biofilms according to substratum material. It is known that microorganisms are able to respond to their environments and change their EPS and adhesion abilities, depending on the properties of the surface onto which they

attach [59]. Differences in EPS production could be a bacterial mechanism to promote hydrophobic interactions to favour irreversible sorption onto solid surfaces [59]. The higher amount of EPS observed on nickel-based alloys compared to stainless steels, could suggest that there is a lower affinity between nickel oxide films and microbial cells compared to oxides formed on stainless steels. EPS could be produced at higher levels to alter the surface interactions and favour irreversible attachment. Likewise, the presence of toxic metals in the environment has been shown to stimulate EPS production by microorganisms [60]. It was shown that higher EPS production also accelerated the corrosion of mild steel. This was attributed to the acidic and metal-binding nature of the EPS that could result in the formation of ion concentration cells. The toxicity of nickel towards microorganisms is well documented [61]. Based on these observational data, our findings could also suggest that EPS production was increased as a bacterial defense mechanism against toxic nickel species from corroding nickel-based alloys.

The relationship between kinetics of biofilm formation and the evolution of E_{corr} in time has been reported previously [47]. The development of biofilms on 316 stainless steel was examined during the increase of ennoblement of E_{corr} after exposure to natural seawater. It was reported that at the onset of ennoblement, the steel surface was covered mainly by single bacterial cells. When E_{corr} had started to increase, some of the initially adhered bacteria had grown into mushroom-like structures which grew in size as the ennoblement increased. Diatoms were seen embedded in these mushroom-like structures. In the same study, non-ennobling biofilms were seen forming uniform mats, rather than individual mushrooms-like structures. Results from the present study indicated that despite the differences in biofilm morphology, structure and composition on the different test alloys, as indicated by microscopy and PCR-DGGE, the extent of ennoblement was comparable between the alloys. However, the mechanisms for ennoblement were unknown and will be the subject of further research.

5. Conclusions

Natural marine biofilms induced localized corrosion on active-passive alloys by shifting E_{corr} into a critical potential for the onset of localized corrosion. Biofilms ennobled E_{corr} of the stainless steels UNS S31603, UNS S31803, UNS S32750, UNS S31254 and the nickel alloys UNS N08825 and UNS N06625 in both raw and treated seawater by as much as 400-500 mV relative to their initial E_{corr} (Ag/AgCl). Treatment of seawater decreased corrosion rates, as determined by weight loss, of all materials but did not confer sterility to the seawater. Ennoblement by marine biofilms triggered localized corrosion on UNS S31603, UNS S31803 and UNS N08825 but did not cause localized corrosion on UNS S32750, UNS S31254 and UNS N06625. These results can be explained on the basis of the potential-temperature dependence of active-passive alloys on the onset of localized corrosion. The localized attack, as determine by pit depths, was more severe on carbon steel, UNS N08825, UNS S31603 and UNS S31803, successively, in both raw and treated seawater. UNS S31603 covered by biofilms suffered intergranular corrosion (IGC) in raw seawater but not in treated seawater. This may be due to differences in biofilm community structure in raw and treated seawater. Biofilm community composition was different on different materials which can be related to the nature of the passive films and the microstructure on the different alloys. In addition, the pattern of colonization, EPS production and physical structure in biofilms differed between materials. On carbon steel, individual microbial cells were encrusted with corrosion products over the surface and on nickel alloys biofilms appeared more abundant, complex and produced more copious amounts of EPS compared to the stainless steels. Despite the differences in biofilm morphology, structure and composition on the different alloys the extent of ennoblement was comparable between the alloys.

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Chapter 10

L.L. Machuca, L. Murray, R. Gubner, S.I. Bailey. Evaluation of the effect of seawater ingress into 316L lined pipes on corrosion performance**.** Materials and Corrosion (2012).

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Evaluation of the effects of seawater ingress into 316L lined pipes on corrosion performance

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Abstract

The potential effects of seawater ingress into 316L lined pipes during subsea tie-in operations on corrosion performance were investigated. Immersion and accelerated corrosion tests were conducted on 316L in different mixtures of treated seawater. In particular, we examined the effect of oxygen and microorganisms in seawater on the performance of the alloy at the different mixtures of treated seawater to assess the risk of localized corrosion in the event of seawater ingress into pipelines. Results showed that oxygen has a negative impact on the biocidal and oxygen scavenging efficiency of the chemical treatments and a detrimental effect on pitting corrosion.

Keywords: stainless steel, pitting corrosion, microbiological corrosion, seawater.

1. Introduction

Commissioning deep-water pipelines is a standard part of any subsea installation. Once a pipeline or infield flowline is laid offshore, it must be tested to ensure that it will perform as designed and that it was not damaged during installation. Pipeline commissioning typically involves flooding with treated seawater, cleaning, gauging and hydrostatic testing. Hydrostatic testing is a routine practice to verify pressure equipment does not leak or have manufacturing flaws. Treated seawater is routinely used in the hydrotesting of subsea pipelines. In the oil and gas industry, it is often the case that hydrostatic test water is left in the system for many months before the system is actually commissioned. Natural seawater contains viruses, prokaryotes (bacteria and archaea), protists and algae. During this holding time, the activity of residual microorganisms can increase, as the effectiveness of the preservation chemicals decays, and also if there was any ingress of raw seawater that mixes with the volumes of treated fluids. In addition, this stagnant water may permit debris such as sand, marine life, and bacteria introduced by poor water treatment or during tie-in operations to settle and form biofilms. Under-deposit corrosion and/or microbiologically influenced corrosion (MIC) may then occur [1-6]. These biofilms may present a serious threat once the pipelines become operational, because fluids transported in pipelines may contain sufficient nutrients for bacteria to flourish [7, 8].

Dissolved oxygen (DO) in the seawater is one of the most aggressive species towards corrosion of metallic materials. The degree to which dissolved oxygen influences corrosion is dependent on the metal or alloy [9]. Corrosion resistant alloys (CRAs) are becoming important structural materials for offshore applications because they possess an appropriate combination of strength and corrosion resistance in marine environments. These materials have negligible general corrosion in aerated water due to a protective, predominantly chromium oxide, film which forms immediately on their surface with exposure to oxygen [9].

High oxygen content is favourable for passive film-forming alloys because it delays the corrosion initiation at surface defects and penetration through oxide protective films. However, in aerated seawater, surface deposits on passive film-forming alloys can create oxygen concentration cells, which can cause pitting and/or crevice corrosion at localized sites [10]. Once pitting is initiated, the propagation rate is accelerated with increasing dissolved oxygen content [11].

Corrosion caused by any of these mechanisms may reduce pipeline and equipment service life. The severity of the problem depends upon the chemistry and microbiology of the water that is used, the length of time that the water remains in the line and the temperature of the system. In order to protect against these adverse effects, seawater used for hydrotesting requires an appropriate preservation treatment [12, 13]. Seawater treatments include filtration and appropriate dosages of chemical treatments. Filtration reduces the amount of sediment and nutrients entering the pipeline, decreasing the risk of MIC and under-deposit corrosion. Chemical treatments may include an oxygen scavenger, a biocide and a corrosion inhibitor. Tetrakishydroxymethyl Phosphonium Sulfate (THPS) is the preferred biocide since it is effective against microorganisms while having a favourable environmental profile that allows easy disposal offshore [13]. Ammonium bisulfite (ABS) is the recommended oxygen scavenger for hydrotesting waters [12]. Biocide and oxygen scavengers dosages are provided by chemical vendors and depend upon predicted times of seawater containment in the system.

During installation of equipment for offshore gas fields, flowlines filled with treated seawater need to have end caps removed to tie-in with subsea equipment. This operation will require the connection point on the flowline to be opened, thus exposing the flowline ends directly to seawater. It is expected that the majority of these operations are within 12 hours exposure time. However, several feasible scenarios such as vessel position loss and vessel breakdown, tie-in tooling failure or damage to subsea hardware or unforseen unfavourable weather conditions can

result in significantly longer exposure and it is possible that a flowline may be exposed to seawater for durations in excess of 24hrs. In the case of breakdown of dive systems or poor weather conditions, e.g. cyclones, it is possible to experience durations of greater than 1 week exposure for the worst case scenario.

This study aimed to investigate the potential effects of the ingress of raw untreated seawater into 316L lined pipes containing hydrostatic test water during subsea tie-in operations on the pitting resistance of the alloy. This used a combination of immersion tests, accelerated corrosion tests and 3D optical surface imaging. In particular, we examined the effect of oxygen and sulphate-reducing bacteria (SRB) on the corrosion performance of the steel. The presence of SRB is of great concern to oil and gas industry worldwide due to its widely recognized involvement in corrosion of materials [14-17]. Microbiological analyses were conducted to evaluate biocide efficiency and to assess the likelihood of MIC in the event of seawater ingress into pipelines. Results from this study will allow a risk assessment on the likelihood of localized corrosion events related to tie-in operations and better definition of the time window of tolerance to seawater ingress to the pipeline in the event of any upsets during tie-in operations.

2. Materials and methods

2.1. Specimens preparation

316L stainless steel coupons used for electrochemical analysis and immersion tests were cut into small coupons having 5 cm2 and 6 cm2 surface active area, respectively. The composition (in wt %) of this material was: Cr 16.92, Ni 10.11, Mo 2.05, N 0.05; Mn 1.38, C 0.016, S 0.001, Fe bal. Prior to exposure, coupons were wet ground using silicon carbide papers of 120, 360, 600 and 1200 grit consecutively. The polished specimens were washed with Milli-Q water, degreased with ethanol

and dried with nitrogen gas. For exposure testing, coupons were sterilized by immersion in ethanol 70% for 1 hour.

2.2. Seawater solutions

Seawater samples were collected at 20 metres depth off Rottness Island (Western Australia) in the Indian Ocean. The chemical composition of the seawater is given in Table 1. 100% treated seawater corresponded to 100 ppm of Ammonium bisulfite (ABS) oxygen scavenger and 550 ppm of tetrakis (hydroxymethyl) phosphonium sulfate (THPS) in 60 μm filtered seawater. From this mixture, 20, 40, 60 and 80% mixtures of treated seawater were prepared. The oxygen scavenger was applied first and allowed to react for 30 min prior to addition of biocide to the system to avoid incompatibilities between chemical treatments [18]. Raw untreated natural seawater was used as experimental control.

Table 1

Analysis of the natural seawater used in this study

Analysis	Composition
Salinity [PSU]	35.58
DO [ml/L]	5.06
Conductivity [mS/cm]	48.79
pH	8.2
Chloride [mg/L]	18500
Magnesium [mg/L]	1340
Sodium [mg/L]	11100
Sulphate [mg/L]	2700

2.3. Electrochemical Testing

For electrochemical testing, the Gamry Intruments Flexcell™, a crevice-free pitting evaluation system was used to evaluate general and pitting corrosion on 316L in the various concentrations of treated seawater. A double junction Ag/AgCl electrode and a platinum coated mesh were used as reference and counter electrode, respectively. Details on the experimental setup to investigate pitting corrosion using this cell are described elsewhere [19]. Solution temperature was achieved internally using a recirculating water bath connected to the cell through a Teflon-coated copper coil. A 150 rpm agitation rate was sustained using a Teflon rotator. A nitrogen blanket was maintained throughout the test to avoid oxygen into the system and to help stabilize the open circuit potential.

The linear polarization resistance (LPR) method was used to monitor corrosion rates over 5 hours. LPR measurements were performed by applying anodic voltage scans at the rate of 0.1 mV/s over a range of ±10 mV around stabilized open circuit potential. The Gamry DC105 software was used to calculate corrosion rates from LPR data. A linear fit of the current vs. voltage data to a standard model yields an estimate of the Polarization Resistance (Rp). Rp is then used to calculate Icorr and corrosion rate.

Cyclic Potendiodynamic Polarization Scans (CPP) tests were conducted to determine pitting potential (E_{pit}) and repassivation potential (E_{rep}) and their relationship to the open circuit potential (OCP) of 316L in the various mixtures of treated seawater. Scans were conducted using a forward and reverse scan rate of 0.167 mV/s. E_{pit} was identified as the potential where the anodic current indicated the onset of stable pitting and E_{rep} was identified as the potential for which the forward and reverse scans intersect, which is where repassivation of pits is considered to take place. CPP tests were conducted in duplicate for each condition.

2.4. Immersion tests

316L coupons were exposed to treated seawater and experimental conditions as described in Table 2. Immersion test A was intended to evaluate the performance of the steel in different concentrations of treated seawater in the event of high levels of oxygen ingress into the system. For immersion test B and D, a nitrogen blanket was formed on top of the exposure cells by continuous injection of high rates of nitrogen gas via a gas flow meter to prevent oxygen contamination and evaluate the efficiency of the specified treated seawater mixtures in the absence of oxygen. The outlet flow of nitrogen from the system was monitored by a second flow meter to ensure there were no leaks in the system. Immersion test B was particularly aimed to evaluate the efficiency of 100% treated seawater on preventing MIC in the event of high loading of SRB into the system and on protecting against pitting corrosion in the absence of oxygen.

Immersion test C was aimed to evaluate the efficiency of the chemical treatments and the performance of the alloy in the event of low concentrations of oxygen ingress into the system which also allows evaluating the effect of the residual biocide on controlling MIC. For this test, the desired DO concentration was controlled to reach maximum values of 2 ppm. The solution was initially flushed with sterile air for 30 min. Then, no gas was bubbled through the water and an air blanket was formed on top of the cells by injection of high rates of air via a gas flow meter for 1 h. The system was then sealed to allow the oxygen in the gas phase to dissolve into the solution. The DO was monitored continuously until its concentration was close to 2 ppm (approximately 2 days). At this point, a nitrogen blanket was formed on top of the cell as described above for tests B and D. No gas was bubbled through the water during the entire exposure to maintain stagnant conditions. All exposure cells were maintained inside an incubator set at 20 °C. Several analyses were conducted after defined exposure periods (Table 2). DO levels were measured using an Orbisphere 3655 oxygen analyser from Hach

Company before and after the exposure periods and pH of testing solutions was monitored using an Orion 5-star plus portable multimeter from Thermo Fisher Scientific, Inc.

Table 2

Summary of the test conditions to investigate the effect of various levels of seawater ingress into 316L lined pipes

Test	**Exposure solution** Treated seawater/ raw untreated seawater	**Conditions**	**Exposure time**	**Analyses**
Accelerated corrosion tests	100/0 80/20 60/40 40/60 20/80 0/100 (control)	Stirring; 20 °C No O_2	5 h	LPR[a], CPP[b]
Immersion test A	100/0 + SRB[c] 80/20 + SRB 60/40 + SRB 40/60 + SRB 20/80 + SRB 0/100 + SRB (control)	Stagnant; 20 °C Free O_2 ingress	7, 10, 14, 21, 28 days	Weight loss surface/pitting analysis DAPI staining for bacterial adhesion
Immersion test B	100/0 + SRB	Stagnant; 20 °C No O_2	7, 14, 28 days	surface/pitting analysis DAPI staining for bacterial adhesion
Immersion tests C	100/0	Stagnant; 20 °C ~2 ppm O_2	7, 14, 28 days	surface/pitting analysis DAPI staining for bacterial adhesion
Immersion tests D	80/20	Stagnant; 20 °C No O_2	7, 14, 28 days	surface/pitting analysis DAPI staining for bacterial adhesion

[a] LPR: Linear Polarization Resistance
[b] CPP: Cyclic Potentiodynamic Polarization Scan
[c] SRB: Sulphate Reducing Bacteria

2.5. SRB inoculum

The SRB population used as inoculum was isolated from natural seawater using the anaerobic nutrient-rich Starkey medium contained in anaerobic jars. Starkey medium contains (g/L): KH2PO4 0.5; NH4Cl 1; Na2SO4 1; CaCl2 * 2H2O 0.1; MgSO4 * 7H2O 2; sodium lactate 50% 10mL; filter-sterilized seawater 1L. The pH of the

medium was adjusted to 7.5. SRB were grown at 20 °C. Active SRB cells were obtained by transferring grown cultures to fresh Starkey medium every 72 hours. The bacterial count in the inoculum was estimated by serial dilution method [20] which indicated a population of 10^8 cell/mL. Treated seawater was inoculated with 10% SRB inoculum when necessary (Table 2). The numbers of SRB in the raw natural seawater used for immersion tests as estimated by standard serial dilution method was 10^3 cell/mL.

2.6. Surface Analysis by 3D optical microscopy

Surface inspection and pit profile measurements to evaluate localized corrosion of the tested alloys were conducted using an optical infinite focus microscope (IFM G4g system, from Alicona Imaging GmbH).

2.7. Evaluation of bacterial adhesion and SRB enumeration

To evaluate bacterial attachment, 316L surfaces were stained with 4,6-diamidino-z-phenylidole (DAPI), a fluorescent stain that binds strongly to DNA. Coupons were removed from the reaction vessel, rinsed with sterile water, stained with DAPI (2µg/mL) and incubated in the dark at room temperature for 15 min. DAPI-stained samples were examined with an epifluorescence microscope (Axio Imager.A1; Carl Zeiss, Germany) equipped with a Plan-Neofluar objective.

Enumeration of sessile SRB (attached to coupons) was conducted only on 28 days exposure coupons. To detach sessile SRB, coupons were removed from the reactions bottles, immersed in sterile seawater containing filter-sterilized Tween 20 solution (0.1% w/v final concentration) and sonicated (series of 90-second sonication steps). Suspensions were then filtered using 0.22 µm membrane filters. Membrane filters with concentrated SRB cells were then placed into SRB enumeration medium (Postgate C medium) [21]. SRB numbers were estimated by the serial dilution method [20]. The growth of bacteria is indicated by the

deposition of a black precipitate in the dilution vial as a result of bacterial metabolic activity.

3. Results and discussion

3.1 Electrochemical testing

Electrochemical testing was conducted to investigate uniform and localized corrosion on 316L in the various mixtures of treated seawater. Average corrosion rates from LPR measurements are shown in Figure 1. It can be seen that the corrosion rates were very low and trended to decrease with time for all the levels of seawater. Corrosion rates in seawater were very similar regardless of the concentration of the chemical treatments. The slight decrease in the corrosion rate observed with longer testing is considered to be due to the enrichment of Cr in the passiviting surface layers. This low corrosion rates indicate that uniform corrosion is insignificant but it is not a measure of the pitting tendency of the alloy.

CPP tests were conducted to determine the susceptibility to localized corrosion of 316L in the various levels of treated seawater [22]. Average corrosion potential (OCP), E_{pit} and E_{rep} obtained from duplicate CPP tests of 316L exposed to the various mixtures of treated seawater at 20°C are shown in Figure 2. The gap OCP-E_{pit} indicates that a high anodic potential is required for 316L to undergo stable pitting. In practice, pitting is often observed after a very long time (months, years) at potentials lower than E_{pit}. In this region of potential, metastable pits can be formed. It appears that during aging periods the protectiveness of the passive film on the alloys deteriorates and transition to stable pitting occurs. Crevice corrosion that occurs more readily than pitting can be expected at potentials close to the E_{rep}. E_{rep} is a very important critical potential indicating the range of potentials below which pitting will not occur [23]. Pitting and crevice corrosion are usually

considered to take place if the corrosion potential of a metal in a given environment surpasses the crevice repassivation potential [24]. It has been demonstrated that when OCP > E_{rep} pitting is expected and when OCP < E_{rep} the material is protected against pitting. Based on the above statements, results from the present study indicate that pitting could easily initiate if a slight anodic potential (E_{rep}-OCP) is attained during exposure. It can be seen that the localized corrosion resistance of 316L SS in seawater is very similar regardless of the levels of chemical treatments. However, these results must be carefully interpreted for assessing the possibility of pitting initiation considering the influence of environmental factors and aging effects on the pitting initiation mechanisms.

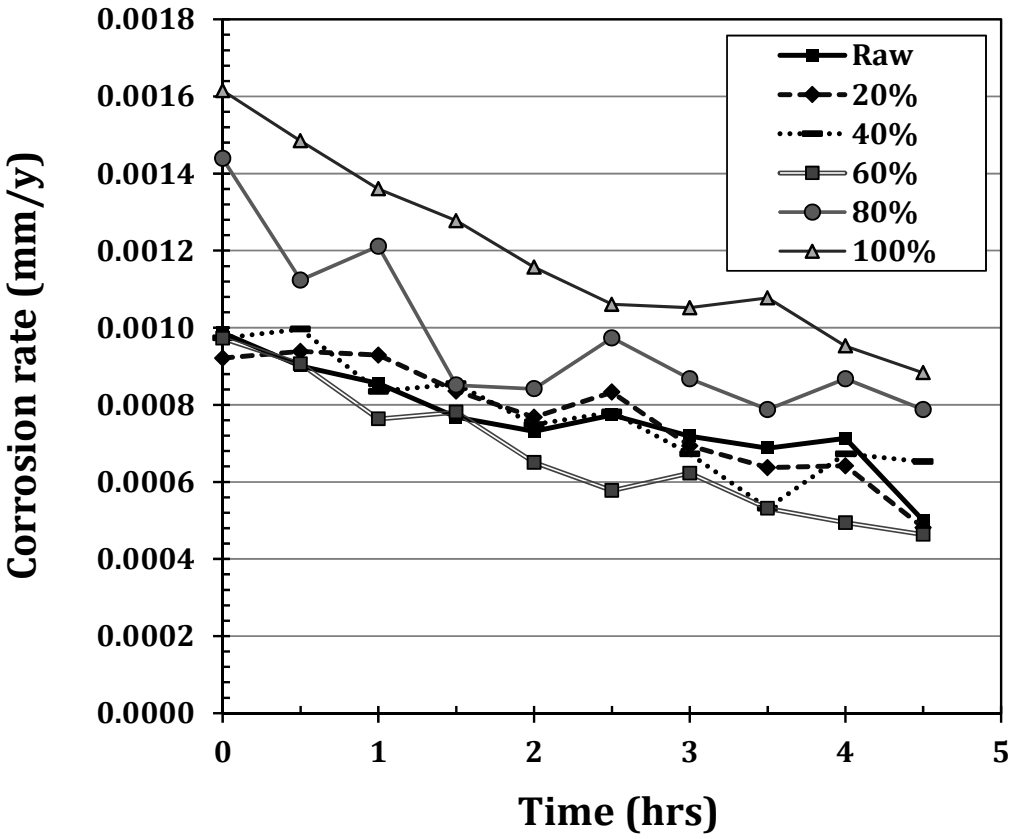

Figure 1. Corrosion rates as a function of time of 316L exposed to different concentrations of treated seawater at 20°C calculated from LPR measurements.

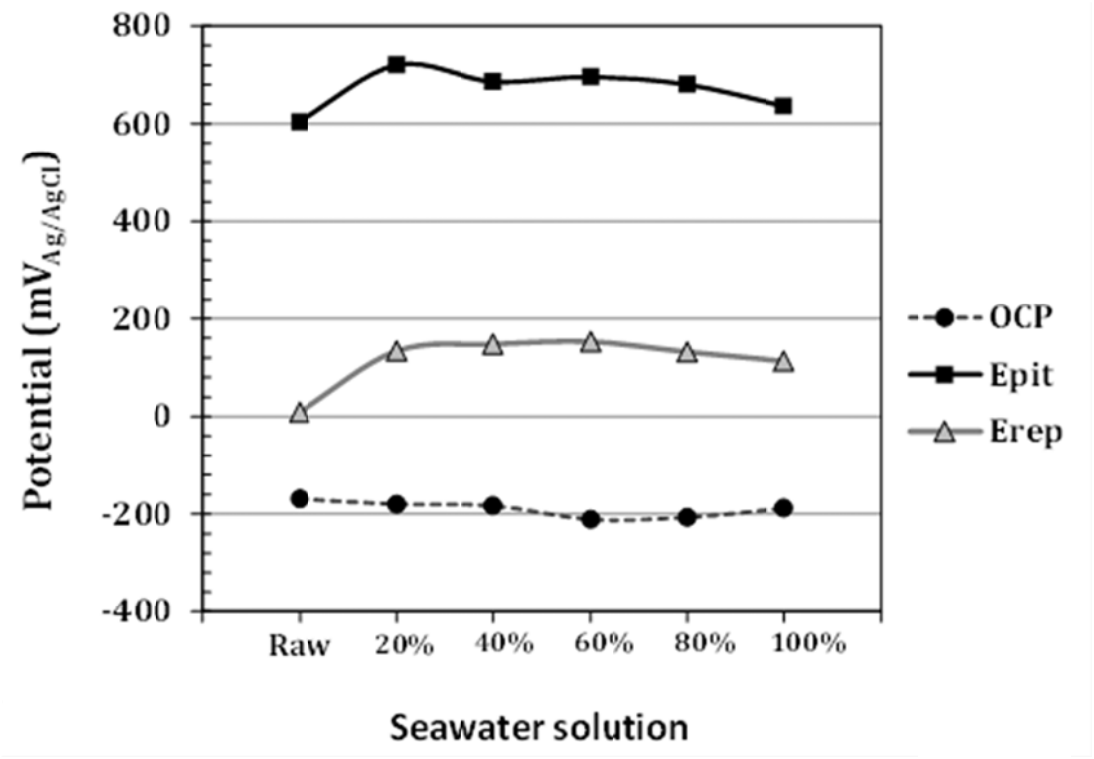

Figure 2. Average OCP, E_{pit} and E_{rep} of 316L exposed to different concentrations of treated seawater at 20°C. Potential were identified from cyclic potentiodynamic polarization tests.

3.2 Immersion tests

3.2.1 Dissolved oxygen (DO) and pH measurements

DO concentration measured throughout exposure for the different immersion tests is shown in Figure 3. For immersion tests A, DO concentration remained high regardless of the chemical treatment (Figure 3 (a)). 316L exposed to raw seawater showed a decrease of DO concentration with time. This oxygen depletion could be due to microbial activity in the raw untreated seawater. Previous studies on the compatibility of THPS and ammonium bisulfite (ABS) showed that THPS had the ability to deactivate the ammonium bisulfite (ABS) [18]. It was shown that the

detection of THPS and ABS residual values after exposure in the presence of oxygen indicated that the two chemicals had not reacted with each other but that the THPS interferes with the oxygen scavenging process by ABS. The authors concluded that the residence time between addition of ABS (to scavenge oxygen) and its subsequent contact with THPS should be maximized in order to prevent the two molecules from having a negative impact on each other. In the present study, ABS was added to the system 30 min prior to the addition of the THPS which was expected to provide enough time for the oxygen scavenging process to take place.

DO measurements for immersion tests B and D are shown in Figure 3(b). DO levels for Test B remained below 20 ppb throughout exposure. DO levels in Test D started at 200 ppb and decreased during the first 5 days of exposure to 40-60 ppb. Afterwards, DO levels remained fairly steady until the completion of the exposure indicating the nitrogen blanket provided an effective mechanism to prevent oxygen ingress into the system so that oxygen scavenging took place effectively. For Test C, where the desired DO concentration was set to maximum ~2 ppm, the solution was initially flushed with sterile air until the dissolved oxygen reached 500 ppb (approx. 30 min). The system was sealed to allow the oxygen in the gas phase to dissolve in the solution. When DO levels reached ~2 ppm, a nitrogen blanket was initiated to avoid any oxygen ingress into the system. When DO levels were below 1000 ppb the nitrogen blanket was stopped and the exposure cells where properly sealed again. This procedure was maintained throughout exposure in order to achieve the specified oxygen levels (Figure 3(c)). Once the nitrogen blanket was stopped, DO levels raised from 0.2 ppm to 2 ppm in 2-3 days.

Figure 4 shows the pH of the seawater in immersion test A. For immersion tests B, C and D, pH values were very similar to those detected on immersion test A at the respective seawater mixtures evaluated. pH slightly decreased during the first 7 days of exposure and then remained fairly steady throughout exposure time.

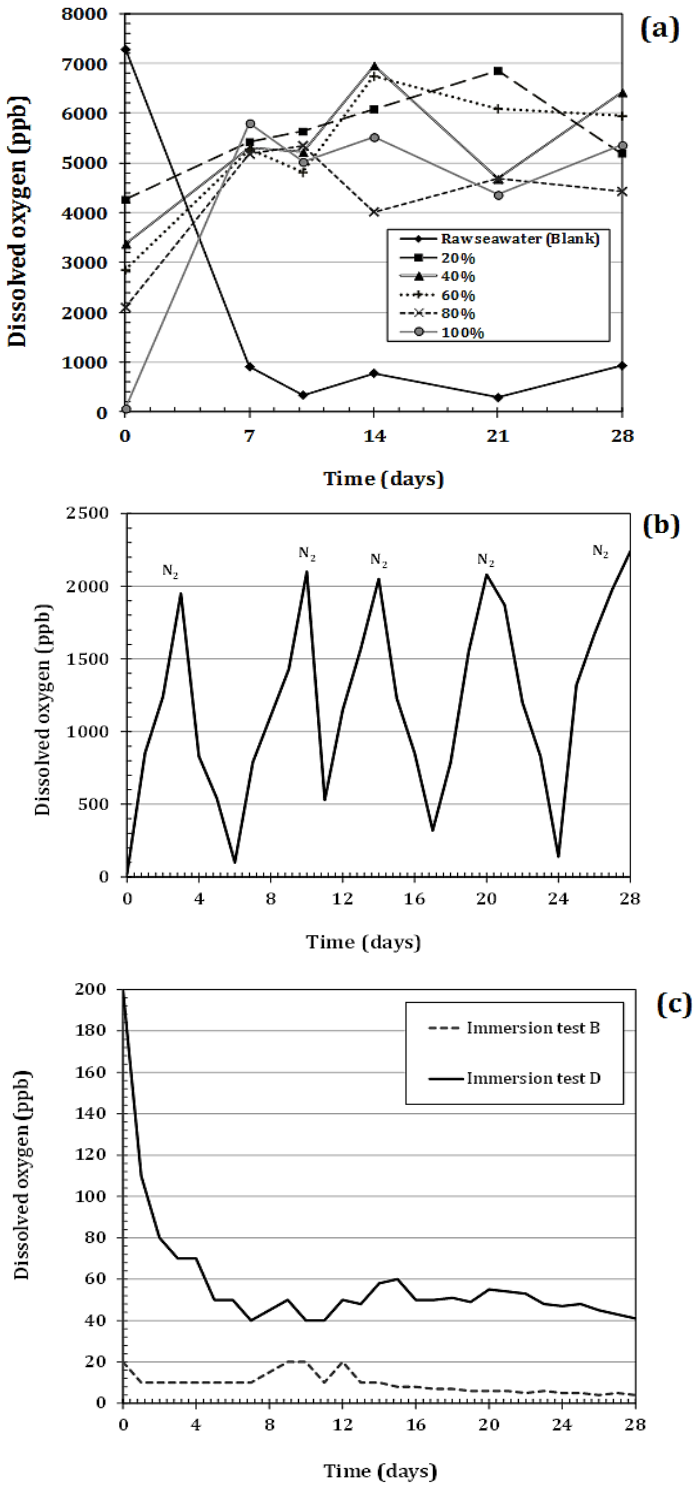

Figure 3. Dissolved oxygen (DO) measurements as a function of time for (a) immersion test A; (b) immersion test C. N2 indicates when a nitrogen blanket was set on top of cells to avoid oxygen ingress and to control the desired levels of DO and (c) immersion test B and D. A nitrogen blanket was maintained throughout exposure to avoid oxygen ingress.

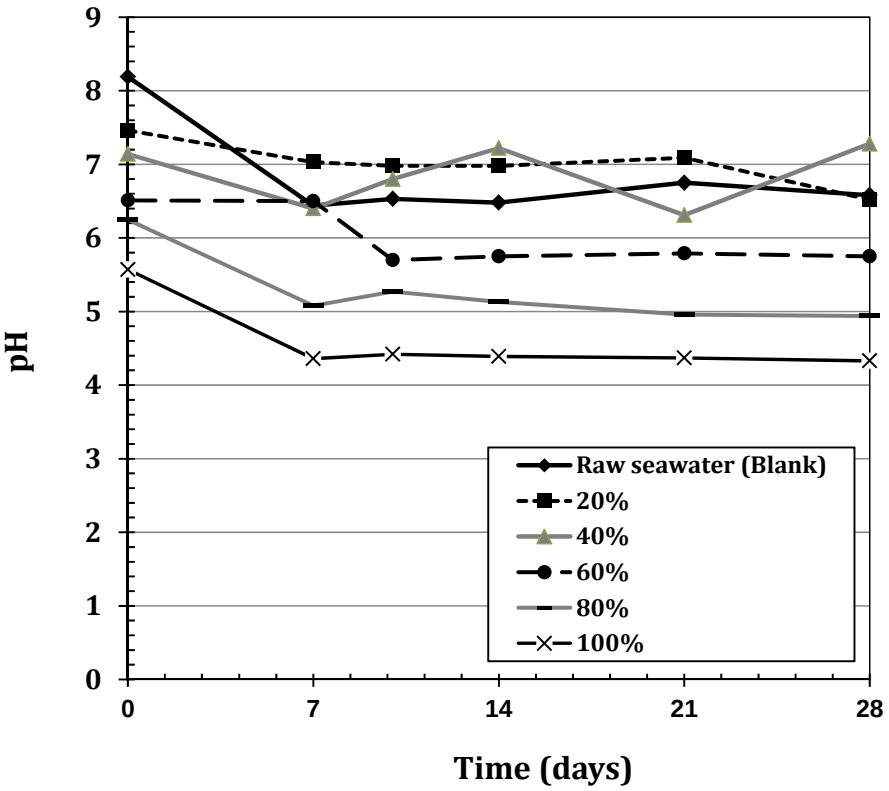

Figure 4. pH measurements of different concentrations of treated seawater throughout exposure.

3.2.2 Surface analysis of microbial adhesion by DAPI fluorescent dye

4'6-diamidino-2-phenylindole (DAPI) fluorescent dye was used to stain bacteria adhered to 316L at the different exposure times. The aim of this analysis was to study the biocidal efficiency against microbial adhesion of the various mixtures of treated seawater and the different exposure conditions. Killing the adhered microorganisms does not necessarily mean their removal from the surface. However, this method can be used to evaluate the first steps of bacterial adhesion therefore providing information on the early killing efficiency of a biocide. Figures 5 and 6 show images of DAPI stained 316L observed under an epifluorescence microscope. Images revealed that microorganisms attached to 316L exposed to the different mixtures of seawater in immersion test A regardless of the exposure time

(Fig. 5). Bacterial cells on 316L were typical rod-shaped bacterial cells mostly forming patches of colonies on the surface. The formation of typical biofilms [25] (cells embedded in self-produced extrapolymeric substance EPS) was not evident. It was noted that bacterial cells on 316L exposed to raw seawater differed in size and fluorescence to cells attached to coupons exposed to treated seawater which can be related to some inhibition effects by residual chemical treatments. Microbial adhesion slightly increased with exposure time for all the different levels of treated seawater in immersion test A. For immersion tests B, DAPI stained 316L coupons revealed microorganisms attached to the surface on 7 and 14 days exposure coupons but microorganisms were not observed on the 28 days coupons. For immersion test B-28 day, whole microorganisms were not observed on DAPI stained coupons but instead, DNA remnants such as microbial debris were observed on the surface. This may indicate microorganisms were affected and disintegrated by the chemical treatment with exposure time (Fig. 6). For immersion tests C and D, microorganisms were not observed on the surface regardless of the exposure time.

3.2.3 Enumeration of sessile SRB by standard serial dilution technique

Enumeration of viable sessile SRB attached to 316L exposed to the various levels of seawater immersion test A, B, C and D for 28 days was conducted using SRB culture enumeration medium and the standard serial dilution method. Results are summarized in Table 3. For immersion test A, viable and cultivable sessile SRB were detected on 316L exposed to raw seawater and 20 to 80% treated seawater but were not detected on 316L exposed to 100% treated seawater. Viable SRB were not detected on coupons from immersion test B, C and D.

The SRB counts on 316L exposed to raw seawater (control) were 10^4 cell/mL. This indicates the SRB numbers inoculated at the beginning of the test (10^8 cell/mL) decreased with time.

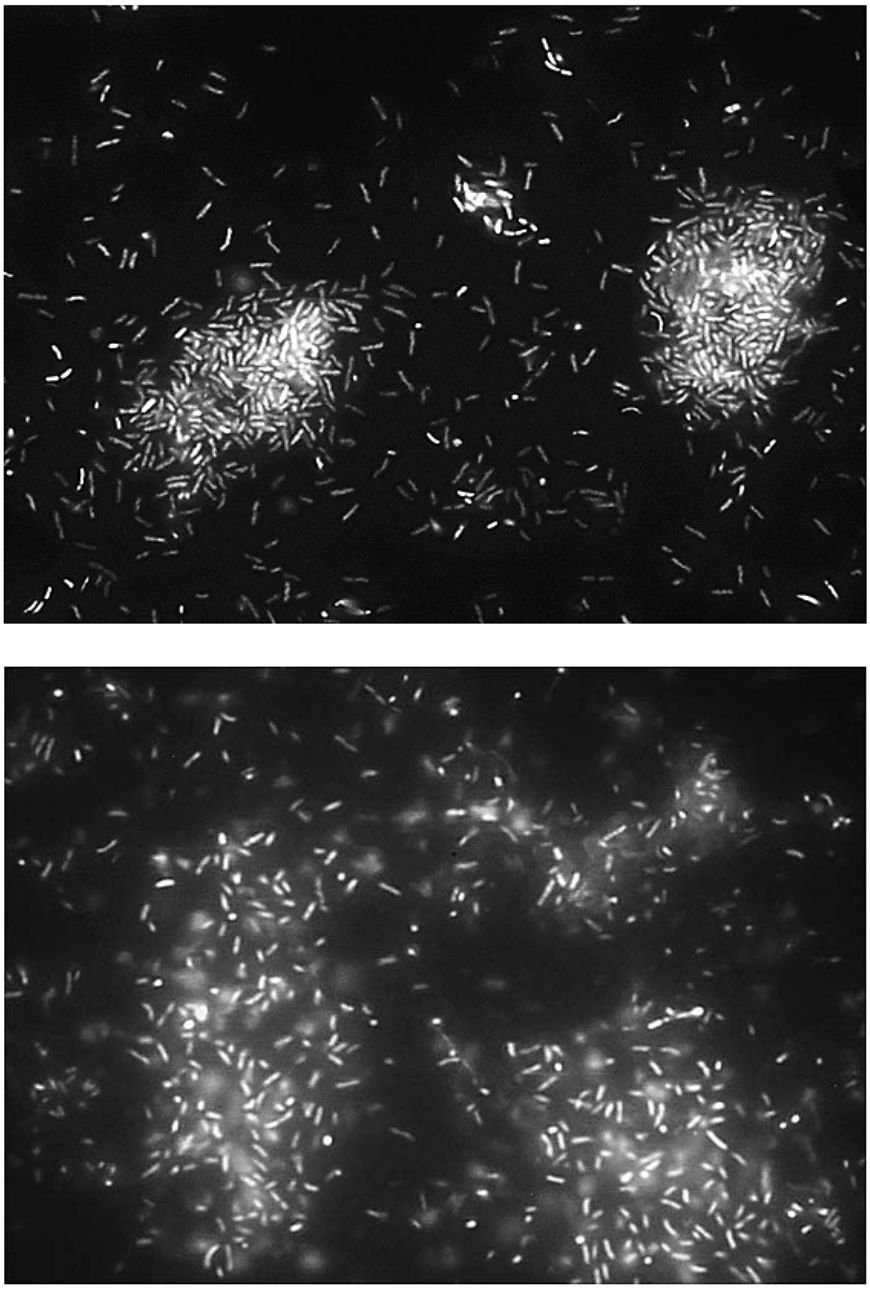

Figure 5. Images of DAPI stained 316L coupons under an epifluorescence microscope for immersion test A. Images show bacterial attached to coupons exposed to (a) raw seawater and (b) 100% treated seawater, for up to 7 days. Bacteria were found attached to coupons from 7 to 28 days exposure regardless of the concentration of the chemical treatment.

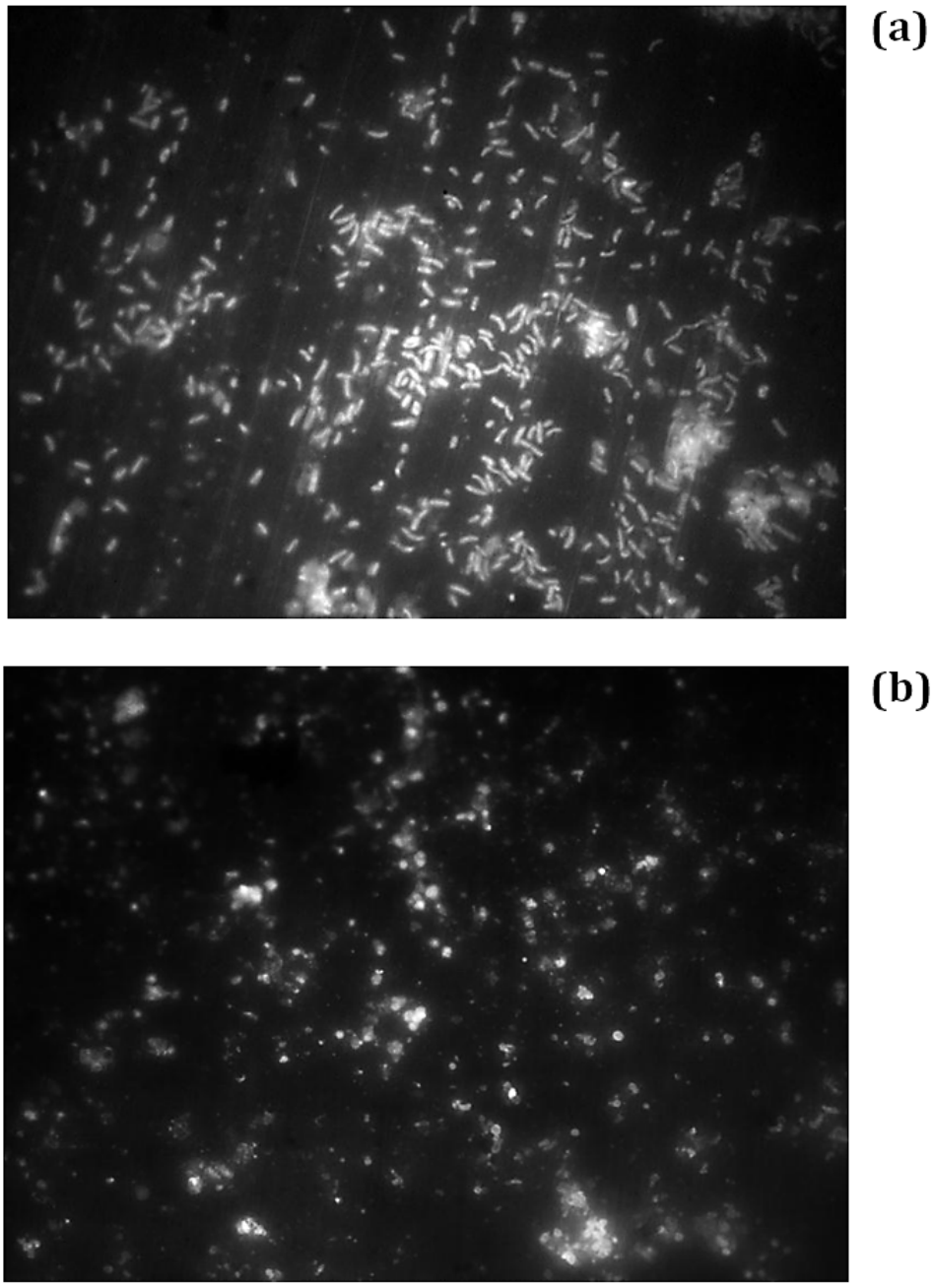

Figure 6. Images of DAPI stained 316L coupons under an epifluorescence microscope for immersion test B. (a) bacteria attached to 316L exposed to 100% treated seawater for up to 7 days. (b) debris and DNA remains on 316L exposed to 100% treated seawater for 28 days.

In seawater, sulphate is reduced to sulphide by the sulphate reducing bacteria (SRB) and this process is coupled to the oxidation of an electron donor. In this study, the SRB populations used to inoculate the various seawater mixtures were growing on lactate as carbon source and electron donor for sulphate reduction. As the test solutions were maintained under stagnant conditions, microorganisms could have consumed a significant proportion of nutrients during the first days of exposure so that nutrients became depleted with exposure time. Primary inhibition of SRB growth could be due to exhaustion of organic and inorganic substrates in the seawater with time. Inhibition could have also resulted from the toxicity of accumulated sulphide in the solution. Sulphide inhibition towards different trophic groups is widely reported in literature [26, 27]. Oxygen and pH of test solutions also could have limited SRB growth. SRB are recognized as anaerobic microorganisms so the presence of oxygen in the system could also have restricted their growth in the different seawater mixtures. However, it has been reported that SRB possess various self-protection enzymes that facilitate survival during periods of oxygen exposure [28]. This is supported by the fact that oxygen levels in the raw seawater remained very low compared to the mixtures of seawater treatments. Since seawater pH was lowered by additions of chemical treatments, pH could have played a major role on the inhibition of SRB growth even if the biocide efficiency was affected by oxygen in the system. For 100% treated seawater pH was lowered to values below 5 which are expected to be very aggressive for SRB given that SRB were growing in culture medium at pH 7.0 before mixing with chemical treatments.

It is important to underline that this enumeration technique is restricted to cultivable microorganisms and usually underestimates the real bacterial numbers in the system [29]. The application of molecular tools has shown that bacteria growing in culture media often represent a minor part of the microbial community: around 99% of microorganisms existing in nature are unable to be cultured by selective enrichment cultures, and they will therefore, be excluded when

enumerated with growth media [30]. This can explain why low numbers of sessile SRB were detected by the serial dilution method whereas abundant bacterial cells were observed on DAPI stained coupons.

Table 3

Enumeration of sulphate reducing bacteria (SRB) attached to 316L after exposure to the various levels of seawater at the four immersion tests for 28 days. SRB enumeration was conducted by serial dilution method.

Immersion test	**Exposure solution** Treated seawater/raw untreated seawater	**SRB count (cell/mL)**
A	100/0	None
	80/20	10_3
	60/40	<10
	40/60	<10
	20/80	<10
	0/100	10^4
B	100/0	None
C	100/0	None
D	80/20	None

3.3. Surface analysis by 3D optical microscopy

3D optical surface imaging and pit profile measurements were conducted to confirm pitting corrosion and to measure pit depths. Pitting corrosion was confirmed on 316L after CPP tests at the different levels of treated seawater. Figure

7 shows pits observed on 316L in 100% treated seawater after completion of the CPP test. Pits depths were very similar for 316L regardless of the concentration of seawater treatment. Table 4 summarizes the surface analysis of 316L exposed to the different levels of treated seawater at the different immersion conditions. In immersion test A, pitting was observed on coupons exposed to raw untreated seawater as well as to all levels of treated seawater from the 7th day to the 28th day of exposure with only few exceptions. Figures 8 shows an optical image and pit profile measurement of a pit on 316L exposed to raw seawater in immersion test A. Overall, pit density tended to decrease with exposure time for 316L exposed to all seawater levels. In most cases, pit density did not exceed 1 pits/cm2.

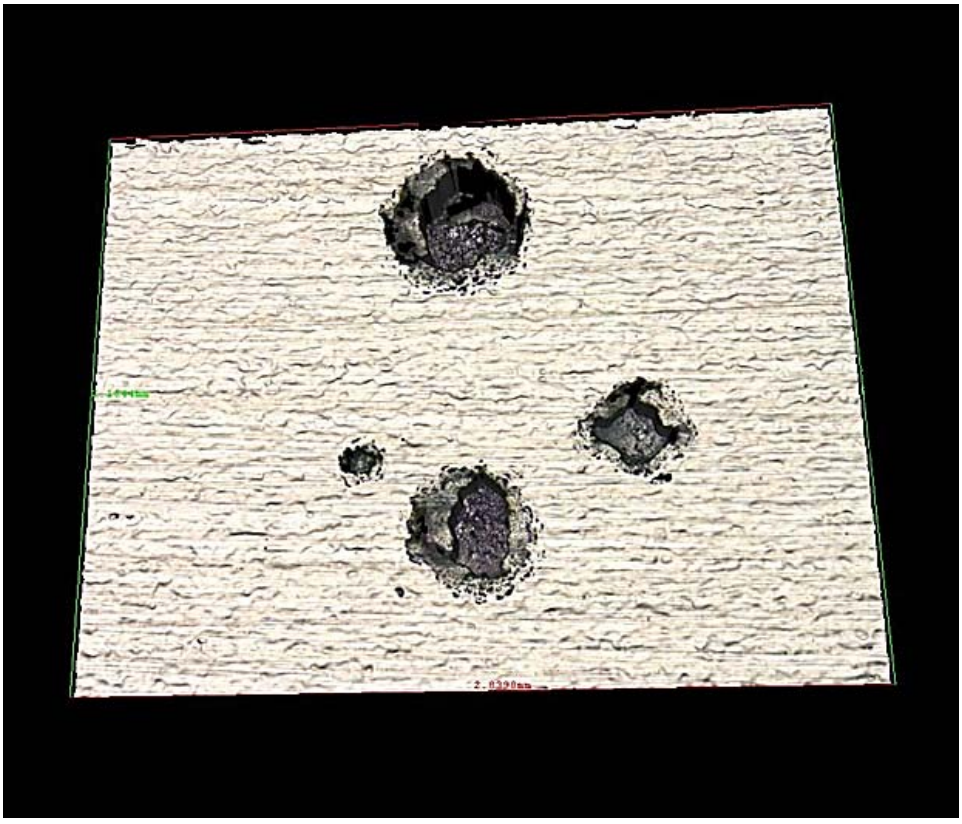

Figure 7. 3D optical surface image of typical pits found on 316L after cyclic polarization tests.

Table 4

Summary of surface analysis results - Immersion Tests

Immersion test	Exposure time (days)	Seawater treatment (concentration)	Pit Density (Pits/cm2)	Average Pit Depth (µm)	Maximum pit depth (µm)
A	7	Raw (Control)	2.98	11.61	21.39
		20%	0.85	6.78	14.73
		40%	0.67	4.42	5.45
		60%	0.85	6.71	11.65
		80%	0.85	4.86	6.15
		100%	2.54	7.81	17.46
	10	Raw (Control)	1.35	6.81	12.64
		20%	0.35	8.16	12.36
		40%	0.34	4.40	5.23
		60%	0.33	7.39	9.43
		80%	0.17	17.62	17.62
		100%	1.74	8.16	16.04
	14	Raw (Control)	0.53	3.21	3.56
		20%	0.68	6.16	14.84
		40%	0.17	4.34	4.34
		60%	0.00	0.00	0.00
		80%	0.68	4.35	5.41
		100%	0.52	5.68	7.09
	21	Raw (Control)	0.00	0.00	0.00
		20%	0.52	14.31	21.45
		40%	0.17	8.43	8.43
		60%	0.17	12.50	12.50
		80%	0.34	4.91	6.97
		100%	0.17	13.98	13.98
	28	Raw (Control)	1.04	6.00	8.36
		20%	0.17	14.96	14.96
		40%	0.17	4.56	4.56
		60%	0.85	7.61	13.29
		80%	1.02	7.26	9.89
		100%	1.20	6.34	9.22
B	7	100%	0.00	0.00	0.00
	14		0.00	0.00	0.00
	28		0.66	3.02	4.80
C	7	100%	0.33	4.23	6.33
	14		0.24	4.64	6.55
	28		0.24	2.86	2.98
D	7	80%	0.00	0.00	0.00
	14		0.00	0.00	0.00
	28		0.00	0.00	0.00

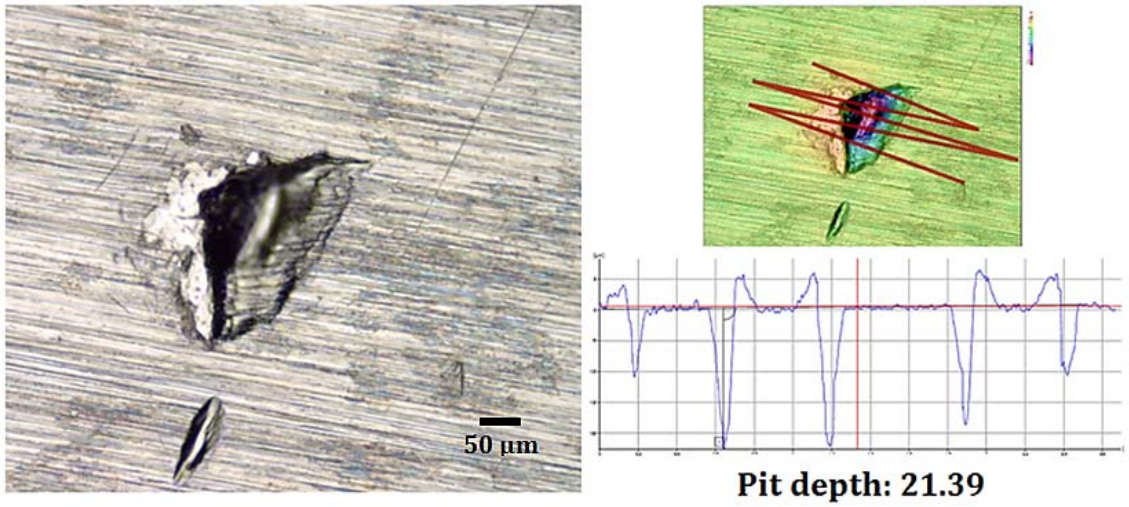

Figure 8. Optical surface image (left) and pit depth measurement (right) of a typical pit found on 316L exposed to raw seawater at 20°C for 7 days in immersion test A.

For immersion test A, pit depths ranged from 3-22 µm, maximum pit density was 2.98 pits/cm2 and maximum pit depth found was ~21 µm. Most of the pits on 316L exposed to immersion test A had pit depths in the range from 0-10 µm. The frequency of the deepest pits decreased with time and was the highest at 7 days of exposure. This finding could indicate that exposure time tended to favour repassivation rather than propagation processes. This is also supported by the fact that the deepest pits observed were wide and shallow, with pit widths typically more than 10 times pit depths. Overall, pitting was randomly observed on 316L exposed to the various levels of seawater for immersion test A and it is not possible to establish any association between seawater treatment concentration and pit depth/density.

For immersion test B, pitting did not take place during the first 14 days of exposure but a few shallow pits were evident on the 28 days exposure coupons. Since for this test, high numbers of SRB were inoculated, these results could indicate that the

biocide activity was not sufficient to supress all microorganisms in the seawater. It is likely that the remaining bacteria could have induced some localized events on the steel surface with time. For immersion test C, pits were observed on the 7, 14 and 28 days exposure coupons. Again, surface analysis of pits indicated that pit widths were about ten times the pit depths. These wide shallow pits are more likely to undergo repassivation rather than propagation. In addition, the overall decrease in pit density and pit depths in all immersion tests with time may indicate that some repassivation processes could have taken place. Pitting was not detected on immersion test D regardless of the exposure time.

Results from immersion tests and DO measurements indicated that DO play a critical role in triggering the initiation of pitting on 316L. DO measurements, surface and microbiological analyses indicated that in the presence of oxygen, chemical treatments become ineffective hence facilitating pitting initiation and microbial growth on steel surfaces. As mentioned earlier, the numbers of SRB in the raw natural seawater used for immersion tests as estimated by the standard serial method was 10^3 cell/mL. Results indicate that, in the absence of oxygen, THPS was efficient in killing SRB in the seawater with ingress of 20% untreated raw seawater. Results also showed that THPS at the recommended dosage for hydrotesting water (100% treatment) is efficient against SRB even at SRB concentrations as high as 10^8 cell/mL.

The use of preservation treatments for hydrostatic test waters is a crucial practice to provide protection of subsea pipelines against corrosion. In the event of seawater ingress into 316L lined pipes during tie-in operations, there is a high risk of localized corrosion particularly from the oxygen and microbial life coming into the system and mixing with the chemical treatments thus increasing their concentration in the treated seawater.

The ingress of oxygen into the system not only can accelerate pitting initiation by creating oxygen concentration cells but it can also interfere in the efficiency of

chemical treatments hence restraining oxygen scavenging and biocidal activity in the system. The entry of seawater into the system can also introduce aggressive species that could affect the efficiency of any residual treatment such as deposits and nutrients that may support bacterial growth and biofilm formation on the steel surface hence increasing the likelihood of MIC. Results from this study indicate that 316L is susceptible to localized corrosion in seawater at 20°C if oxygen is not effectively removed from the seawater. If oxygen is restricted to minimal levels in the system, 316L is protected against pitting even if the recommended dosages of chemical treatments are mixed with raw seawater in the proportion treated/raw seawater 80:20. However, this result must be carefully interpreted as pitting could be easily initiated under these conditions if the physicochemical and biological conditions in the system are slightly changed during exposure, e.g. ingress of high loading of bacteria and nutrient sources for biofilm growth, increase in exposure temperatures and oxygen ingress into the system, among others.

In the event of long exposure of lined pipes to raw seawater during tie-in operations, the removal of oxygen is crucial to ensure appropriate protection and subsequent preservation of the flowline against pitting. Particular consideration should be given to the potential ingress of deposits or sand into the system which could increase the likelihood of MIC. Filtration is essential to remove such particulates.

4. Conclusions

1. Cyclic potentiodynamic tests indicated that in the absence of oxygen, an anodic driving potential is required to trigger pitting corrosion on crevice-free 316L in seawater at 20°C regardless of the concentration of the chemical treatments.

2. Immersion tests indicated that, in the presence of high concentrations of oxygen, the efficiency of the THPS, regardless of the concentration, is considerably reduced. This was demonstrated by the large bacterial colonization on the steel surface observed at all the levels of chemical treatments. Under these conditions, oxygen scavenging no longer takes place, most likely because of inactivation by THPS, bacteria growth is favoured and pitting can easily initiate after as little as 7 days of exposure.

3. Immersion tests showed that if oxygen is restricted to levels below 20 ppb in the system, 316L is protected against pitting at the recommended dosages of chemical treatment for up to 14 days exposure even at SRB concentrations as high as 108 cell/mL. At concentrations of DO below 60 ppb, which were achieved by mixtures of 80% treated seawater:20% raw seawater, and provided the ingress of oxygen is restricted during exposure, 316L is also protected against pitting corrosion for up to 28 days. A concentration of 2 ppm of dissolved oxygen was sufficient to induce pitting corrosion on 316L from the first 7 days of exposure even at the recommended dosage of chemical treatments.

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Appendices

Appendix 1

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Chapter 2

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Systematic study of the corrosion properties of selected high-resistance alloys in natural seawater

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ABSTRACT

Electrochemical measurements were conducted to evaluate localized corrosion on UNS S31603, UNS S31803, UNS S32750, UNS S31254 and UNS N08825 in natural seawater. Critical pitting and crevice temperatures were assessed using a potentiostatic technique and critical potentials for pitting and crevice corrosion initiation and repassivation were identified using potentiodynamic polarization at temperatures from 5 to 40 °C. Passivity breakdown always occurred through pitting and crevice growth above a transition temperature. Below this temperature, pitting corrosion was not observed on any of the alloys regardless of the applied potential, but initiation of crevice corrosion occurred after the alloys reached a transpassive potential.

1. Introduction

Material selection is a pivotal task to identify which type of alloys will have the appropriate corrosion resistance required for application in any given environment when corrosion resistance is the major requirement. During the past decades several alloys have been developed to suit particular environments but often their high costs are a limiting factor when considering their actual application, especially for larger structures. High resistance alloys have been used for a wide range of applications in marine environments due to a number of factors including strength and corrosion resistance. The corrosion resistance of these alloys is attributed to their high alloying content of Mo, N and Cr [1]. When exposed to oxygen, these elements allow the formation of a passive film on the alloys surface which protects it from aggressive chemical species such as chlorides in marine environments. Nonetheless, under certain circumstances this protective passive film can break down locally and localized corrosion is likely to take place.

Crevice and pitting are the most typical forms of localized corrosion in high resistance alloys in seawater. The resistance of a particular steel grade to pitting and crevice corrosion has been described by a Pitting Resistance Equivalent number, or PREN. The PREN has been widely used to rank high-resistance alloys in their resistance to localized corrosion. It can be calculated from the alloying composition as PREN = %Cr + 3.3%Mo + 16%N for stainless steels and PREN = %Cr + 1.5 (%Mo + %W + %Nb) for nickel based alloys [2]. Clearly, grades with high content of the alloying elements chromium, molybdenum and nitrogen form more stable protective films and are more resistant to corrosion. Corrosion resistance has also shown to be influenced by the microstructure of a metal [3] and the physical conditions of the surface [4].

Both crevice and pitting corrosion are influenced by environmental factors such as halide concentration, pH, chemical species, potential and temperature [5]. However, contrary to crevice corrosion, pitting can initiate at different surface areas where the corrosive medium has free access. In contrast, crevice corrosion preferentially occurs at occluded areas where the access of the environment is restricted [6]. Crevice corrosion is sometimes viewed as a more aggressive form of corrosion as it will occur at less severe conditions than does pitting [7] and it does not necessarily require the presence of an aggressive ion such as chloride [5].

The resistance against pitting or crevice corrosion has been expressed via the critical potential and the critical temperature required for pitting or crevice corrosion initiation and repassivation in chloride-containing solutions. This is because several alloys have shown to have both a temperature and a potential dependence on the onset of localized corrosion with these two parameters being related to each other [8].

Critical potentials and temperatures related to localized corrosion can be determined under laboratory conditions to predict the performance of the alloys in an actual service and can be used as criteria for ranking the corrosion resistance of alloys in seawater. Several different methods have been used to determine temperature and potential limits for alloys towards their application in different seawaters. These approaches include a variety of

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immersion tests as well as accelerated electrochemical methods in seawater simulating solutions such as sodium chloride and ferric chloride solutions as well as artificial seawaters [9,10]. However, these substitute ocean water solutions do not contain the complex chemical and biological species found in natural seawaters and accordingly natural seawater should be the preferred environment for laboratory testing. Although paramount contributions to the knowledge of marine corrosion have derived from studies using synthetic seawater solutions, the lack of information in the available corrosion literature regarding the performance of alloys in natural seawaters may lead to inaccuracies in the integrated analysis of the localized corrosion resistance of the alloys in marine environments. Furthermore, a comparative analysis of both the pitting and crevice corrosion resistance of different steel grades in natural seawater requires a consistent test methodology across all tests.

This paper is a comparative study of the localized corrosion resistance of bare and artificially creviced alloys of different grades in natural seawater from Western Australia. A unified analysis of both pitting and crevice corrosion of high resistance alloys in natural seawater appears not to have been conducted previously. This study aimed to determine critical potentials for pitting and crevice corrosion initiation and repassivation using potentiodynamic polarization tests at temperatures from 5 to 40 °C which are typical exposure temperatures for offshore assets. In addition, critical pitting temperatures (CPT) and critical crevice temperatures (CCT) of selected alloys in natural seawater were evaluated using a potentiostatic technique. Alloys UNS S31603, UNS S31803, UNS S32750, UNS S31254 and UNS N08825 were selected for this study since they represent a wide range of categories and grades with a distinct set of general properties, including corrosion resistance, chemical composition and microstructure. The alloy characteristics were then correlated to the resistance of the alloys to both crevice and pitting corrosion in natural seawater in order to examine the possible corrosion mechanisms that operate at different temperatures, which may help establish essential guidelines for design criteria, risk assessment and asset integrity management based on materials selection for seawater applications.

2. Experimental procedure

2.1. Specimen preparation

Square specimens were cut from the supplied plate samples and had a thickness of ~5–7 mm. The chemical composition of alloys in weight percent as well as their calculated PREN value is presented in Table 1. For pitting and crevice corrosion evaluation, working electrodes had a surface area of 5 cm^2 and 40–50 cm^2 respectively. For crevice corrosion evaluation, crevice assemblies were prepared based on the design of the CrevCorr Round Robin test [11] and a brief description is provided here. Fig. 1 shows the crevice former mounted on a steel specimen and describes all parts of the crevice assembly. For crevice testing, specimens were drilled in the centre with a 7 mm diameter hole. The crevice formers were made of PVDF (polyvinylidene difluoride). The outside diameter was

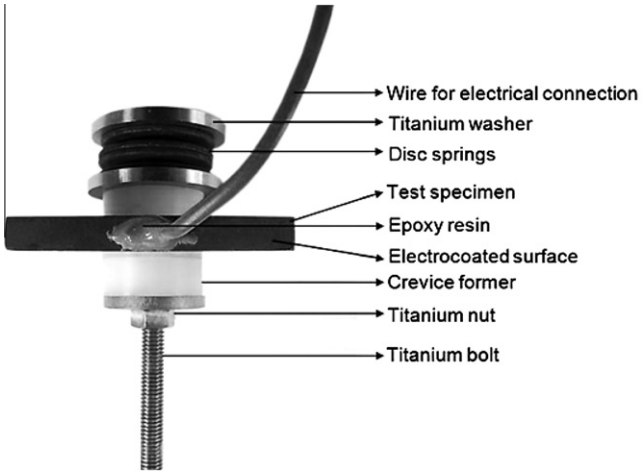

Fig. 1. Crevice assembly used to evaluate crevice corrosion of alloys. (Design based on the CrevCorr Round Robin test.)

20 mm and the inside diameter was 7 mm and height was 15 mm. Before assembling, the surface of the crevice formers was wet ground up to 1200 grit finish, cleaned with acetone and dried. The nut, threaded bolt and washers in the crevice assembly were made of titanium. Around the bolt a PVC (polyvinylchloride) hose was mounted to prevent electrical contact between specimen and the assembly. Four disc springs (nylon coated steel) were used to maintain the constant load corresponding to the applied torque during the experiments. A torque of 3 N.m was applied. This arrangement for crevice evaluation and the torque applied has proved to form an ideal uniform crevice at the surface that yields for reproducible results [11].

For preventing crevice corrosion in areas other than the crevice formers, specimens were first electrocoated using a protective epoxy (Powercron® 6000CX, PPG Industrial coatings) at the surface area where the spot weld was made for electrical connection. Any exposed area after electrocoating was further covered by epoxy resin (Belzona 1391, Belzona polymerics Ltd.). Prior to assembling, alloy specimens were wet ground up to 600 grit finish (SIC grinding paper), degreased with acetone and dried with nitrogen gas.

For pitting experiments, bare square coupons were wet ground up to 600 grit finish (SIC grinding paper), degreased with acetone and dried with nitrogen gas. For pitting testing, specimens were held in place through a metal thumbscrew against a sample holder underneath the electrochemical cell. The thumbscrew also worked as electrical contact for electrochemical measurements so that welding to an electrical wire was not required.

2.2. Test conditions

The electrolyte solution used to carry out the electrochemical measurements was natural seawater collected from 20 m depth in the Indian Ocean off Rottnest Island (Western Australia) using a rosette sampler. The chemical composition of the seawater is

Table 1
Test materials and alloying composition.

Alloy	Type	C (wt.%)	Mn (wt.%)	Fe (wt.%)	Cr (wt.%)	Ni (wt.%)	Mo (wt.%)	N (wt.%)	S (wt.%)	PREN[a]
UNS S31603	Austenitic	0.022	1.76	bal	17.4	10	2.03	0.05	0.001	24.83
UNS S31803	Duplex	0.015	1.53	bal	22.35	5.72	3.16	0.18	0.001	35.65
UNS S32750	Super duplex	0.019	0.82	bal	24.74	6.61	3.73	0.26	0.0003	41.23
UNS S31254	Super austenitic	0.01	–	bal	20.18	18.15	6.10	0.20	0.010	43.51
UNS N08825	Nickel base alloy	0.05	0.85	22	22.50	bal	3	–	0.03	27

[a] **PREN = %**Cr + 3.3% Mo + 16% N (stainless steels)%Cr + 1.5 (% Mo + % W + % Nb) (nickel base alloys).

Table 2
Analysis of the natural seawater used as electrolyte in the experiments.

Analysis	Composition
Salinity [PSU]	35.58
DO [mg/L]	5.06
Conductivity [mS/cm]	48.79
pH	8.2
Chloride [mg/L]	18500
Magnesium [mg/L]	1340
Sodium [mg/L]	11100
Sulphate [mg/L]	2700

given in Table 2. An agitation rate of 150 rpm was sustained using rotating Teflon stirrers. All experiments were carried out using a conventional three electrode cell assembly [12]. A double junction Ag/AgCl electrode with a commercial chloride based (approximately 0.67 M) filling solution designed to give a potential of 0.244 V vs S.H.E. was used as reference electrode. The potential was verified against a saturated calomel electrode. Platinum mesh was used as counter electrode. The Gamry Intruments Flexcel™, a cell based on the Avesta cell design [13], in conjunction with a Gamry potentiostat (Gamry Instruments, Inc.) was used for pitting corrosion evaluation. The cell has the advantage of preventing crevice corrosion at the seal between specimen and its holder. For crevice experiments, a modified version of this pitting cell was used. The sample area in the cell was sealed and a machined Teflon disc was placed inside the cell to hold the crevice assembly. Test temperatures were obtained using a circulating water bath connected to a coated coil inserted into the cell and a heating jacket. Temperature was measured by a temperature probe placed adjacent to the working electrode. All tests were carried out in triplicate separately.

2.3. Electrochemical measurements

2.3.1. Breakdown potential (E_b) and repassivation potential (E_r) measurements by cyclic potentiodynamic polarization scans

Cyclic polarization scans were conducted at five temperatures (5, 10, 20, 30 and 40 °C) to identify the breakdown potential (E_b) and the repassivation potential (E_r) for pitting and crevice corrosion of alloys in seawater at each temperature. Before conducting the scans, samples were allowed to stabilize in the seawater at open-circuit for 1 h. The scans were carried out using a forward and reverse scan rate of 0.167 mV/s. The sweep direction was reversed when either a current density of 1.5 mA/cm^2 or a potential of 1.5 V vs. Ag/AgCl was reached. The initial and final point of the scan was set at a potential of −0.1 V vs. E_{corr}. The breakdown potential, E_b, was identified as the potential where the anodic current indicated the onset of transpassivity or stable pitting or crevice (current increase at the passive region). The repassivation potential, E_r, was identified as the potential for which the forward and reverse scans intersect, which is where repassivation of pits is considered to take place. Polarization scans were carried out for each alloy at each temperature starting from the highest temperature value (40 °C) and continuing with the next lower. When triplicates showed passivity breakdown by transpassive dissolution instead of pitting or crevice at certain temperature and when surface inspection confirmed this result, the same experiment was not carried out at lower temperatures as those would not be expected to be detrimental for the material at the same tested conditions [14].

2.3.2. Determination of critical pitting (CPT) and crevice (CCT) temperatures of alloys is seawater

Potentiostatic determination of CPT and CCT was conducted using the Flexcel Critical Pitting test cell Kit (Gamry Instruments, Inc) following the standard recommendations [15]. This procedure involved polarization of the working electrode to a potential more noble than the pitting potential [8]. An Initial temperature of 1 °C was set at which the sample was allowed to stabilize at open-circuit for 1 h. An anodic potential of +700 mV was then applied and held for the duration of the test while the solution temperature was increased at a defined scan rate until stable pitting or crevice initiated. The scan rate was set at 1 °C/min and 0.5 °C/min for CPT and CCT evaluation respectively. Continuous stirring of the test solution was used to minimize the temperature lag between solution and specimen. Current versus temperature curves were recorded at the maintained applied potential. The CPT and CCT were defined as the temperature at which the current density exceeded 100 μA/cm^2 for 60 s indicating the onset of localized corrosion. This value was chosen because it corresponded to a marked deviation from the passive current density of the alloys at the test temperature.

2.4. Surface analysis

At the completion of the tests, specimens were removed from the electrochemical cell and cleaned according to the ASTM standard [16]. Pitting or crevice corrosion was confirmed by surface inspection using optical microscopy (IFM G4g system, Alicona Imaging).

3. Results and discussion

3.1. Breakdown potential (E_b) and repassivation potential (E_r) measurements by cyclic potentiodynamic polarization tests

Crevice and pitting corrosion of high resistance alloys in natural seawater were investigated by conducting potentiodynamic polarization scans to identify the breakdown and repassivation potentials of the alloys at different temperatures. Fig. 2 shows the E_b (Fig. 2(a)) and the E_r (Fig. 2(b)) of bare and artificially creviced alloys immersed in natural seawater at 40 °C. It can be observed that alloys UNS S31803, UNS S32750, UNS S31254 and UNS N08825 exhibited high resistance to pitting corrosion by having high E_b and E_r values, which were related to transpassive dissolution rather than pitting [17]. In addition, UNS S32750 and UNS S31254 displayed excellent resistance to crevice corrosion in seawater at 40 °C. These results were confirmed by microscopic examination of specimens after testing (Fig. 3(a)). The outstanding resistance of UNS S31254 and UNS S32750 to crevice corrosion in natural seawater at temperatures up to 40 °C has been previously reported [11].

UNS S31803 and UNS N08825 proved to be susceptible to crevice corrosion in seawater at 40 °C as indicated by their low breakdown potentials and very active repassivation potentials (Fig. 2). Fig. 3(b) reveals crevice corrosion at the surface of UNS N08825 after testing. The same extent of crevice attack was observed on UNS S31803. Furthermore, it was noted that the localized attack in artificially creviced alloys was concentrated at the outer circumference of the crevice area and progressed outward towards the metal exposed to solution.

UNS S31603 showed very poor resistance to both pitting and crevice corrosion in seawater at 40 °C (Fig. 2). Since UNS S31603 was the only material that exhibited pitting corrosion in seawater at 40 °C, further studies were conducted to evaluate its resistance to pitting in seawater at temperatures below 40 °C. Breakdown (E_b) and repassivation (E_r) potential versus temperature values for crevice-free UNS S31603 in natural seawater are illustrated in Fig. 4. It is clear that the increase in the electrolyte temperature decreased the breakdown and repassivation potentials of the alloy

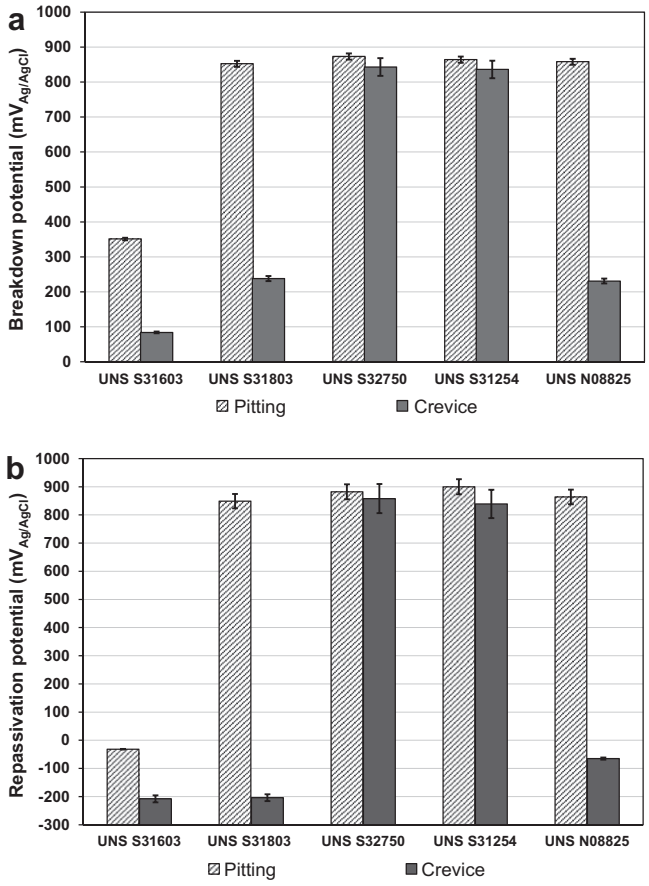

Fig. 2. Pitting and crevice corrosion evaluation of alloys in natural seawater at 40 °C. (a) Breakdown potential (E_b) and (b) repassivation potential (E_r) values obtained from potentiodynamic polarization tests. Errors bars indicate standard deviations from measurements in triplicate.

and hence its resistance to pitting corrosion which is in agreement with other reports [18]. UNS S31603 exhibited pitting corrosion in seawater at temperatures from 10 to 40 °C. However, no pitting but transpassive dissolution was observed when this alloy was tested in seawater at 5 °C, as reflected by its high breakdown and repassivation potentials at this temperature.

Crevice corrosion was further evaluated on alloys UNS S31603, UNS S31803 and UNS N08825 at temperatures below 40 °C as these alloys exhibited crevice corrosion at this temperature. Fig. 5 depicts the breakdown and repassivation potential versus temperature values for artificially creviced UNS S31603, UNS S31803 and UNS N08825 in natural seawater at 5, 10, 20, 30 and 40 °C. Similar to the pitting evaluation, it was noted that the breakdown potential of the creviced alloys decreased with increasing the temperature of the electrolyte (Fig. 5(a)). Moreover, it can be seen that there is a temperature range where the breakdown potential falls abruptly, which most likely indicates the transition temperature from transpassive dissolution to crevice corrosion initiation [19]. For UNS N08825 and UNS S31803 this transition temperature was in the range from 10 to 20 °C and 30 to 40 °C, respectively. For UNS S31603 this temperature was found to be in the range from 5 to 10 °C. Above this transition temperature, E_b decreased more gradually with increasing the seawater temperature for the three alloys. A similar finding was observed for the repassivation potential versus temperature data as illustrated in Fig. 5(b). However, the transition temperature in this case, here named as T_{tr} (repassivation transition temperature) refers to the evolution from a positive repassivation potential after transpassive dissolution in the

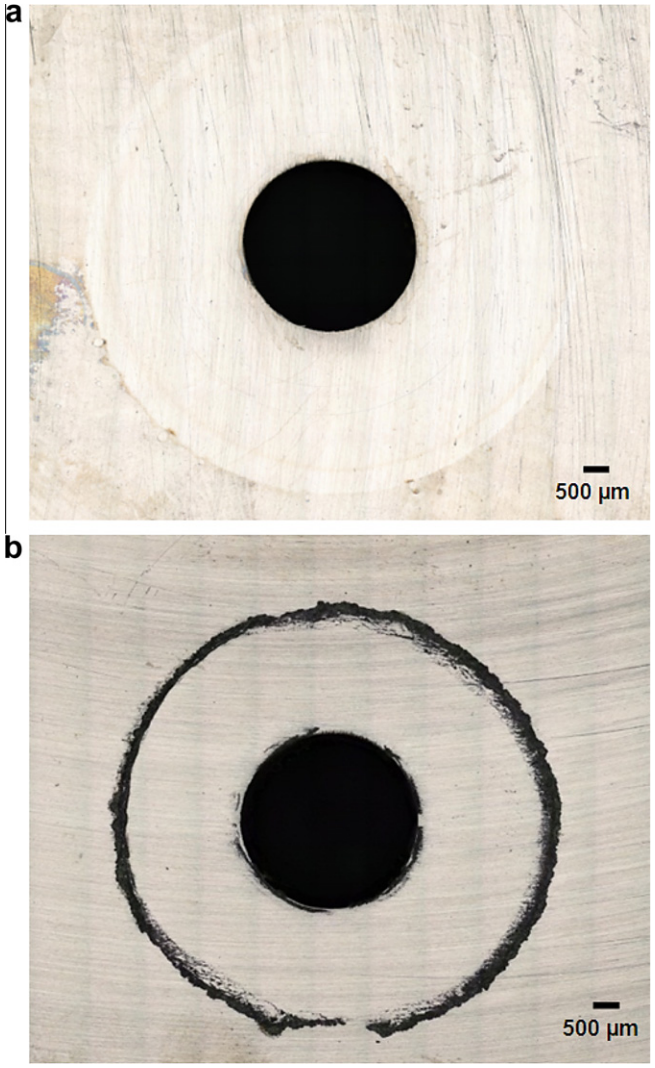

Fig. 3. Optical microscope images of creviced alloys after potentiodynamic polarization test in seawater at 40 °C. (a) UNS S32750 surface showing some etching at the crevice former boundaries but no crevice corrosion was detected; (b) severe crevice corrosion on UNS N8825 surface. Attack was concentrated on the crevice former boundaries.

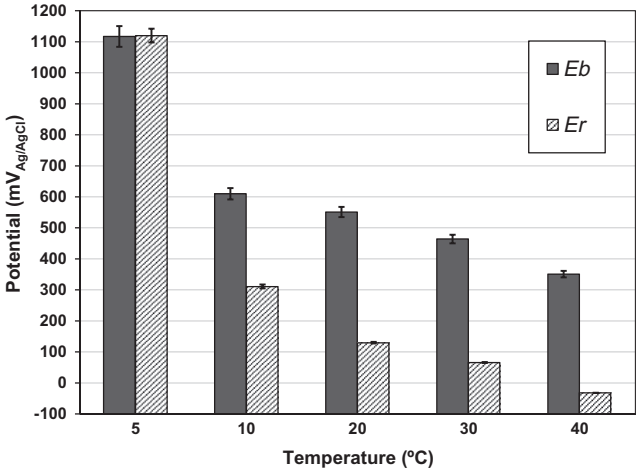

Fig. 4. Average breakdown potential (E_b) and repassivation potential (E_r) values for pitting corrosion of UNS S31603 in natural seawater at temperatures from 5 to 40 °C. Potential values were obtained using potentiodynamic polarization tests. Errors bars indicate standard deviations from measurements in triplicate.

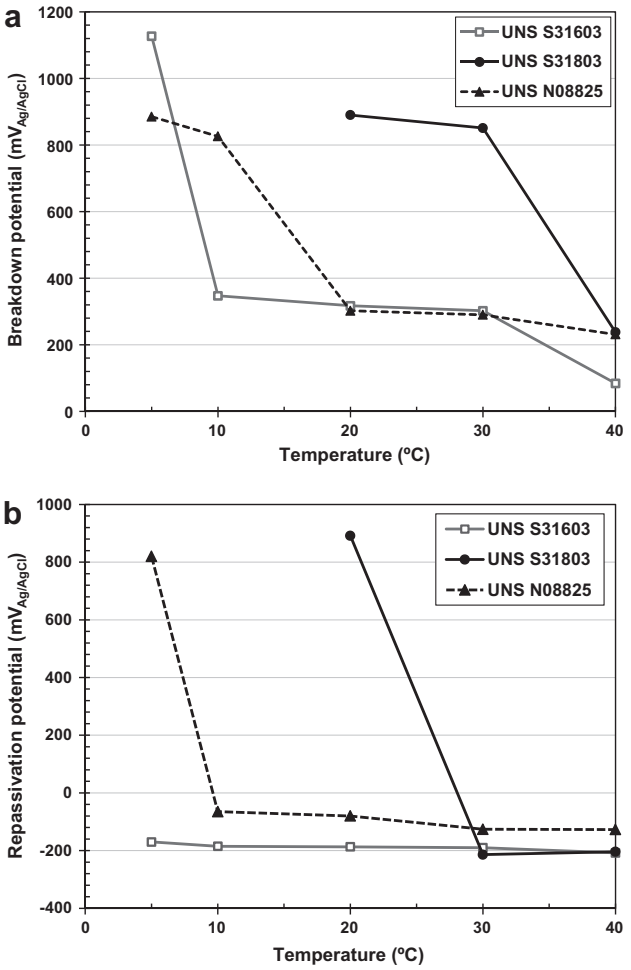

Fig. 5. Crevice corrosion evaluation of alloys in natural seawater as a function of temperature. (a) Breakdown potential versus temperature; (b) repassivation potential versus temperature. Potential values were obtained using potentiodynamic polarization tests.

absence of crevice corrosion to an active repassivation potential after crevice propagation once transpassive dissolution has first taken place. For N08825 and UNS S31803, T_{tr} was reached in the range from 5 to 10 °C and 20 to 30 °C, respectively. This means that for these two alloys the T_{tr} was approximately 10 °C below the transition temperature for crevice initiation. For UNS S31603 the T_{tr} could not be identified since a very negative repassivation potential was observed even at the lowest temperature tested (5 °C). In all cases crevice corrosion was very severe at temperatures above the T_{tr}. Fig. 6(a) shows the cyclic polarization scan of UNS N08825 in seawater at 40 °C, a temperature above the transition temperature for crevice initiation of the alloy. It can be seen that a typical polarization scan for active passive alloys under aggressive conditions [20] is obtained at this temperature. Fig. 6(b) is a 3D optical surface image showing the severe crevice attack on UNS N08825 after potentiodynamic polarization testing. The same results were found for UNS S31803 and UNS S31603 when evaluated at temperatures above the transition temperature for crevice initiation.

It is also worth mentioning that during crevice corrosion evaluation of UNS N08825 and UNS S31803 in seawater at their respective T_{tr} and of UNS S31603 at 5 °C (presumed to be or to be closed to its T_{tr}), these alloys exhibited dissimilar potential versus current transients to those observed at temperatures above their T_{tr}. It was observed that alloys displayed very high breakdown potentials, at the transpassive potential region, which was expected to be due to transpassive dissolution rather than crevice formation. However, during the reverse scan of the polarization curve, and before the intersection between the forward and reverse scan, the current started to increase and then remained constant while the potential decreased, finally reaching a very active repassivation potential (Fig. 7(a)). This resulted in very active metal dissolution (Fig. 7(b)). This pattern of cyclic polarization scan and crevice attack was similar for UNS N08825, UNS S31803 and UNS S31603 when evaluated at their respective T_{tr}. This is why negative repassivation potentials were observed at certain temperatures where high breakdown potentials had been identified for those alloys, as mentioned previously. It is important to note that this observation is the result of crevice corrosion evaluation by potentiodynamic polarization scans using a maximum applied potential of 1.5 V vs. Ag/AgCl which is well above the breakdown potential for transpassive dissolution, and that it is possible that at lower potentials crevice corrosion may not have taken place. The reason for this is unclear. However, in one previous report it was proposed that below the transition temperature for crevice initiation, crevice growth is likely to take place after a gradual acidification of the environment in the crevice which results from the formation of soluble transpassive corrosion product species and once an iron-rich film is formed from secondary passivation in the crevice [19]. It was proposed that a high over-potential (sufficient to cause the first transpassive dissolution and the secondary passivation) is necessary for crevice corrosion stabilization and propagation at electrolyte temperatures below the transition temperature for crevice corrosion initiation. Results of that work were obtained from crevice corrosion testing on 316 stainless steel in 1 M NaCl using potentiodynamic polarization. Those results are quite in agreement with the results from the present study on the evaluation of creviced UNS S31603 and UNS S31803 stainless steels and the nickel-base alloy UNS N08825 in natural seawater. To the best of our knowledge, this is the first published data related to this phenomenon taking place in high resistance alloys in natural seawater. Nonetheless in practical terms, this high overpotential is very unlikely to be reached by the alloy in natural environments. This phenomenon therefore may not be observed under real field conditions and then would not signify a real threat to the stability of high resistance alloys in natural seawater.

It is important to highlight that this phenomenon was not observed during pitting evaluation where at temperatures below the transition temperature for pitting initiation, no pitting was detected on bare crevice-free alloys regardless of the applied potential. However, this observation only applies to the evaluation of UNS S31603 as only this material showed pitting at the temperatures evaluated here. Nonetheless, it has been reported previously that below the CPT stable pits do not occur at any potential up to the onset of transpassivity [14]. It was suggested that although metastable pitting can occur well below the CPT, the transition to stable pitting is only possible once a CPT is reached.

The results outlined here reveal an important similarity in the performance of artificially creviced UNS S31603, UNS S31803 and UNS N08825 in response to the temperature changes in the electrolyte. UNS S31603, UNS S31803 and UNS N08825 which are austenitic stainless steel, duplex stainless steel and austenitic nickel-base alloy, respectively, have different content of alloying elements and different microstructures. Austenitic steels are characterized by having a face-centered cubic structure, high chromium content and always contain nickel. Duplex stainless steels have both ferritic (body-centered cubic) and austenitic (face-centered cubic) phases typically having high chromium content and some amounts of nickel. The use of nitrogen as alloying element is mostly restricted to austenitic and duplex steels. Molybdenum

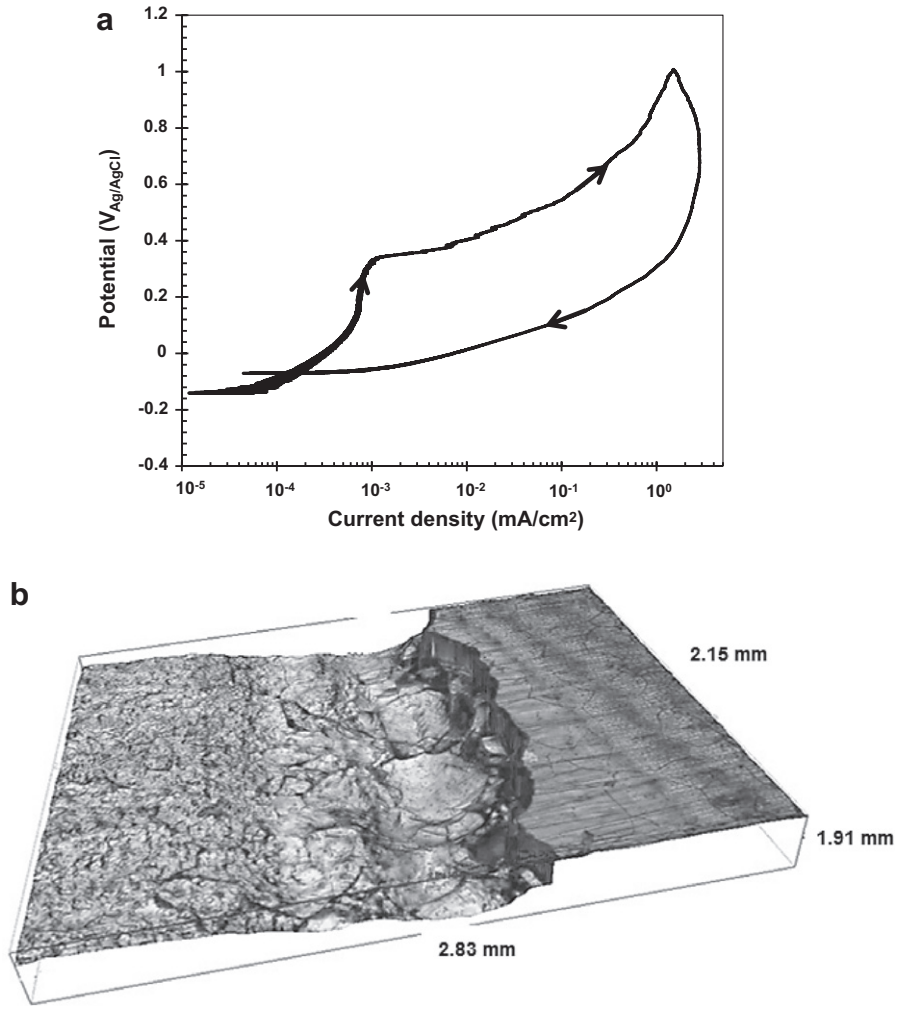

Fig. 6. Crevice corrosion evaluation of UNS N08825 in seawater at 40 °C, a temperature above the transition temperature for crevice initiation for this alloy. (a) Potentiodynamic polarization curve showing that the alloy exhibited localized corrosion; arrows indicate the direction of the scan. (b) Surface image obtained using the three dimensional imaging functionality of the optical Alicona infinite focus microscope exhibiting severe crevice attack at the crevice former boundaries after the potentiodynamic polarization test.

is also typically added to these alloys. The specific effects of the alloying elements on the structure and properties of alloys have been widely investigated [5]. From Table 1 it can be seen that these alloys have similar content of iron, chromium and molybdenum. Nickel is also present as an alloying element in the steels except for UNS N08825 where nickel is the major constituent. From our results, it is interesting to see that regardless of their chemical and microstructural differences, UNS S31603, UNS S31803 and UNS N08825 exhibited similar polarization behavior when evaluated at their critical temperatures for crevice corrosion initiation in seawater. Investigation and characterization of the surface chemistry of these creviced alloys during polarization at their critical temperatures was beyond the scope of this work but since these alloys depend for corrosion resistance upon the formation of a passivating oxide film, our results suggest that the stability of these films during sample polarization in seawater was equally affected by temperature changes in the electrolyte solution. The composition and electronic structure of oxide films formed on nickel alloys and stainless steels have been investigated [21]. It was reported that mixtures of iron, chromium and nickel oxides formed at the surface of 316 stainless steel and two nickel base alloys exhibited electronic properties that were equally altered by applied potential and temperature.

3.2. Potentiostatic determination of the critical pitting (CPT) and crevice (CCT) temperatures of alloys in seawater

The investigation of the CPT and CCT of high resistance alloys in aerated natural seawater was conducted by a potentiostatic method at a potential of 700 mV vs. Ag/AgCl while the temperature was ramped at a gradual scan rate. Fig. 8 shows the average values of critical pitting (CPT) and crevice (CCT) temperatures of the alloys in seawater. It can be seen that UNS S32750 and UNS S31254 had the highest CPT and CCT values and hence showed superior resistance to crevice and pitting corrosion. A lower CPT value for UNS S31254 than the one found in this study has been previously reported [22]. However those studies were conducted using synthetic seawater and longer test exposure times than the one used here.

UNS S31803 and UNS N08825 displayed good resistance to pitting corrosion at temperatures below 50 °C. However, crevice corrosion occurred at temperatures below 50 °C in those alloys under the same test conditions. CCT values of 20 °C lower than the CPT for alloys in an acidified ferric chloride solution have been reported [7]. Our results show that the lowest CPT and CCT values were obtained from UNS S31603 specimens. This result again underlines the poor resistance of this alloy to localized corrosion in seawater. Considerably lower values of CPT and CCT for UNS S31603 than

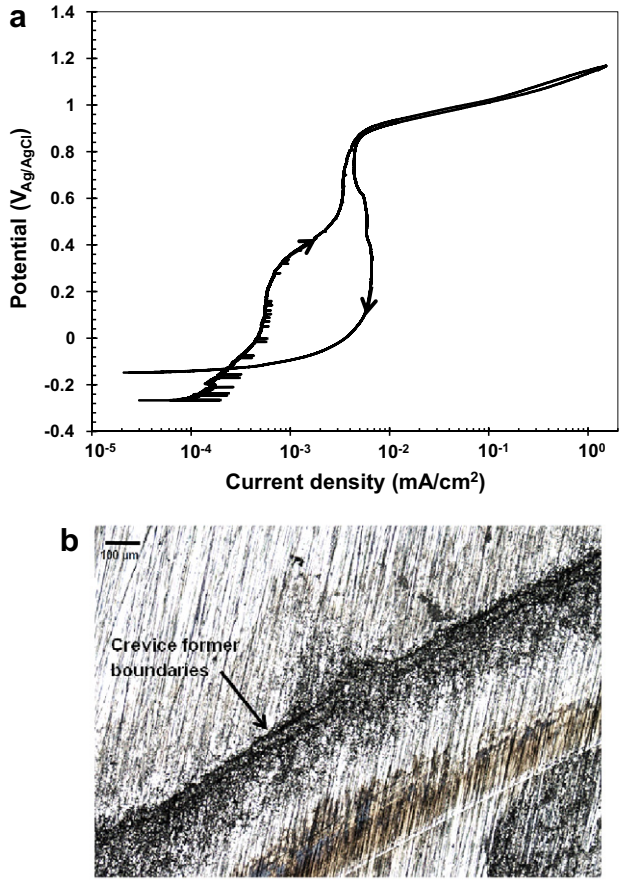

Fig. 7. Crevice corrosion evaluation of UNS S31803 in seawater at 30 °C. This temperature was found to be the repassivation transition temperature T_{tr} for this alloy. (a) Potentiodynamic polarization test displaying an atypical potential versus current transient; arrows indicate the direction of the scan. (b) Crevice attack on the alloy surface restricted to the crevice former boundaries after the potentiodynamic polarization test. Surface inspection was conducted using optical microscopy.

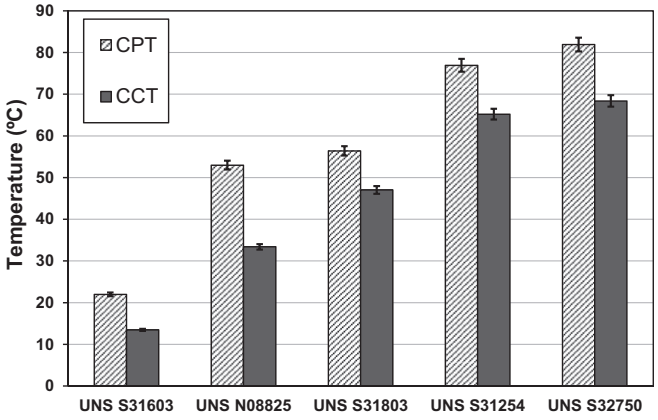

Fig. 8. Average values of critical pitting temperature (CPT) and critical crevice temperature (CCT) of high-resistance alloys in natural seawater. Values were obtained using a potentiostatic technique (700 $mV_{Ag/AgCl}$ applied potential). Errors bars indicate standard deviations from triplicate measurements.

those obtained in this study have been published previously [7]. However, those results were acquired from longer exposure times and more aggressive testing solutions as compared to the ones used in this study.

Fig. 9 shows an optical surface image of a pit typically found on UNS S31603 examined directly after CPT testing. This pit exhibited

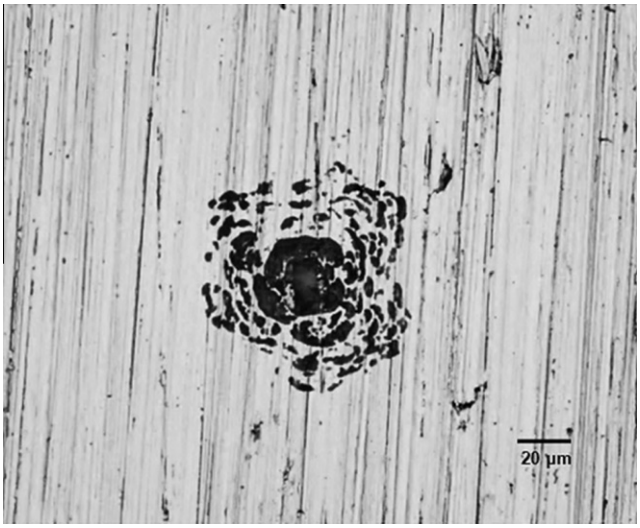

Fig. 9. Optical microscope surface image of a pit typically found on UNS S31603 after potentiostatic evaluation of CPT (700 $mV_{Ag/AgCl}$ applied potential) exhibiting a lazy metallic cover.

a typical pit cover that has been previously defined as a lacy metal cover probably as a remnant of the passive film during stable growth of the pits on stainless steels [4]. Similar pits were observed on the surface of UNS N08825 and UNS S31803. Overall, after CPT testing pits were randomly distributed over the surfaces and exhibited diverse morphologies. Fig. 10 shows some surface images of creviced UNS N08825 after CCT evaluation. Crevice corrosion was concentrated at the crevice former boundaries on the surface (Fig. 10(a)). Same pattern of crevice attack was observed on the surface of all tested alloys after CCT evaluation. Grain structures were found next to the deepest creviced areas at the crevice former boundaries on the UNS N08825 surface (Fig. 10(b)).

Nickel–iron alloys have been mostly used for their special physical properties related to expansion or magnetic characteristics, where corrosion resistance may not be a principal reason for their selection but may sometimes be an added advantage [23]. Alloy UNS N08825 is an austenitic nickel–iron–chromium alloy with additions of molybdenum. Its nickel content makes it particularly resistant to chloride stress-corrosion cracking but the presence of chromium and molybdenum provides good resistance to localized corrosion in different environments. From this investigation it can be concluded that UNS N08825 has superior corrosion resistance in seawater as compared to the conventional 316L stainless steel. However, its actual application should be cautiously considered when seawater temperatures above 10 °C are to be expected.

Results from the present study indicate that the method to investigate CCT and CPT is highly reproducible (Table 3). The fact that the CPT and CCT values of alloys in seawater measured by the potentiostatic technique were rather higher than the temperatures found to be detrimental for pitting and crevice corrosion of the same alloys using polarization curves can be explained by the fact that the two methods used different applied potentials. In addition, the temperature scan rate of 0.5/min and 1 °C/min for CPT and CCT respectively, may not always allow enough time for nucleation processes to occur, which can yield higher CCT and CPT values than those obtained from immersion tests at longer exposure times or from potentiodynamic measurements. The use of different applied potentials and temperature scan rates to evaluate CCT and CPT may generate different CCT and CPT values but those results will certainly still reflect the propensity of different grades to suffer localized corrosion as a function of temperature and are still suitable for comparative studies.

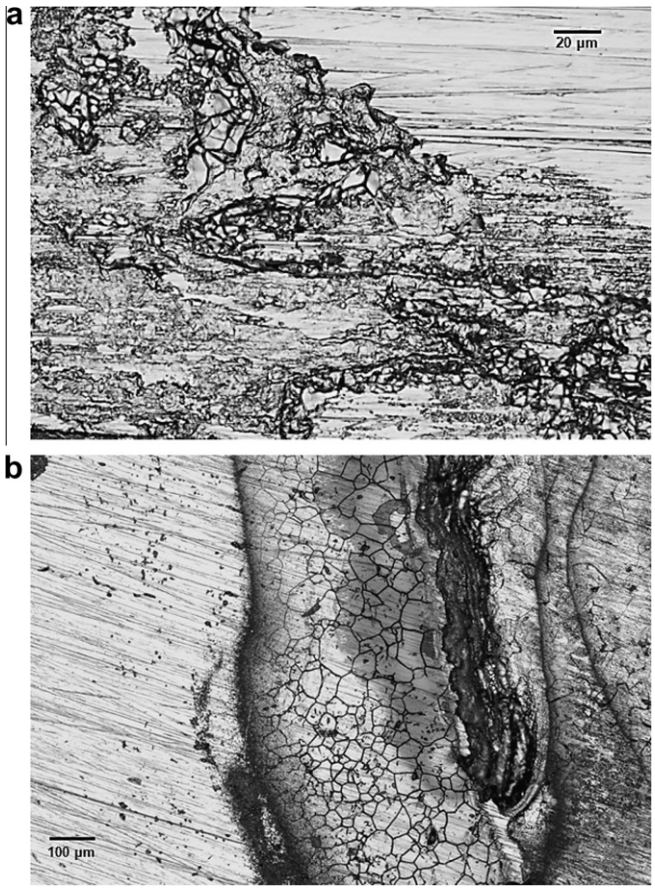

Fig. 10. Optical microscope surface images of UNS N08825 after potentiostatic evaluation of CCT (700 $mV_{Ag/AgCl}$ applied potential). (a) Crevice corrosion attack localized at the crevice former boundaries on the surface; (b) crevice corrosion at the crevice former boundaries exhibiting grain structures next to the deepest creviced areas.

Table 3
Reproducibility of the critical pitting (CPT) and crevice (CCT) temperature of high resistance alloys in seawater. Average values of triplicate measurements.

Alloy	Parameter	Average (°C)	Standard deviation (°C)
UNS S31603	CPT	22	0.96
	CCT	13	0.60
UNS S31803	CPT	57	1.39
	CCT	47	1.03
UNS S32750	CPT	82	1.54
	CCT	68	0.61
UNS 31254	CPT	77	1.83
	CCT	65	0.81
UNS N8825	CPT	53	2.30
	CCT	33	1.27

Clearly, measurements to evaluate pitting and crevice corrosion by different methods are quite subject to test conditions and should not be used to predict long term field exposure. Nonetheless, they provide valuable information about the alloys resistance to particular environments and allow ranking the materials according to specific conditions. Our potentiostatic method to evaluate CPT and CCT proved to be a very practical, rapid and reproducible approach to evaluate the tendency of an alloy to suffer localized corrosion as a function of temperature. However, this method could underestimate the real aggressiveness of an environment towards the material. Resistance to localized corrosion should always be established in combination with additional complementary techniques.

The investigation of critical potentials for pitting and crevice corrosion initiation and repassivation was shown to be a powerful approach to perform a more complete analysis of the localized corrosion resistance of the alloys in seawater. Crevice corrosion is usually considered to take place if the corrosion potential of a metal in a given environment surpasses the repassivation potential [24]. Moreover, the autocatalytic nature of crevice corrosion indicates that the minimum conditions for crevice initiation will trigger a continued propagation of metal dissolution until a repassivation condition is reached [25]. Our results highlight that the evaluation of marine crevice corrosion, mainly through the investigation of repassivation potentials is perhaps the best approach to characterize the alloys resistance to localized corrosion. Ideally, potentiostatic and potentiodynamic methods should be combined to accurately characterize the localized corrosion resistance of different alloy grades. Furthermore, natural seawater should be the preferred testing solution for marine corrosion evaluation as it contains chemical and biological species that are not found in seawater synthetic solutions.

The experimental data presented here indicate that the alloys investigated can be ranked in their resistance to pitting and crevice corrosion in natural seawater as follows: UNS S32750 > UNS S31254 > UNS S31803 > UNS N08825 > UNS S31603. These results are mostly in good correlation with the PREN values shown in Table 1. However, UNS S31254 exhibits the highest PREN values and hence its corrosion resistance might be expected to be superior to UNS S32750. The PREN number has been used as a guide to localized corrosion and indicates the strong effect of chromium, molybdenum and nitrogen as alloying elements on pitting corrosion. The higher this number the more resistant the steel is to pitting. In general, it is well known that alloying elements such as chromium (Cr), molybdenum (Mo), nitrogen (N) are very beneficial additions to iron and nickel alloys, since these elements greatly improve their resistance to localized corrosion in seawater [5,26].

The empirical PREN formula (PREN = %Cr + 3.3% Mo + 16% N) was established based on experimental results on conventional stainless steels and its application to duplex grades has been argued by many authors [27]. Duplex stainless steels are iron-based alloys that possess a two phase microstructure: austenite and ferrite in approximately similar percentages. The ferrite phase contains more chromium and molybdenum than the austenitic phase but most of the nitrogen moves to the austenitic phase. It has been previously demonstrated that higher content of nitrogen in duplex steels contribute to several properties including the stability of the two-phase microstructure and improved resistance to localized corrosion [28,29]. As the austenite phase is the lower-allowed phase, some authors propose the use of the PREN number formula with 30 instead of 16 for the nitrogen (PREN = %Cr + 3.3% Mo + 30% N) when considering duplex stainless steels. Our results support this argument. Since PREN only takes into consideration the effect of alloying on the resistance of materials, the role of microstructure and its interaction with the additions of specific alloying elements should always be considered in categorizing alloys in their resistance to localized corrosion.

4. Conclusions

1. Passivity breakdown of alloys in seawater always occurs through pitting and crevice corrosion at temperatures above the transition temperature for pitting and crevice initiation. Pitting corrosion does not take place below that transition temperature on any of the alloys regardless of the applied potential but crevice corrosion occurs below that transition temperature after the alloys reach a transpassive potential.

2. A repassivation transition temperature T_{tr}, which refers to the evolution from a positive repassivation potential after transpassive dissolution in the absence of crevice corrosion to an active repassivation potential after crevice propagation once transpassive dissolution has first taken place, is approximately 10 °C below the transition temperature for crevice initiation. However, alloys will need to first reach a transpassive potential to undergo crevice corrosion at their T_{tr} which in real environments is unlikely to happen.
3. UNS S32750 and UNS S31254 have excellent resistance to crevice and pitting corrosion in natural seawater at temperatures below 40 °C at a maximum potential of 1.5 V vs. Ag/AgCl. In addition, these alloys have CCT and CPT values higher than 60 °C at 700 mV vs. Ag/AgCl under potentiostatic conditions.
4. UNS S31803 and UNS N08825 are resistant to pitting in seawater at temperatures below 40 °C and UNS S31603 undergoes pitting corrosion in seawater at temperatures from 10 °C above. UNS S31603, UNS N08825, and S31803 display poor resistance to crevice corrosion at seawater temperatures above 5, 10 and 30 °C, respectively.
5. High resistance alloys are ranked according to their resistance to pitting and crevice corrosion in aerated natural seawater as follows: UNS S32750 > UNS S31254 > UNS S31803 > UNS N08825 > UNS S31603. This investigation indicates that the PREN is a very useful guide to rank the alloys resistance to localized corrosion. However, the effect of microstructure and its interaction with the additions of specific alloying elements should always be considered when selecting the correct PREN formula to compare the localized corrosion resistance of different steel grades.
6. Accurate evaluation of localized corrosion resistance of alloys requires a combination of different testing methods to avoid underestimating the aggressiveness of a given environment towards the pitting and crevice corrosion of the alloys. Our results highlight that the evaluation of marine crevice corrosion mainly through the investigation of repassivation potentials is the best approach to characterize the resistance of the alloys to localized corrosion.

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Appendix 2

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Chapter 3

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Microbiologically influenced corrosion of high resistance alloys in seawater

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ABSTRACT

Microbiologically influenced corrosion (MIC) of construction alloys used for subsea applications was evaluated. Susceptibility to MIC of UNS S31603, UNS 31803, UNS S32750, UNS 31254, UNS N06625 and UNS N08825 was assessed by measuring the open circuit potential evolution in time, cyclic polarization tests, surface analysis and biofilm composition. Fresh-ground specimens were tested in seawater as well as after aging in aerated natural seawater at 30ºC for up to four weeks where natural marine biofilms were allowed to develop. Test controls consisted of experiments using filter-sterilized seawater. In order to identify the relationship between electrochemical activity and biofilm composition, DGGE (denaturing Gradient Gel Electrophoresis) and sequencing were conducted to examine the diversity of the microbial consortia in the marine biofilms covering the materials surface. Results are presented to show the effect of immersion time and the presence of marine biofilms on the electrochemical behavior of the corrosion resistant alloys and hence the resistance to localized corrosion. These results also showed the degree to which material composition may affect the bacterial community and shift the microbial diversity in biofilms.

Key words: microbiologically influenced corrosion, stainless steels, seawater, localized corrosion, biofilms, DGGE, microbial community structure.

INTRODUCTION

Microbiologically influenced corrosion (MIC) of materials and alloys has been reported for many years. Microorganisms may profoundly influence, initiate or accelerate, the interfacial process leading to surface degradation through the interaction with physical and chemical variables including pH, redox potential, conductivity, material composition and temperature among others[1, 2]. Moreover, a particular system can become more susceptible or resistant to MIC depending upon factors such as, water composition and material composition[3]. Regardless of the particular reactions and processes governing MIC in different environments, there is agreement in the literature that microbial adhesion and the consequent biofilm formation is the determining step in microbiologically influenced metal deterioration which has produced significant economic losses and serious environmental repercussions[4]. Examples

include pipe and rig corrosion, blockage of filtration equipment and oil spoilage. Microorganisms growing on metal surfaces influence the fate of those materials in the environment, with results ranging from localized corrosion to significant decrease in corrosion rate. It has been suggested that approximately 20% of all corrosion damage to metallic materials is microbiologically influenced but generally costs are underestimated[5].

High resistance alloys have been used increasingly for off shore applications. The excellent corrosion resistance of these construction materials is due to the formation of a stable passive layer on the surface. Nevertheless, stainless steels are susceptible to localized corrosion by chloride ions and reduced sulfur compounds[6]. The corrosion rate and pitting potential of stainless steels in seawater are the functions of Cr and Ni content. Also, the presence of alloying elements such as Mo and N has significant and beneficial influence on the pitting and crevice corrosion resistance of stainless steels[7]. The related mechanisms of pitting and crevice corrosion are very largely controlled by the presence of chlorides in the environment, exacerbated by elevated temperatures[8]. The resistance of a particular grade of stainless steel to pitting and crevice corrosion is indicated by its Pitting Resistance Equivalent number, or PREN. The PREN for stainless steels can be calculated from the composition as PREN = %Cr + 3.3%Mo + 16%N and for nickel based alloys as PREN= %Cr + 1.5 (%Mo + %W + %Nb)[9]. Clearly, grades with high content of the alloying elements chromium, molybdenum and nitrogen are more resistant.

It is well-known that active-passive alloys such as standard grade stainless steels immersed in natural seawater undergo a shift of the open circuit potential in the noble or anodic direction a phenomenon termed ennoblement and attributed only to microbial-related activities[10]. It has been shown that ennoblement of the corrosion potential can affect both the initiation and propagation of localized corrosion of these alloys[11, 12]. Moreover, the breakdown potential, repassivation potential and other parameters used to investigate the localized corrosion resistance of alloys can also be affected by biofilm formation[13]. However, the mechanism for this is still uncertain. The inherently higher localized corrosion resistance of the high performance alloys suggests that they should also be more resistant to MIC. There are no known instances of MIC having occurred during actual service with the 6% Mo stainless steels. The open-circuit potential has been used for many years as an important parameter to describe MIC and other corrosion mechanisms. In addition, potendiodynamic scans have been used to evaluate the effect of microbial films on corrosive behavior[14, 15].

Traditional enumeration of viable bacteria by culture techniques has always been of major importance as a standard method for routine monitoring of MIC. However, these techniques are time consuming and restricted to cultivable microorganisms rather than for the complexity of microbial communities present in a particular environment. The application of molecular tools in environmental microbiology and research studies of microbial adhesion has become crucial to advance the understanding of MIC. These culture independent molecular and DNA fingerprinting methods offer a more accurate identification of microbial communities present in a particular environment or sample[16] and accordingly they could reveal complex metabolic processes involved in MIC.

This study aimed to evaluate the corrosion performance of high resistant alloys in seawater at a typical exposure temperature for offshore assets using open circuit potential measure, potendiodynamic polarization scans and surfaces analysis. In addition, this work investigated the microbial diversity in natural marine biofilms developed on these materials under laboratory closed conditions. Of particular interest was the evaluation of qualitative (community composition of sessile microorganisms) changes in marine biofilms as a function of substrate material composition. Microbiological analysis of biofilms in association with electrochemical findings and surface features will be used to establish which bacteria may play a key role in MIC processes.

EXPERIMENTAL PROCEDURE

Seawater collection and specimen preparation

Seawater samples from 20 metres depth were collected from the Indian Ocean off Rottnest Island (Western Australia). Samples were kept at 4°C before they were used for experiments. Square coupons were ground to 600 grit finish (SiC grinding paper), degreased with acetone and dried with nitrogen gas prior to each experiment. Chemical analysis data for alloys are described in Table 1. For long term immersion experiments, coupons were soaked in Decon® 90 (Decon laboratories Limited) for 5 hours and sterilized by immersion in 70% ethanol for 3-4 hours.

Test conditions

Tests were performed on fresh-ground materials coupons as well as during and after 4 weeks aging in seawater. For all tests, fully aerated natural seawater was used as testing solution and maintained at 30°C. A 150 rpm agitation rate was also sustained throughout the tests. In all cases, a platinum coated mesh and Ag/AgCl electrode were used as counter and reference electrode, respectively. For testing fresh-ground materials, the Gamry Intruments Flexcel^{TM} of 2 L capacity was used and working electrodes had a nominal surface area of 5 cm^2. For aging experiments, laboratory closed-systems using suspended-substratum vessels without water renewal were designed to allow microbial films to develop on the alloys' surfaces during four weeks immersion time. Working electrodes having a test surface area of 50 cm^2 were used for these experiments. Coupons were immersed in the testing solution by hanging using a coated copper wire via a spot weld which also functioned as electrical connection between sample and potentiostat. Any exposed wire was covered with epoxy resin. Three replicate samples were immersed in 2 L vessel filled with 1.5 L of unfiltered natural seawater (named as NSW). For not-biologically active water (Controls), three replicate samples were immersed in 1.5 L of filter-sterilized (0.22 µm polycarbonate filters) natural seawater (named as FSW). Condensers were used to maintain constant levels of test solution over all the experiments. The influence of light/algae activity was minimised by covering the vessels with aluminium foil.

Electrochemical measurements

For testing fresh-ground specimens, open-circuit potential was recorded for 1hour before cyclic potentiodynamic polarization scans were conducted. These tests were carried out in triplicate separately. For aging tests, the electrochemical activity was monitored measuring the open circuit potential (OCP) signal every four hours and cyclic polarization scans were carried out at the end of the exposure time. The long-term potential monitoring was performed using a multichannel potentiostat (ACM Instruments Potential 20). All potendiodynamic scans were performed using Gamry Instruments DC-105 software (Gamry Instruments, Inc.). The cyclic polarization scans were conducted using a forward and reverse scan rate of 0.5 mV/sec. The sweep direction was reversed when either a current density of 1.5 mA/cm^2 or a potential of 1.5 V vs. RE was reached. The final point of the scan was set at a potential of 0 V vs. Eoc. Low currents were selected as stop criteria for potendiodynamic scans since high currents can greatly alter the material surfaces and thus disturb the established biofilm. Additionally, 1 out of the 3 replicates alloy samples was kept in OCP conditions and no potendiodynamic scans were carried out on this sample. This was done to help establish to what extent the increase in current density can affect the microbial composition of marine biofilms.

Biofilm sample collection for microbial analysis

After aging in seawater, coupons were retrieved from the electrochemical cell, washed with sterile seawater (to wash out planktonic cells) and immersed in containers with 100 mL of sterile seawater. In order to facilitate cell detachment, filter-sterilized Tween 20 (0.1% w/v final concentration) was added to the seawater. Biofilms were aseptically recovered by 60 sec-sonication steps until no microbial cells were observed in the suspensions.

Analyses of microbial community composition

Seawater samples as well as the sonicated solutions from biofilmed coupons were analysed to characterize the microbial composition. Seawater was microbiologically analysed in order to compare the free-living bacteria communities with the sessile microorganisms attaching to the alloy surfaces. Microbial cells were harvested by centrifuging at 13,000 rpm for 20 min. Concentrated cells were resuspended in 200 µL phosphate buffer solution (PBS) and DNA was extracted from the cells with a

commercial soil kit (PowerSoil™ DNA Isolation Kit, MO BIO Laboratories Inc). The V2-V3 region of the bulk 16S rRNA genes was amplified for denaturing gradient gel electrophoresis (DGGE) by PCR using bacterial specific primers (27f-1492r). Prior to DGGE analysis, PCR products were purified using the Ultraclean™ PCR clean up kit (MO BIO Laboratories Inc.). To run the DGGE, PCR products were used as templates to amplify a subregion of the 16S rRNA by using primers BacV3f with G-C clamp and 907r. Denaturing gradient gel electrophoresis was performed using the DCode Universal Mutation Detection System (BIORAD) as per the manufacturer's instructions. The PCR product was mixed with DNA loading buffer (BIOLINE) and then loaded onto 7%(w/v) polyacrylamide gel (40% acrylamide/bis solution, 37.5:1) with a denaturing gradient of 30-70% urea-formamide in 1x TAE buffer. DGGE was performed at 60°C overnight at 150V. DNA bands were visualized and excised from each lane using sterile scalpel blades. The bands were resuspended in 40µL of sterile RNase-free water overnight. Eluted DNA from individual bands was used for reamplification by PCR with primers BacV3f no G-C clamp and 907r. Finally PCR products were sequenced at Macrogen, South Korea. To identify the microbes, the sequence data was compared with 16S rRNA gene sequences in the GenBank database using the basic local alignment search tool (BLAST; http:/www.ncbi.nlm.nih.gov/blast/).

Surface Analysis

Coupons were cleaned with an acid solution[17] degreased with acetone and kept in desiccators until microscopic analysis was done. The test coupons were examined for their surface features using optical microscopy. Images and pit profiles were obtained using an Infinite focus microscope and Alicona IFM software.

RESULTS

Open circuit potential measurements (OCP)

Average values of OCP of specimens exposed to aerated natural seawater at 30°C measured as a function of time are shown in Figure 1. Specimens were labeled as F (aged in filter-sterilized seawater) and N (aged in natural seawater). The OCP of all specimens showed a slight shift to more noble values in the first days which is related to passive film thickening and stabilization. OCP of 316-N coupons showed a slight ennoblement during the first 15 days after which the OCP remained stable for the entire duration of the test. The OCP of duplex grades (2205 and 2507) was very unsteady throughout the test and no clear differences were observed between specimens exposed to sterile and natural seawater (Fig 1-a). The OCP of 316L, 2205, 2507 and 254 specimens never reached values higher than +50 mV. These results showed that no appreciable ennoblement was observed on any of the stainless steels evaluated under the particular conditions of this study. The absence of an active biofilm able to naturally polarize the materials surface to a potential close to E_b can be due many factors. First of all, these samples were sourced from relatively clean and deep seawaters where nutrients may be scarce. Also, this study used a closed system without seawater renewal and this was maintained for up to four weeks. It is possible that after a couple of days nutrients became limited in their capacity to keep bacteria metabolically active to a level necessary to shift significantly the OCP toward values close to E_b. This is in agreement with the fact that most of the studies where the ennoblement phenomenon has been reported have been conducted on open systems where seawater is regularly replaced or on culture based experiments where bacteria are maintained in nutrient-rich media. Only a few studies have reported ennoblement on closed systems but even the external supplements of nutrients, enzymes or bacteria were added[18].

In the case of nickel based alloys, (Fig 1-b), 825-N OCP showed a more apparent ennoblement during the first 10 days reaching a maximum value of +19 mV. Then, OCP values fell significantly and then remained fairly steady until the end of the immersion time. This decrease in potential has been attributed to either a decrease in film thickness or a deficiency in the production of one of its constituents[19]. The OCP of 625-N showed also a slight shift to more positive values throughout the test reaching its maximum at the 15th day of the experiment. 625 and 825 specimens exposed to sterile seawater showed no ennoblement of their OCP at any time during the immersion period.

Cyclic potentiodynamic polarization scans (CPS)

Cyclic polarization scans obtained from freshly ground specimens in natural seawater at 30ºC (referred to as unaged) as well as specimens aged in sterile seawater (FSW) and natural seawater (NSW) at 30ºC for up to four weeks are shown in Fig. 2 and 3. Each graph illustrates these three conditions for each type of alloy. The unaged samples show the typical well-defined CPS curve observed in active-passive alloys where a stable passive region is formed followed by a passivity breakdown at a breakdown potential (E_b) where a sudden current increase is observed. During the reverse scan a repassivation potential (E_r) is reached at the intersection between the forward and the reverse scan which is associated with a drop in current caused by repassivation of pits.

Fig. 2-a shows the CPS of 316L specimens. In unaged specimens, a stable passive region is formed after anodic polarization from the OCP. E_b is then reached at about +450 mV. A hysteresis loop is formed during the reverse scan until the repassivation potential (E_r) is reached at about +210 mV. CPS of 316L-FSW reveals a poorly defined passive region and E_b is reached at a potential of +600mV. A hysteresis loop is also formed during the reverse scan and E_r is reached at a potential (-200 mV) much lower than the E_r observed on the unaged sample. This potential indicates that aging in seawater for up to 4 weeks increases the likelihood of propagation of localized corrosion for the 316L alloy. Also, a critical pitting temperature (CPT) well below 30°C has been reported for 316L stainless steels in saline solutions [20]. Below the CPT stable pitting did not occur at any potential up to the onset of transpassivity. Only above the CPT is stable pitting likely to take place. This indicates that the conditions evaluated here are very aggressive for this material and pitting is expected to take place. The polarization curve of 316L-NSW showed higher passive current densities (more than an order of magnitude) as compared with the sterile controls. Also, a more active (negative) E_b (~+210 mV) was observed in 316-NSW as compared with the other two conditions tested. Again, a hysteresis loop is observed and the E_r value is much the same as obtained in the 316-FSW. These findings highlight that 316L was susceptible to MIC under the conditions tested here.

The CPS pattern of alloys 2205, 2507 and 254SMO is shown in Fig. 2-b. These three materials exhibited the same pattern in the polarization scan for the three different conditions tested. Again, a wide passive range is observed on unaged surfaces. E_b was then reached at very high potentials and no hysteresis loop was observed at the reverse scan on any of these materials under any of the conditions tested. Specimens aged in NSW and FSW also followed the same trend in the polarization scan as compared to unaged specimens, the only difference being that aged specimens reached higher passive current densities. Similar E_b values were found for all the specimens at all the experimental conditions. These E_b values are more related to transpassive corrosion rather than pitting. These results suggest that alloys 2205, 2507 and 254SMO have a good resistance to MIC and seawater under the conditions evaluated here.

CPS for 825 nickel alloy are shown in Fig. 3-a. In fresh-ground specimens E_b is reached at ~+908 mV after a wide passive region. No hysteresis loop is formed. For 825-FSW coupons, E_b is much the same as observed in unaged specimens (~850 mV) but a hysteresis loop is formed during the reverse scan. The E_r value was -121 mV which is notably more active than observed on 825 unaged samples. In the CPS of 825-NSW coupons, again no well-defined formation of a passive film is observed. 825-NSW showed a more active E_b as compared with the other two tested conditions. This value was ~ +321 mV about 500 mV more active (negative) than the E_b observed in the other two conditions tested. Nonetheless, E_r was the same for NSW and FSW specimens (-121 mV). From these results it can be seen that 825 nickel alloy may be susceptible to MIC. Finally alloy 625 showed a significantly different polarization scan compared with the other materials evaluated here (Fig. 3-b). However, no localized corrosion was observed on this material under any of the conditions tested. These results underline the high resistance of alloy 625 to seawater and MIC for the typical marine environment evaluated in this study.

Microbial composition by PCR-DGGE

The PCR-DGGE approach was used to analyze the16S rRNA gene fragments amplified from the DNA extracted from the seawater and coupon samples after four weeks aging in fully aerated seawater. No DNA was detected in extracts from either control specimens or filter-sterilized seawater used for controls. DGGE profiles of natural seawater samples and natural marine biofilms are shown in Fig. 4. Fig. 4-a. shows the results from DGGE 1 (S1, 316L, S2, 2205 and 2507) and Fig. 4-b shows the results from DGGE 2 (S3, 254SMO, alloy 625 and alloy 825). Results are shown in triplicate for each material. The number of individual bands obtained in a DGGE analysis is related to the number of bacterial populations in the tested sample. The detected bands were excised from the gel and sequenced. Tables 2, 3 and 4 describe the microbial populations identified in seawater samples and biofilms on each type of material. Numerous bands were observed in the seawater samples (S1, S2 and S3) which reflect the diversity of free-living communities. It can be seen that a sub set of the bacterial populations seen in the seawater were also found in the biofilms. The sequence data showed that most of the identified free-living bacteria correspond to Proteobacteria specifically Alphaproteobacteria and Gammaproteobacteria. Populations identified on biofilm samples of 316L experiments are similar to populations identified by others under similar experimental conditions[21].

Results from this study clearly reflect the shift in microbial communities into biofilms according to material composition. Although different communities were found on different types of alloys, some of the bacteria populations were commonly encountered on different materials. *Marinobacter* was the only population commonly indentified on all grades of materials evaluated, including stainless steels and nickel alloys. Also, it is important to underline that *Erythrobacter* was characterized on biofilms of each of the iron-base alloys (316L, 2205, 2507 and 254SMO) but not on any of the Nickel base alloys. This suggests either a selective adhesion capability of *Erythrobacter* to high iron content alloys or a noxious effect of nickel on its attachment to surfaces. In addition, *Marinobacter flavimaris* was found in biofilms of 254SMO stainless steel, 625 and 825 alloys which demonstrates its ability to attach to both iron and nickel base alloys.

Among stainless steels grades, *Thalassobius* was commonly identified on the austenitic steels 316L and 254SMO but not on any of duplex grades 2205 and 2507 which could suggest an influence of the two-phase microstructure on the microbial adhesion. Microbial diversity in duplex stainless steel varied significantly between the two different grades which was unexpected since material composition of these two alloys are rather similar. This suggested that material composition is not the main conditioning factor in the settlement of microbial populations on surfaces. *Janniashia rubra* was unique to 625 nickel alloy and two populations, *Flexibacter sp.* and *Oceanibaculum indicum* were unique to 825 alloy surfaces. These former three populations were exclusive to nickel base alloy surfaces and were not found on 254SMO stainless steel. Since these populations were not forming part of the free-living bacteria identified in S1 and S2 (seawater samples used for 316L, 2205 and 2507 experiments) no conclusions can be made about the attachment of those bacteria to those surfaces.

Low bacterial diversity in natural marine biofilm samples of nickel alloys was observed as compared to stainless steels. However, this observation must be carefully interpreted since three different seawater samples were utilized in these experiments. Even though the seawater source was the same (same sampling point and depth but different sampling date) for the three seawater samples, the microbial analysis of the free-living populations revealed differences in microbial communities present in the samples. These fluctuations in bacterial communities in the three seawater samples are not surprising since samples were collected one month apart. During this period, water currents, oxygen levels and temperature changes may alter the biodiversity in specific sampling points in the open sea.

Microorganisms identified on materials where MIC was observed (316L SS and 825 nickel based alloy) are of particular interest. These populations present on corroded material surfaces are expected to play a key role on the enhanced localized corrosion observed on these materials. Further investigation and laboratory experiments using these bacteria will help elucidate if the effect on materials is achieved by individual populations or by several selective species inside the community. It is important to highlight

that apparently non-aggressive species identified on materials where MIC was not observed, may be able to play a crucial role on influencing corrosion mechanisms under different environmental conditions. The metabolic activities of these microorganisms could be associated with corrosion processes although the precise role of individual populations in each community and its aggressiveness to MIC will need further investigation.

One of the aims of this work was to evaluate the suitability of using a limit current density of 1.5 mA/cm^2 during potentiodynamic polarization scans that permit to evaluate localized corrosion in biofilmed specimens without disturbing their biofilm structure. DGGE profiles and sequencing showed that this limit current density did not affect the microbial diversity and composition in biofilms when comparing with samples kept under open circuit potential.

Surface Analysis

Figure 5 shows some surface images of specimens after 4 weeks aging in seawater and subsequent removal of biofilms. Surface inspection using an infinite focus light microscope was done to confirm the findings from electrochemical testing. Images from 316L specimens after potentiodynamic polarization revealed the presence of numerous pits distributed regularly on the whole surface (Fig. 5-a). Similar round and irregular shaped pits were observed on those specimens. Figure 5-b shows one of the typical shallow micropits observed on 316L coupons kept under freely corroding conditions. No pits were observed on any of the 2205, 2507, 254SMO and 625 specimens under any of the experimental conditions tested here. A few small irregularly shaped pits were observed on 825 coupons kept at their OCP. Images from polarized 825 coupons revealed a larger attack which was concentrated at the edges in contact with the epoxy resin which is more likely to be a crevice-type of attack. Images reflect that the corrosion damage was more pronounced in polarized coupons after exposure to natural seawater than the ones exposed to sterile seawater.

TABLE 1.
Materials evaluated and nominal compositions

Material	UNS number	Type	C wt %	Mn wt %	Fe wt %	Cr wt %	Ni wt %	Mo wt %	N wt %	Nb wt %	PRE-N*
316L SS	S31603	Austenitic	0.022	1.76	bal	17.4	10	2.03	0.046	-	24.835
Alloy 2205	S31803	Duplex	0.015	1.53	bal	22.35	5.72	3.16	0.18	-	35.658
Alloy 2507	S32750	Super duplex	0.019	0.819	bal	24.74	6.61	3.73	0.2619	-	41.2394
Alloy 254SMO	S31254	super austenitic	0.01	-	bal	20.18	18.15	6.1	0.2	-	43.51
Alloy 625	N06625	Nickel base alloy	0.1	0.45	5.00	22.5	bal	9		3.85	41.27
Alloy 825	N08825	Nickel base alloy	0.05	0.85	22	22.5	bal	3		-	27

*** PRE-N=** %Cr + 3.3 %Mo + 16 %N (stainless steels) %Cr + 1.5 (% Mo + % W + %Nb) (nickel base alloys)

TABLE 2.
Microbial populations identified on seawater and biofilm samples of 316L stainless steel experiments.

316L Stainless steels experiments		
Band in DGGE	**S1-seawater sample (free-living populations)**	**316L SS (populations in biofilm samples)**
1	*Balneola alkaliphila*	
2	*Alteromonas sp.*	
3	*Glaciecola sp.*	
4	*Haliea sp.*	***Haliea sp.***
5	*Thalassobius sp.*	***Thalassobius sp.***
6	*Marinobacter sp.*	***Marinobacter sp.***
7	*Bacteroides*	
8	*Mucus bacterium*	
9	*Erythrobacter sp.*	***Erythrobacter sp.***

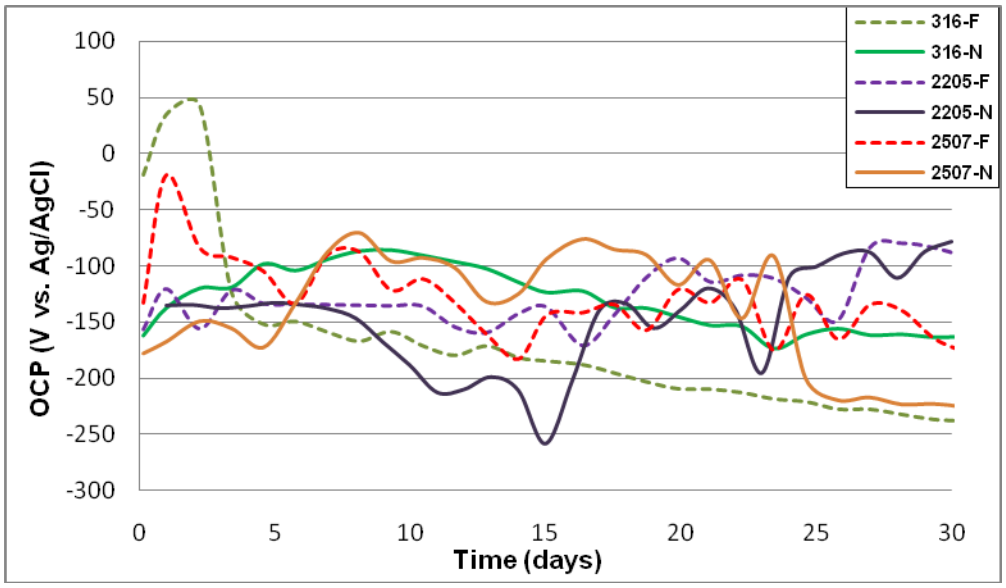
TABLE 3.
Microbial populations identified on seawater and biofilm samples of 2205 and 2507 stainless steel.

2205 and 2507 Stainless steel			
Band In DGGE	**S2-seawater sample (free-living populations)**	**2205 SS (populations in biofilm samples)**	**2507 SS (populations in biofilm samples)**
1	*Thalassobius sp.*		***Thalassobius sp.***
2	*Thalassobius mediterraneus*		
3	*Thalassobius aestuarii*		
4	*Alcanivorax sp.*	***Alcanivorax sp.***	
5	*Alteromonas dieselolei*		
6	*Novosphingobium sp.*		
7	*Novosphingobium subterraneum*	***Novosphingobium subterraneum***	
8	*Marinobacter hydrocarbonoclasticus*		***Marinobacter hydrocarbonoclasticus***
9	*Marinobacter flavimaris*		***Marinobacter flavimaris***
10	*Marinobacter sp.*	***Marinobacter sp.***	
11	*Ruegeria mobilis*	***Ruegeria mobilis***	
12	*Halomonas sp.*	***Halomonas sp.***	
13	*Erythrobacter sp.*	***Erythrobacter sp.***	***Erythrobacter sp.***
14	*Flavobacterium sp*		***Flavobacterium sp***

TABLE 4.
Microbial populations identified on seawater and biofilm samples of 254SMO stainless steel and the nickel alloys 625 and 825.

254SMO, alloy 625 and alloy 825 experiments				
Band In DGGE	**S3-seawater sample (free-living populations)**	**254SMO SS (populations in biofilm samples)**	**625 Ni alloy (populations in biofilm samples)**	**825 Ni alloy (populations in biofilm samples)**
1	*Roseobacter sp.*			
2	*Pseudoalteromonas sp.*			
3	*Marinobacter hydrocarbonoclasticus*			
4	*Marinobacter flavimaris*	***Marinobacter flavimaris***	***Marinobacter flavimaris***	***Marinobacter flavimaris***
5	*Marinobacter taiwanensis*	***Marinobacter taiwanensis***		
6	*Vibrio chagasii*			
7	*Alteromonas addita*			
8	*Polibacter sp.*			
9	*Marinovium algicola*	***Marinovium algicola***		
10	*Thalassobius aestuarii*		***Thalassobius aestuarii***	
11	*Thalassobius mediterraneus*	***Thalassobius mediterraneus***		
12	*Erythrobacter sp.*	***Erythrobacter sp.***		
13	*Alcanivorax sp.*	***Alcanivorax sp.***		
14	*Janniashia rubra*		***Janniashia rubra***	
15	*Flexibacter sp.*			***Flexibacter sp.***
16	*Oceanibaculum indicum*			***Oceanibaculum indicum***

a)



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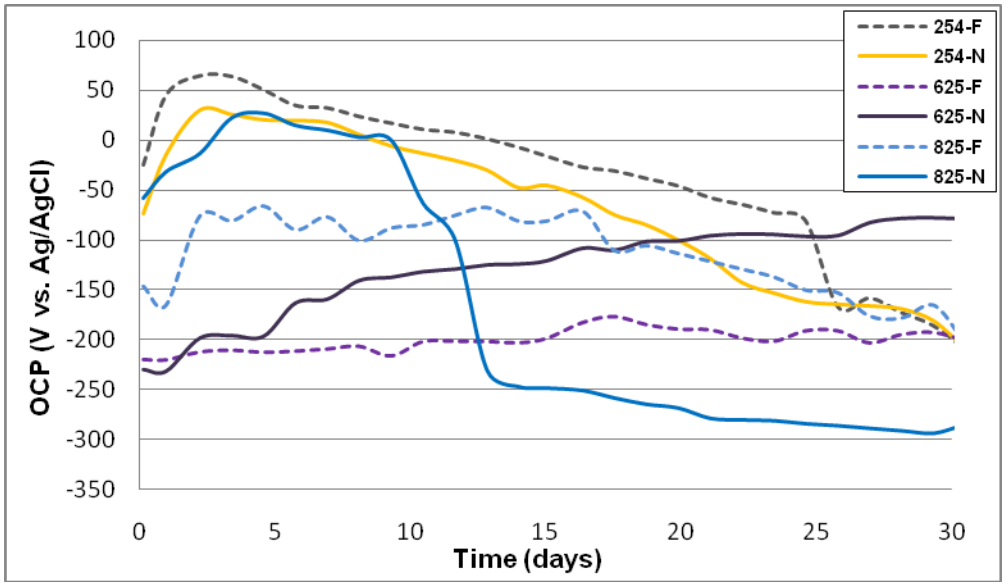
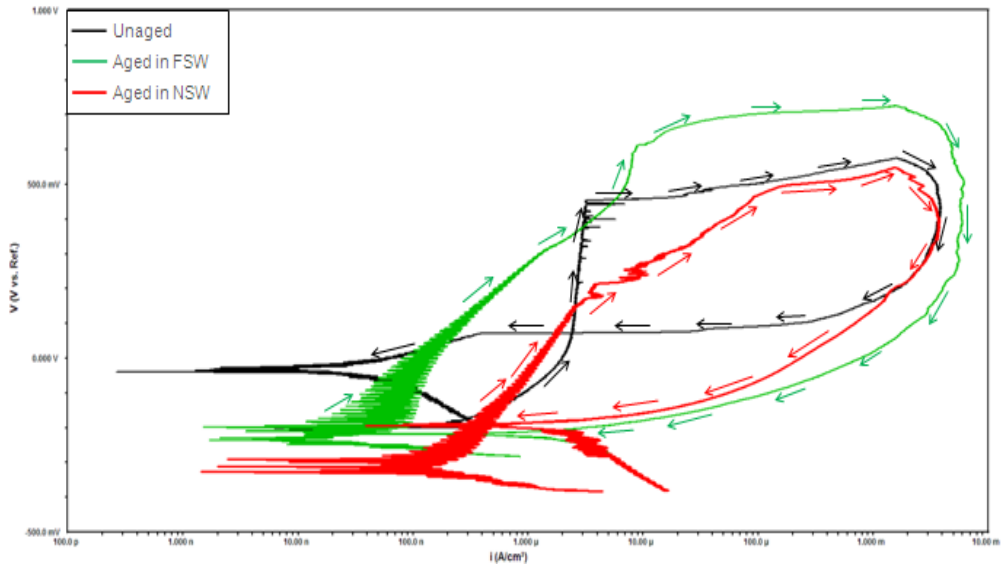


Figure 1: Average OCP (vs. Ag/AgCl) over time values for a) 316L, 2205, 2507stainless steels and b) 254SMO, 625 alloy, 825 alloy in aerated seawater at 30°C. F= specimens aged in filter sterilized seawater and N= specimens aged in natural no filtered seawater.

a)



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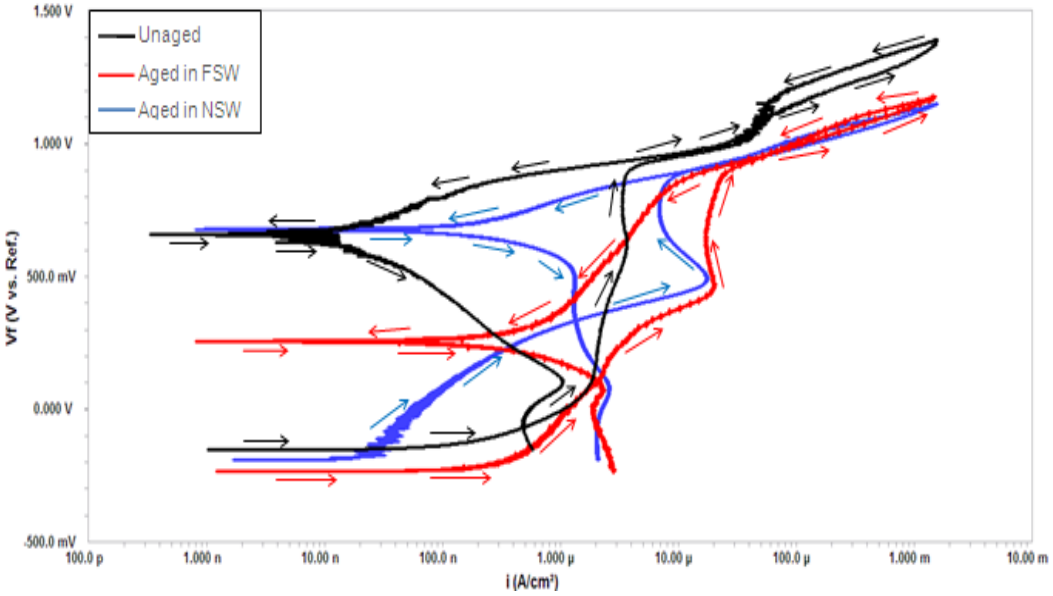
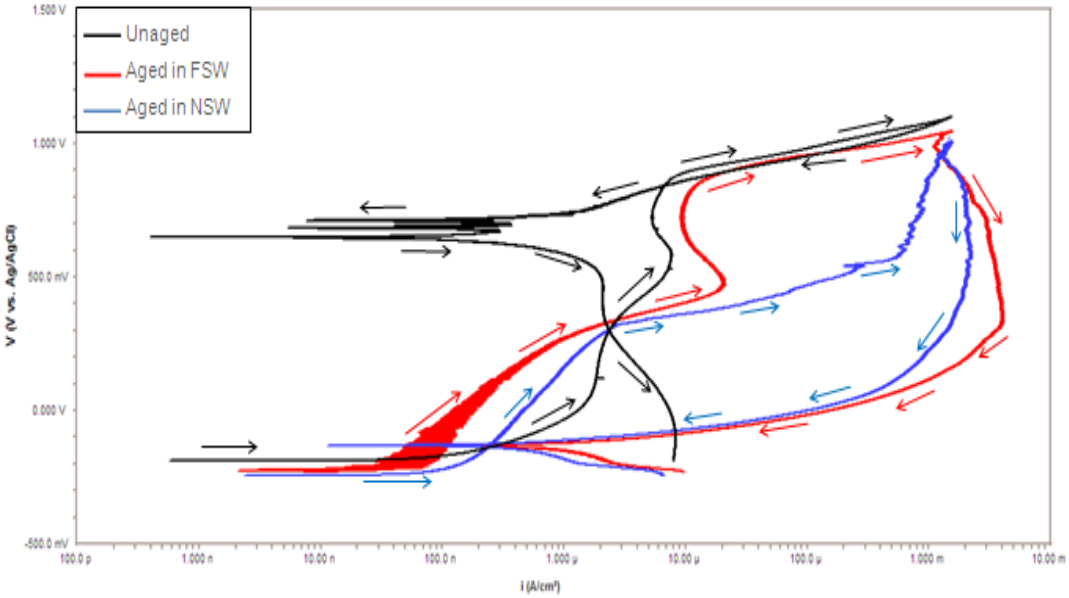


Figure 2: Cyclic polarization scans of a) 316L; b) 2205, 2507 and 254SMO stainless steel (CPS pattern). Unaged= fresh ground specimens; Aged in FSW= specimens aged in filter sterilized seawater; Aged in NSW= specimens aged in natural no filter seawater.

a)



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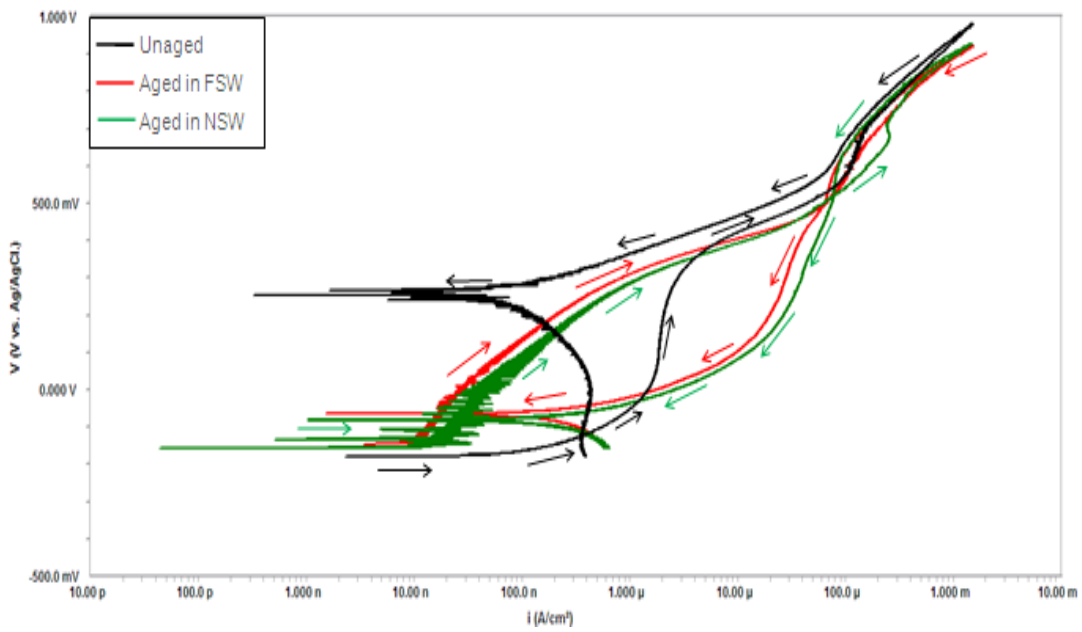

Figure 3: Cyclic polarization scans of a) 825 nickel alloy and b) 625 nickel alloy. Unaged= fresh ground specimens; Aged in FSW= specimens aged in filter-sterilized seawater; Aged in NSW= specimens aged in natural no filtered seawater.

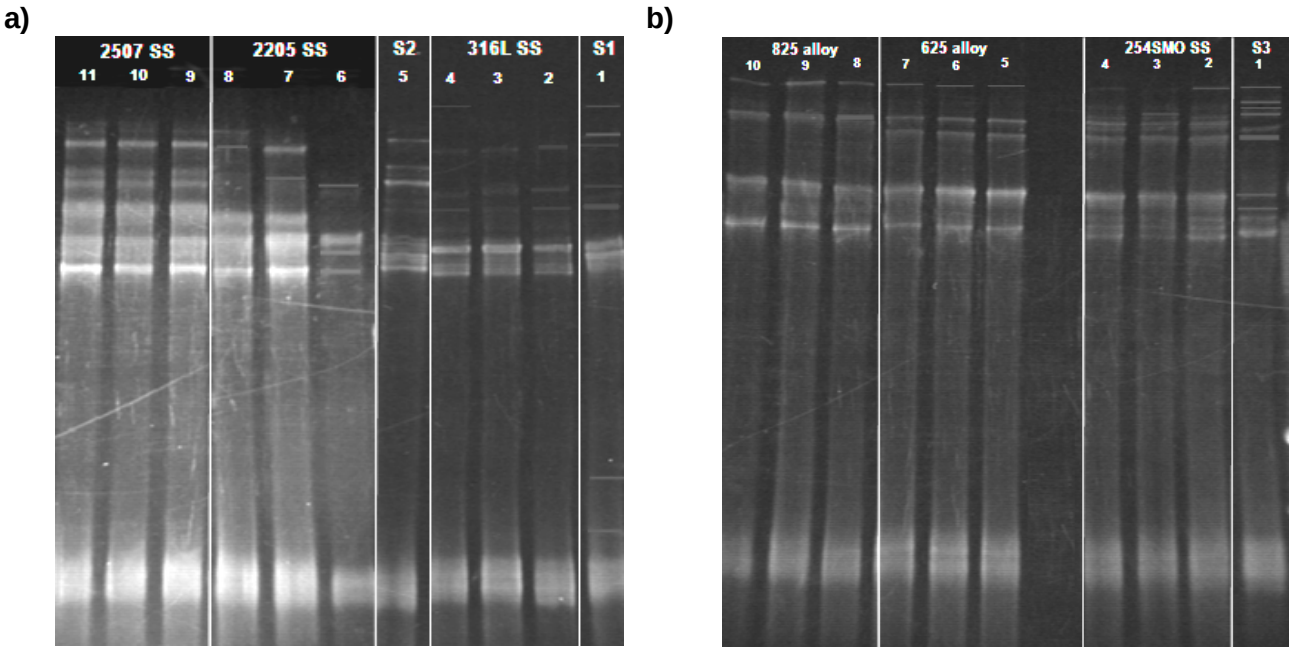

Figure 4: DGGE profiles of 16S rRNA bacteria genes from Biofilms developed on alloys' surfaces aged in aerated natural seawater for up to 4 weeks. a) DGGE 1: S1, 316L, S2, 2205 and 2507 stainless steels and b) DGGE 2: S3, 254SMO, 625 alloy and 825 alloy. Each lane corresponds to one sample and reflects the diversity in the biofilm community. Each band within a lane corresponds to one microbial population into the community.

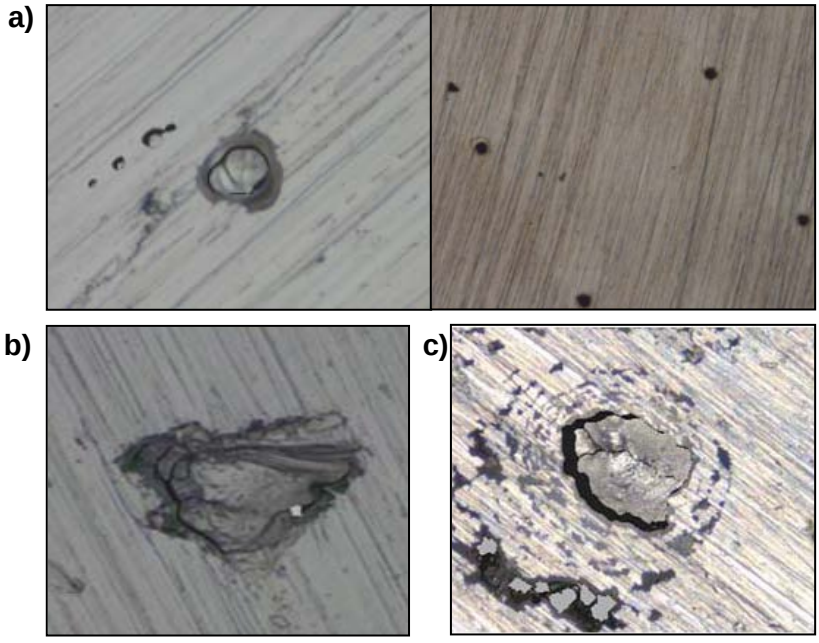

Figure 5: Optical images of specimens aged in natural unfiltered seawater and after biofilm removal. a) pits on 316L coupons after potendiodynamic polarization scan. b) shallow pit on 316L coupon kept under open circuit potential. c) crevice type of attack beneath the epoxy resin in 825 coupons after potendiodynamic polarization scan.

CONCLUSIONS

- Average values of open circuit potential showed no appreciable ennoblement on any of the UNS S31603, UNS S31803, UNS S32750 and UNS S31254 stainless steels specimens. In the case of nickel alloys, UNS N08825 and UNS N06625 showed a slight ennoblement that was not observed on control specimens.
- UNS S31803, UNS S32750, UNS S31254 and UNS N08825 exposed to seawater at 30°C showed the same behavior when tested by using the potendiodynamic polarization method. Results from electrochemistry and surface analyses indicated that these materials render good resistance to localized corrosion even when accelerated corrosion methods are applied. Moreover, no microbial-related attack was observed on any of these materials.
- UNS S31603 and the nickel alloy UNS N08825 exhibited poor resistance to localized corrosion under all the conditions evaluated here. In addition to the effect of halide ions and temperature on the localized corrosion resistance of these materials, results from this work show an exacerbated effect due to the presence of microbiological components.
- Localized corrosion resistance can be compared to the PREN values where UNS S31603 and alloy UNS N08825 have comparatively lower PREN as compared with the higher alloyed materials, UNS S31803, UNS S32750, UNS S31254 and UNS N08825.
- A limit current density of 1.5 mA/cm^2 during potentiodynamic scans was found to be suitable for testing localized corrosion without disturbing the microbial community composition of biofilmed specimens. This current did not affect the microbial diversity in biofilms of polarized specimens as compared to their replicates kept under open circuit potential.
- Microbiological analysis showed the complexity in microbial communities present in biofilms of high resistance alloys. Microbial populations were diverse and on the whole selective to different type of materials. *Marinobacter* was the only population commonly identified on all the materials including stainless steels and nickel alloys while *Erythrobacter* was only characterized on biofilms of iron-base alloys but not on any of the nickel base alloys.
- Microorganisms identified on materials where MIC was observed (UNS S31603 SS and UNS N08825) are expected to play a role on the enhanced localized corrosion observed on these materials. Nonetheless, it is difficult to establish any precise association between these microbial populations and the localized corrosion on these materials. Further investigation will help elucidate the role of these populations on MIC.
- The PCR-DDGE molecular approach used to evaluate microbial communities within biofilms was shown to be a powerful method to investigate the shift in microbial community structure in biofilms as a function of material composition. To the best of our knowledge this is the first study where microbial community analyses are used to investigate marine biofilm composition of high corrosion resistance alloys exposed to natural seawater.

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Appendix 3

Original reprint of publication

Chapter 4

L.L. Machuca, Bailey, R. Gubner, E. Watkin, M.P. Ginige, A. Kaksonen, Bacterial community structure in natural marine biofilms and the corrosion of carbon steel in seawater, in: *18th International Corrosion Congress,* Paper 371, Perth, Australia, 2011.

BACTERIAL COMMUNITY STRUCTURE IN NATURAL MARINE BIOFILMS AND THE CORROSION OF CARBON STEEL IN SEAWATER

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SUMMARY: The application of molecular tools to the investigation of microbiologically influenced corrosion (MIC) has become crucial in the advancement of understanding the complexity and mechanisms of microbial interactions with materials and the environment. In this study, carbon steel specimens were evaluated for MIC under laboratory closed conditions by conducting corrosion tests and biofilm community structure analysis. Material coupons were immersed in natural seawater under aerobic and anaerobic conditions for up to 4 weeks where natural marine biofilms were allowed to develop. Experimental controls consisted of tests using aerobic and anaerobic filter-sterilized seawater. All experiments were carried out at 20ºC. Corrosion of carbon steel specimens was assessed using weight loss measurements, surface inspection, pit profile analysis and surface roughness measurements. The bacterial community structure of biofilms on the carbon steel surfaces was characterized using a molecular microbiology approach. Total DNA was extracted from biofilms and used as a template for amplification of 16S rRNA genes followed by denaturing gradient gel electrophoresis (DGGE) and DNA sequencing. Results are presented to show the diversity in microbial communities in biofilms covering carbon steel surfaces. In addition, these data show the relationship between carbon steel corrosion and biofilm community structure changes associated with the presence and absence of oxygen in seawater.

Keywords: Microbiologically influenced corrosion, seawater, carbon steel, biofilms, bacteria community structure, denaturing gradient gel electrophoresis.

1. INTRODUCTION

Carbon steel is one of the most widely used materials in marine applications. After exposure to seawater carbon steel surfaces typically corrode uniformly and are less likely to corrode in a localized manner. These corrosion reactions have been widely studied and several explanations have been proposed to the mechanisms of carbon steel corrosion in the presence and activities of microorganisms [1-3].

It is well-known that microbial adhesion to metallic surfaces immerse in seawater is a natural process that takes place after only a few hours of exposure and that this process results in biofilm formation [4]. Within this biofilm microorganisms can alter both the rate and type of the electrochemical reactions leading to metal deterioration [5, 6]. In addition to biological components, corrosion processes can also be influenced by many factors such as temperature, water chemistry and velocity, material composition and oxygen content. The complexity involved in these reactions has generated controversy over the precise mechanism of both aerobic and anaerobic corrosion.

A number of microorganisms including species of sulphate reducing bacteria, thiosulphate reducing bacteria, methanogens, and iron oxidizing bacteria have been identified as corrosion-enhancing microorganisms [7]. However, as these populations exist in a biofilm matrix, the interactions of this matrix with substratum surfaces and the environment are most likely the result of the synergistic effect of a whole microbial community present in the biofilm rather than of individual microbial populations [8, 9].

The traditional enumeration of viable bacteria by culture techniques as standard method for routine monitoring in field and for laboratory studies has always been of major importance in the study of MIC. However, these techniques are restricted to cultivable microorganisms and do not reveal the complexity of microbial communities in environmental samples. The application of molecular tools has shown that bacteria growing in culture media often represent a minor part of the microbial community: around 99% of microorganisms existing in nature are unable to be cultured by selective enrichment cultures and they will, therefore, be excluded when enumerated with growth media [10].

New molecular culture-independent techniques such as fluorescent *in situ* hybridization (FISH), cloning, polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE) and DNA sequencing have been used extensively to investigate the diversity of microbial communities in environmental samples as well as the influence of environmental conditions on the composition, diversity and dynamics of biofilm communities [11, 12]. Furthermore, these methods have proved to be a powerful tool to accurately identify microbial populations present in natural biofilms on material surfaces [13, 14].

Previously, it has been shown that physicochemical variables influence not only the material/solution electrochemistry but also the microbial community composition and structure in biofilms developing on material surfaces [15, 16]. The complexity of these interactions suggests a non-unified mechanism of MIC and highlights the importance of investigating the relationship between the biofilm population dynamics and electrochemically active surfaces in different environments. Despite increasing recognition of the importance of the analysis of microbial populations in natural biofilms to advance in the understanding of MIC mechanisms, the role of biofilm microbial communities in the corrosion of materials remains elusive.

The purpose of this study was to investigate the composition of microbial communities present in marine biofilms developed on corroding carbon steel surfaces exposed to both aerobic and anaerobic seawater to gain better insight into the interactions between different groups of microorganisms and the corrosion of carbon steel. This was assessed using PCR–DGGE and DNA sequence analysis of 16S rRNA gene fragments of DNA extracted from the biofilm samples. Corrosion of carbon steel was evaluated using weight loss and surface analysis.

2. EXPERIMENTAL

2.1 Seawater samples and specimens preparation

Seawater samples were collected from 20 metres depth in the Indian Ocean off Rottnest Island (Western Australia). Carbon steel specimens (10x10x5mm square coupons) were suspended in the test solution. The composition (in wt %) of this material was (obtained from the supplier): C 0.22, Al 0.100, Mn 1.70, P 0.040, S 0.040, and Si 0.55. Prior to immersion, specimens were wet ground using silicon carbide papers of 120, 360 and 600 grit consecutively, degreased with acetone, dried with nitrogen and weighted in triplicate. Total coupon areas were measured using a digital gauge. Coupons were finally soaked in Decon® 90 (Decon laboratories Limited) for 3 hours and sterilized by immersion in 70% ethanol for 1 hour. This procedure was evaluated prior to the experiment to ensure specimens did not undergo any weight loss from sample pre-treatment before immersion in the test solution.

2.2 Test conditions

Laboratory closed-systems using suspended-substratum vessels without water renewal were designed to allow microbial films to develop on the carbon steel surfaces during four weeks immersion in seawater. Figure 1 shows an illustration of these reaction vessels. Four reaction vessels were setup, two as biofilm cells and two as control cells. For aerobic experiments, fully aerated natural seawater was used as the test solution and for anaerobic experiments the vessels were purged with nitrogen to achieve anaerobic conditions. A continuous filter-sterilized (0.20 µm syringe filter) gas inflow was maintained to ensure consistent pressure conditions for the duration of the experiment. Dissolved oxygen was monitored using an orbisphere 3655 oxygen analyser (Hach Company). Temperature was maintained at 20°C using a circulating water bath. Coupons were immersed in the solution by hanging using a nylon string. Five replicates were immersed in each cell which filled with 2 L of test solution. Three specimens were used for weight loss measurements and the remaining two for microbiological analysis. For non-biologically active water (controls), specimens were immersed in filter-sterilized (0.22 µm polycarbonate filters) natural seawater. Light was excluded from the system to minimise the influence of light/algae activity. At the completion of the immersion period, coupons were cleaned by following the standard procedure [17]. Average values of triplicate weights were used to calculate weight loss and corrosion rates of each sample [17] using the following formula:

$$CR = kW/DAT$$

Where CR= corrosion rate, mm/y; k= constant, 8.76×10^4; W=weight loss, grams; D=density, g/cm^3; A= area in cm^2; T= time of exposure in hours.

Surface inspection, pit profile analysis and surface roughness measurements were conducted using the Alicona infinite focus microscope (IFM G4g system, Alicona Imaging).

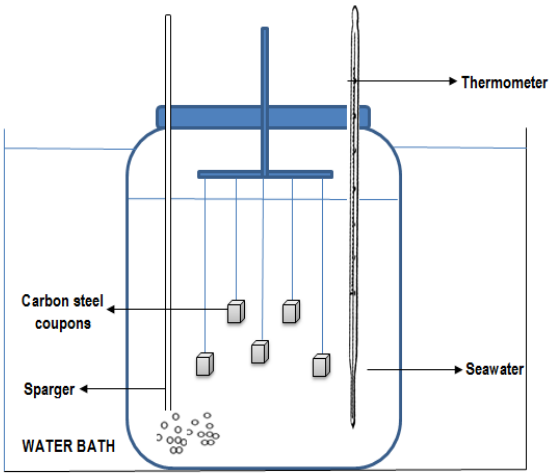

Figure 1 Schematic illustration of the reaction vessel used for experiments

2.3 Biofilm sample collection and analysis of microbial community composition

After the immersion period, coupons were retrieved from the electrochemical cells, washed with sterile seawater (to wash off planktonic cells) and immersed in 100 mL of sterile seawater containing filter-sterilized Tween 20 (0.1% w/v final concentration) [18]. Biofilms were aseptically recovered using 60 second-sonication steps (solution was refreshed between sonication steps) until no microbial cells were observed in the solution under a phase contrast microscope. The seawater was microbiologically analysed in order to compare the free-living bacterial communities present before the immersion period with the biofilm communities on the steel surface. Sonicated biofilm samples were centrifuged at 700 x *g* for 1 min to separate corrosion products from microbial cells which were then harvested by centrifuging at 10,000 x *g* for 20 min and resuspended in 200 µL phosphate buffer solution (PBS). DNA was extracted from the cells with a commercial soil kit (PowerSoil™ DNA Isolation Kit, MO BIO Laboratories Inc). The bulk 16S rRNA gene was amplified for denaturing gradient gel electrophoresis (DGGE) using nested PCR approach, which includes two consecutive PCR reactions with different primers. The primer pairs were 27F and 1492R, and BacV3f-GC and 907R, for the first and second PCR respectively (Table 1). Prior to DGGE analysis, PCR products were purified using the Ultraclean™ PCR clean up kit (MO BIO Laboratories Inc.). DGGE was performed using the DCode Universal Mutation Detection System (BIORAD) as per the manufacturer's instructions. The PCR product was mixed with DNA loading buffer (BIOLINE) and then loaded onto 7% (w/v) polyacrylamide gel (40% acrylamide/bis solution, 37.5:1) with a denaturing gradient of 30-70% urea-formamide in 1x TAE buffer. The DGGE was run at 150V (60°C) for at least 5 hours. DNA bands were visualized and excised from each lane using sterile scalpel blades. The bands were resuspended in 40µL of sterile RNase-free water overnight (4°C). Eluted DNA from individual bands was re-amplified by PCR using primers BacV3f no G-C clamp and 907R. Finally PCR products were visualised by electrophoresis and sequenced at Macrogen, South Korea. To identify the microbes, the sequence data was compared with 16S rRNA gene sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST; http:/www.ncbi.nlm.nih.gov/blast/) [19].

Table 1. List of primers used for PCR

primer	Amplification target	Sequence
27F	Bacterial 16S rRNA gene	5'- GAG TTT GAT CCT GGC TCA G -3'
1492R[#]	Universal 16S rRNA gene	5'- ACG G5T ACC TTG TTA CGA CTT -3'
BacV3f*	Bacterial 16S rRNA gene	5'- CCT ACG GGA GGC AGC AG -3'
907R	Universal 16S rRNA gene	5'- CCG TCA ATT CMT TTG AGT TT -3'

[#]Mixed base codes: 5 = deoxyribose inosine modification (universal base). (GeneWorks Pty Ltd).

*Primers BACV3f-GC had a GC rich clamp (5'- GCCCGCCGCGCGCGGCGGGCGGGGCGGG-3') in the 5'-end.

3. RESULTS AND DISCUSSION

3.1 Measurement of corrosion rates and surface analysis of corroded carbon steel

Average corrosion rates calculated from weight loss of triplicate specimens are shown in Figure 2. The highest corrosion rates were observed under aerobic conditions, regardless of the presence of bacteria. This was approximately ten times higher than the corrosion values observed under anaerobic conditions. In the presence of biofilms, carbon steel showed higher corrosion rates than those observed in controls under both aerobic and anaerobic conditions. Macroscopic analysis by visual inspection revealed that coupons exposed to aerobic seawater with and without bacteria exhibited uniform corrosion and a thick layer of accumulated orange iron oxides that fully covered the electrode surface (Figure 3). Once the outer oxides were removed from the coupon a thin oxide layer which was black in colour was evident closest to the surface. Specimens exposed to anaerobic conditions, in the presence and absence of bacteria, remained rust-free with some minor corrosion products unevenly distributed on the surface (Figure 4). Although none of these deposits was characterized, studies have shown that in seawater environments goethite (α-FeOOH) and lepidocrocite (γ-FeOOH) iron oxides as well as Fe (II–III) hydroxysulfates such as the sulphated green rust GR (SO_4^{2-}) are key compounds found in these layers and it is suggested that these compounds are likely to be the result not only of abiotic reactions but also of microbiological activity [20, 21].

It is generally accepted that the progressive formation of a thick rust layer on steel immersed in seawater tends to slow down its corrosion rate during the early stages of the corrosion process. During later stages, these passivating layers can be partially broken and pitting can take place. However, the formation and transformation of different compounds into rust layers is influenced by the ions presents in the solution, the oxygen content and the metabolic products resulting from microbial activity so that minor changes in these species may shift the properties of the rust from protective to aggressive at different times of exposure [22].

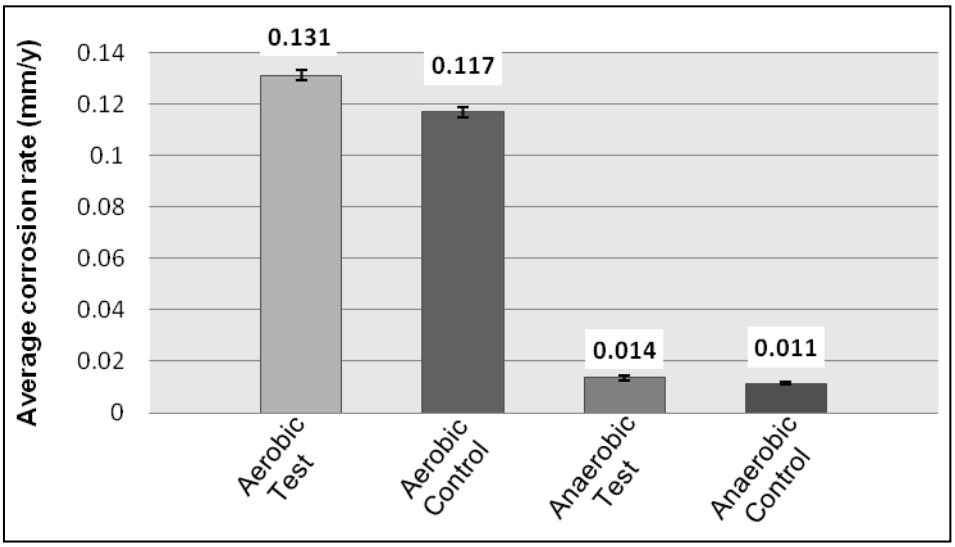

Figure 2 Average corrosion rates of carbon steel specimens exposed to seawater under aerobic and anaerobic conditions for 4 weeks. Bars indicate the standard deviation of three replicate results.

Figure 3 Macroscopic analysis of carbon steel coupon after 4 weeks exposure to seawater under aerobic conditions. Same pattern was observed in both test and control.

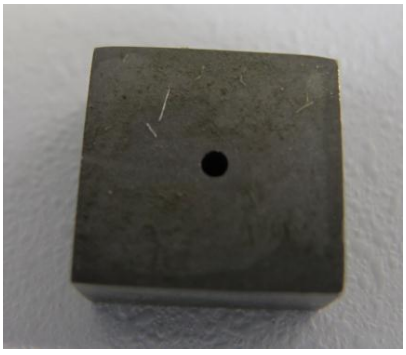

Figure 4 Macroscopic analysis of carbon steel coupon after 4 weeks exposure to seawater under anaerobic conditions. Same pattern was observed in both test and control.

Carbon steel surfaces were studied using the Alicona infinite focus microscope and software. Figures 5-10 show images of corroded surfaces after 4 weeks immersion in seawater under aerobic and anaerobic conditions. Coupons exposed to fully aerated seawater revealed uneven surfaces with a typical uniform corrosion pattern throughout the surface (Figure 5 and 6). The presence of bacteria in the seawater made no difference to this corrosion pattern. Although etching was accentuated in certain areas no pitting was detected on these surfaces regardless of the presence of bacteria. Specimens exposed to sterile seawater under anaerobic conditions (control) revealed a grit-polished surface with fewer irregular areas of uniform corrosion when compared to specimens exposed to aerobic conditions (Figure 7). Conversely, specimens exposed to seawater with bacteria under anaerobic conditions showed some areas of uniform attack, however several pits were observed regularly distributed on the surface (Figure 8-10). Profiles measurements of these pits (Figure 11) were obtained from 3D images of pits observed on the surface of coupons exposed to seawater containing bacteria under anaerobic conditions (anaerobic test). Pits were irregularly shaped and some of them had many invaginated areas. Pits depths ranged from 7 to 15 µm. Surface roughness profiles of corroded coupons after 4 weeks immersion period were also obtained. Figure 12 shows surface roughness of specimens exposed to seawater under aerobic and anaerobic conditions. Roughness of specimens exposed to aerobic seawater with bacteria (aerobic test) was rather the same to the roughness seen in the control (Figure 12.a) Surfaces roughness of coupons exposed to anaerobic seawater without bacteria (anaerobic control) showed similar patterns to those for aerobic test and control (Figure 12.b). Specimens exposed to anaerobic seawater with bacteria (anaerobic test) showed a distinctive pattern of surface roughness in which higher mean values of roughness depths (deepest peaks) were detected as compared to the other tested conditions (Figure 12.c).

In this study, higher corrosion rates were obtained in coupons immersed in continually aerated seawater than in coupons exposed to anaerobic conditions. It is well known that once a freshly ground steel surface is immersed in neutral aerated seawater oxygen reduction is the main oxidation reaction at the surface with the consequent build-up of oxide layers that gradually cover the surface. If the surface is not homogeneously covered by these layers, uncovered areas can remain in contact with the electrolyte and oxidation of iron can take place constantly. As this study observed corrosion at a single time point it is not possible to predict the phenomenon that took place under aerated conditions. However the absence of pits on the surface suggests that a very uniform mechanism predominated over the surfaces until the end of the immersion time. In the absence of oxygen, corrosion mechanisms can result from secondary cathodic reactants such as those related to microbial activities. Examples include the production of ferrous sulphide, volatile phosphorus compounds or even a direct oxidation of cathodic hydrogen from the surface [23].

Previous studies have shown that the formation of an evenly distributed biofilm on carbon steel surfaces may act as a barrier to protect the steel from aggressive species in the electrolyte resulting in lower corrosion rates [1]. In this study we observed that biofilms were not protective but actually enhanced the corrosion process but only to a small extent. Nonetheless, observed differences in surface attack patterns under anaerobic conditions in the presence of bacteria but not in controls are of major interest as it suggests a possible key role played by anaerobic bacteria in biofilms. However, as these results only represent a single time-point, caution must be taken in extrapolating to long-term applications. The increase in corrosion rates under the presence of biofilms observed in this study could be attributed to several mechanisms. For instance, the effect of a direct bacteria-metal interaction where bacteria may extract electrons directly from the steel surface as it has been observed before [24], the formation of patches of biofilm growth and extracellular polymeric substance (EPS) [25] or the result of microbial metabolic pathways. Any of these mechanisms could result in a more localized type of attack and a faster perforation of the steel.

3.2 Analysis of bacterial community composition in biofilms on carbon steel surfaces

The DGGE fingerprinting of the 16S rRNA gene PCR products of both seawater and biofilm samples that developed on the carbon steel specimens is shown in Figure 13. Duplicate DGGE patterns were obtained for the seawater (Figure 13, lanes 1 and 2) and biofilms from both aerobic (Figure 13, lanes 3 and 4) and anaerobic (Figure 13, lanes 5 and 6) conditions. No DNA was detected in extracts from control samples using the same DNA extraction procedure described above. The number of individual bands obtained in a DGGE analysis is related to the number of bacterial populations in the samples with each band theoretically corresponding to one microbial population in the community. All detected bands were excised from the gel and sequenced. Duplicates samples showed good reproducibility in DGGE patterns. Numerous bands were observed in the seawater sample which reflects the diversity of free-living communities. From the DGGE profiles, it is seen that although a sub set of the bacterial populations detected in the seawater were also found in the biofilms some of the bands identified on biofilms samples were not detected in the seawater. One explanation for this could be that these populations were present in low numbers in the seawater and their DNA failed to be recovered. However once they colonized the coupon surfaces the biomass increased in biofilms thus enabling them to be detected in the biofilms after the immersion period. This was particularly apparent in the anaerobic populations whose metabolism and growth are significantly favoured by the absence of oxygen.

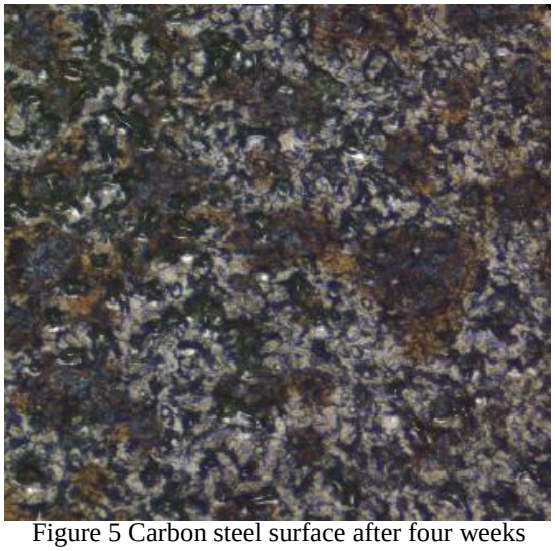

Figure 5 Carbon steel surface after four weeks exposure to sterile aerated seawater (Aerobic control) (100X magnification)

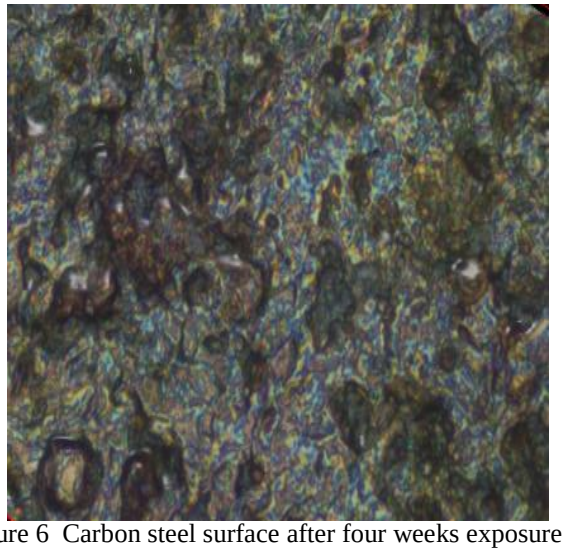

Figure 6 Carbon steel surface after four weeks exposure to aerated seawater containing marine bacteria (Aerobic test) (100X magnification)

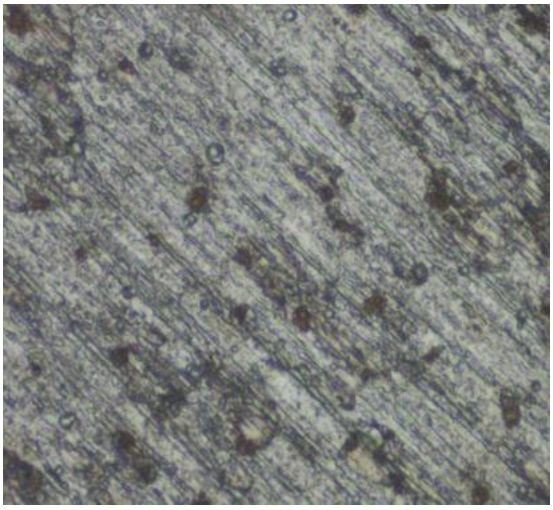

Figure 7 Carbon steel surface after 4 weeks exposure to sterile seawater under anaerobic conditions (Anaerobic control) (100X magnification)

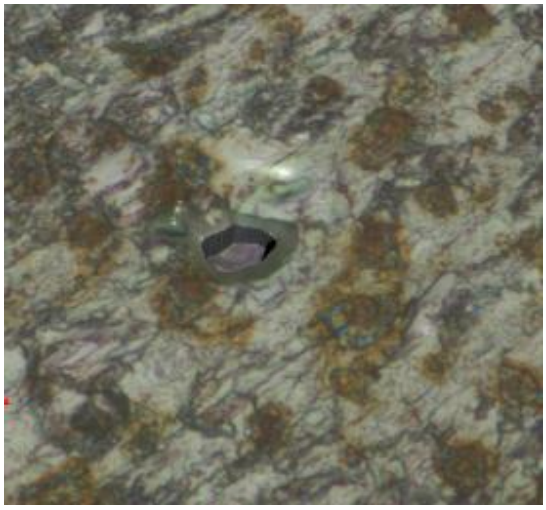

Figure 8 Typical pits observed on carbon steel surfaces after 4 weeks exposure to anaerobic seawater containing marine bacteria (Anaerobic test) (100X magnification)

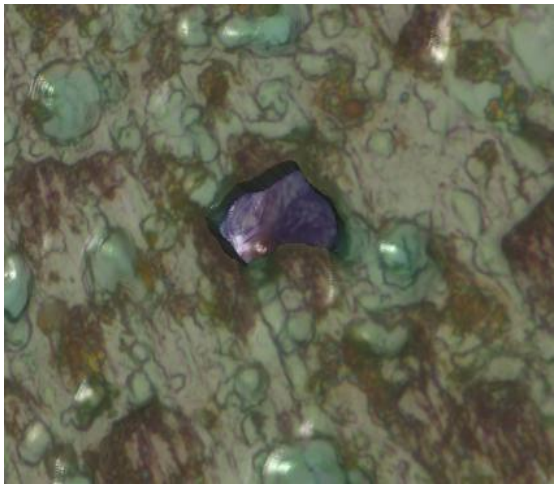

Figure 9 Typical pits observed on carbon steel surfaces after 4 weeks exposure to anaerobic seawater containing marine bacteria (Anaerobic test) (100X magnification)

Figure 10 Typical pits observed on carbon steel surfaces after 4 weeks exposure to anaerobic seawater containing marine bacteria (Anaerobic test) (100X magnification)

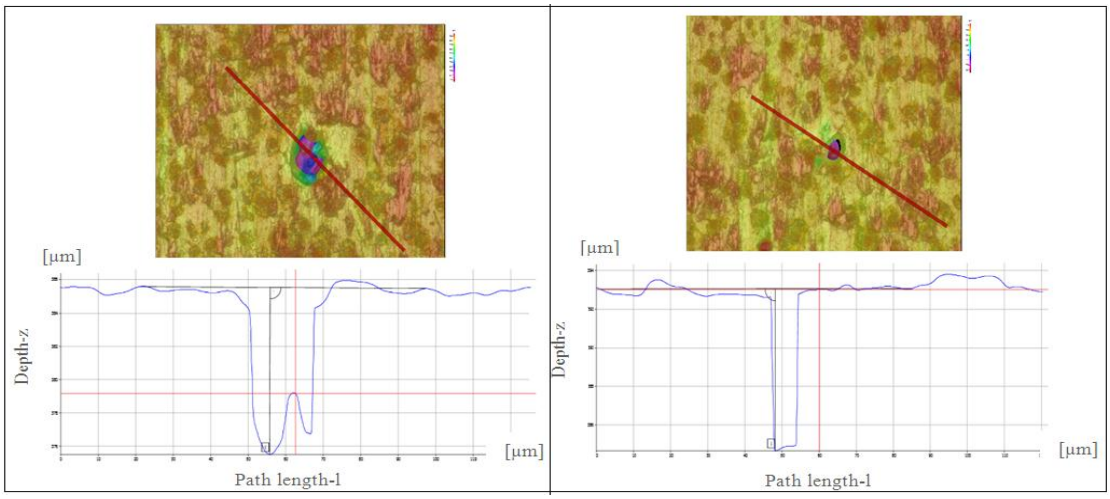

Figure 11 Pit profile measurements of two different pits observed on carbon steel surfaces exposed to seawater containing marine bacteria under anaerobic conditions (Anaerobic test). Analysis performed by Alicona Infinite Focus Microscope (100X magnification).

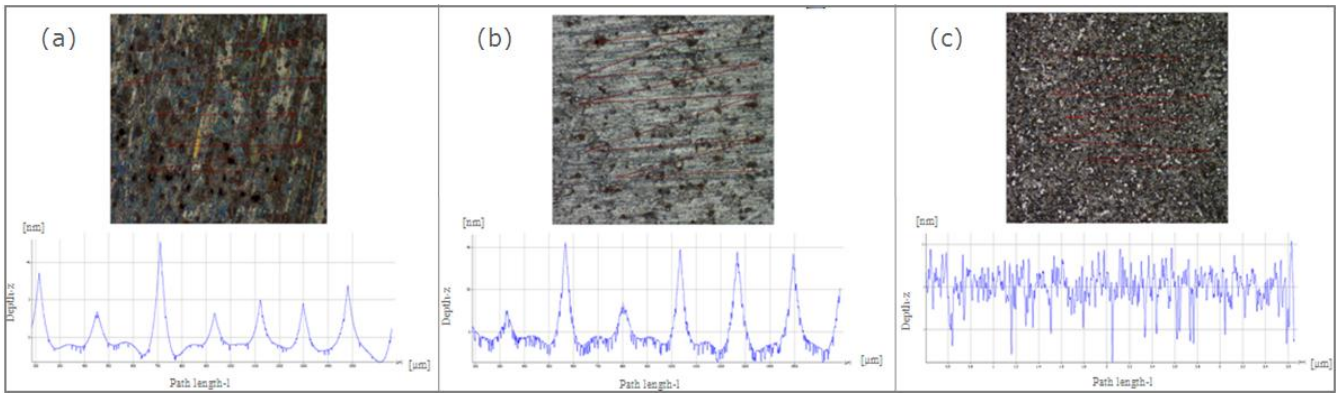

Figure 12 Surface roughness profiles of carbon steel exposed to seawater for up to four weeks. Analysis performed by Alicona Infinite Focus Microscope (50X magnification). (a) Aerated seawater (same pattern in test and control); (b) Anaerobic control; (c) Anaerobic test (with bacteria).

The DGGE profiles also showed a higher microbial diversity in aerobic biofilms than in anaerobic biofilms as reflected by the number of bands per sample in the gel. However, in some cases two different bands detected in the DGGE were characterized as from the same bacterial population once the sequence analysis was performed (Figure 13, aerobic tests: bands 1 and 2 corresponded to *Pseudomonas* sp.). This has been reported in earlier studies and the occurrence of multiple copies of the 16S rRNA gene in some bacterial chromosomes has shown to result in several bands for one single bacterial population [26]. This highlights the importance of conducting analysis of DGGE profiles in conjunction with DNA sequencing in order to ensure a proper interpretation of the microbial diversity in biofilm samples.

In this study, populations identified in aerobic biofilms included *Sulphitobacter* sp., *Caulobacter* sp., *Rhodobacter* sp. and *Alcanivorax* sp. *Sulphitobacter* sp. has shown to be able to use energy from the oxidation of thiosulfate, sulphur and sulphite to sulphate during grow in the presence of an organic carbon source under aerobic conditions and has been found to be one of the dominant sulphate-forming population in the Black Sea [27]. Therefore a sulphur cycle involving *Sulphitobacter* sp. and sulphate reducing bacteria is likely to occur in nature. *Alcanivorax* spp. have been previously identified in marine biofilms grown on stainless steel surfaces but no localized corrosion was observed on those steels [15]. C*aulobacter* sp. have demonstrated unique adhesion properties that enable the formation of stable monolayer biofilms on surfaces but their role in biofilm-related corrosion has not been previously reported. In a previous study *Rhodobacter* spp. were shown to be the most important surface-colonizing bacteria during early stages of colonization of submerged surfaces in coastal marine environments (28).

While there appeared to be discrete microbial populations in aerobic and anaerobic environments some commonly encountered populations were identified in both aerobic and anaerobic biofilms. These were *Pseudomonas* sp. (Figure 13. aerobic test: band 1 and 2; anaerobic test: band 1) and *Sphingomonas* sp. (Figure 13, aerobic test: band 3; anaerobic test: band 2). *Sphingomonas* spp. have been classified as strictly aerobic bacteria and their identification in anaerobic environments has not been reported previously. *Sphingomonas* sp. was reported as an acid producing microorganism and extracellular heteropolysacarides were copiously produced under aerobic conditions [27]. *Pseudomonas* spp. have been

classified as facultative anaerobes with the ability to grow and form robust biofilms in both aerobic and anaerobic environments [27]. In the presence of oxygen facultative bacteria favour aerobic respiration; however when oxygen is depleted alternative electron acceptors such as nitrates and sulphates can be used [29]. Under anaerobic conditions, *Pseudomonas* sp. has shown to be an important denitrifying bacterium [27. In adittion, *Pseudomonas* spp are usually associated with MIC processes especially through their ability to produce exopolysaccharides that facilitate microbial attachment to surfaces [30].

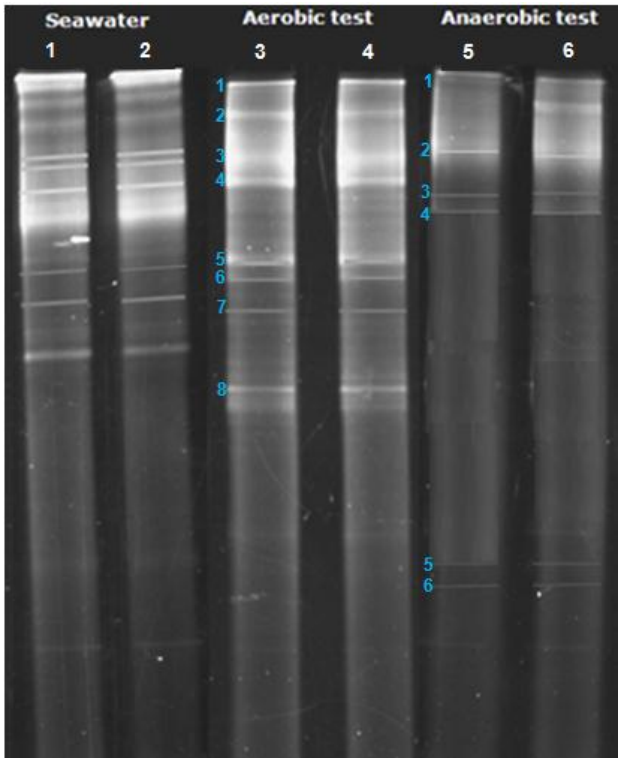

Figure 13 DGGE profile of partial 16S rRNA genes from DNA extracted from seawater (lanes 1 and 2) and biofilm samples developed on carbon steel surfaces exposed to seawater under aerobic (lanes 3 and 4) and anaerobic conditionsr (lanes 5 and 6). Numbers next to the lanes refers to the number of bands per sample.

In this study, *Agrobacterium* sp. was the only bacterial population exclusively identified in anaerobic biofilms (Figure 13, anaerobic test: bands 5 and 6). *Agrobacterium* spp. are facultative anaerobes and their growth on carbohydrate-containing media has shown to be typically accompanied by copious amounts of extracellular polysaccharides [27]. Also *Agrobacterium spp.* have been recognized as denitrifying bacteria [31]. Activities of denitrifying populations have shown to promote pitting corrosion in specimens exposed to media with nitrate when testing microbial souring control by the biocompetitive exclusion method (BCX) [32, 33]. One study also reported two denitrifying bacteria as the most frequently found species in natural gas pipelines [26]. However the mechanism of anaerobic corrosion in the presence of denitrifying bacteria has not been previously explained. Unexpectedly, sulphate-reducing bacteria (SRB) the most commonly identified microorganisms in studies of anaerobic corrosion, were not identified in this study. There are a number of possible explanations for the absence of SRB in biofilms developed on carbon steel under anaerobic conditions. One of those could be the predominant occurrence of denitrifying bacteria as detected from the PCR-DGGE. Since nitrate reduction is more energetically favourable than sulphate reduction, competitive exclusion may have been a factor [32].

Metabolic activities that have been previously reported for the microbial populations identified in this study may be used to suggest their potential role in MIC. However, the specific corrosion mechanisms taking place in the presence of these groups of microorganisms and the electrochemical reactions at the carbon steel/electrolyte interface are not likely to be explained without conducting specific mechanistic studies. In this study the denitrifying capability of bacterial populations identified in anaerobic biofilms was not investigated therefore no associations can be established between nitrate metabolism and the localized corrosion observed on the material surface beneath these biofilms. Nevertheless, as SRB populations were not identified as part of the anaerobic biofilm community and given that facultative anaerobes with a potential to carry out a denitrifying metabolism were identified in those biofilms it can be suggested that the enhanced corrosion observed on specimens covered by anaerobic biofilms could have a relationship with bacterial denitrifying activities.

These results have shown that the presence of microbial populations in biofilms on carbon steel surfaces strongly depend upon the oxygen pressure conditions. A higher number of populations detected in aerobic biofilms reflect that aerobic

metabolism is the preferred respiration pathway in most of populations in seawater where oxygen is mostly available and distributed throughout the ocean and where a high energy-efficient aerobic metabolism could be more advantageous. Also, rusting might provide a more heterogeneous surface that possibly will favour diversity in biofilms. As minor changes in water chemistry can selectively enhance or suppress particular microbial metabolic activities it is expected that multiple corrosion mechanisms operate at different exposure conditions. As facultative microorganisms can control transcriptional activation of alternative electron transport pathways when responding to changing levels of oxygen and electron acceptors, facultative populations could be essential in biofilm communities as they possibly provide a continuous active metabolic pathway especially when oxygen concentrations are extreme for the growth of obligate aerobic or anaerobic bacteria. For the results of this study indicating the dominance of denitrifying bacteria in biofilms on surfaces that underwent localized corrosion and given that denitrifiers are metabolically diverse in terms of alternative energy-generating mechanisms they can be expected to play an important role in MIC.

4. CONCLUSIONS

1. Carbon steel specimens immersed in aerated seawater for four weeks exhibited higher corrosion rates than specimens under strict anaerobic conditions both in the presence and absence of microorganisms.. Biofilms on carbon steel surfaces exposed to seawater under aerobic and anaerobic conditions could enhance the corrosion rate of the material to a small extent as compared to the controls.
2. Biofilms that developed on carbon steel surfaces under anaerobic conditions induced a pitting type of attack and exhibited surface roughness patterns that were not observed in abiotic controls or on specimens covered by biofilms formed under aerobic conditions. Irregular pits with depths in the range of 7-15 µm were distributed on the entire surface of those specimens tested under anaerobic conditions. Thus, the presence or activities of anaerobic bacteria can be linked to the corrosion of carbon steel immersed in deaerated seawater.
3. Analysis of the microbial communities in the biofilms colonizing the carbon steel surfaces revealed that microbial diversity was higher in the aerobic biofilms when compared to the anaerobic biofilms. *Pseudomonas* sp. and *Sphingomonas* sp. were commonly encountered in biofilms developed under aerobic and anaerobic conditions. Populations identified in anaerobic biofilms are expected to have taken part in the observed localized corrosion of carbon steel exposed to seawater under anaerobic conditions. As those populations have previously shown denitrifying capabilities under anaerobic conditions, the nitrate metabolism is suggested to play a key role in MIC mechanisms.
4. PCR-DGGE and DNA sequencing analysis of the microbial composition of biofilms proved to be a powerful method to show the selectivity of microbial populations in response to environmental factors such as the oxygen content and its interaction with corroding surfaces in seawater. However, the phenomenon of MIC is complex and further investigation of biofilm populations involving mechanistic studies needs to be conducted to better understand microbial-metal interactions in marine environments.

5. ACKNOWLEDGMENTS

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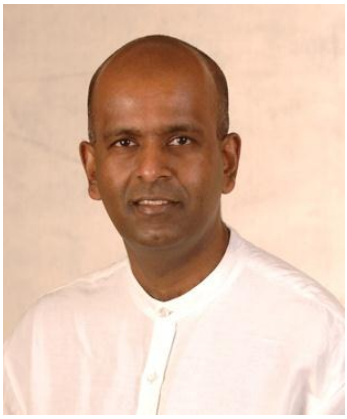

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Appendix 4

Original reprint of publication

Chapter 5

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Crevice corrosion of duplex stainless steels in the presence of natural marine biofilms

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ABSTRACT

The evaluation of crevice corrosion of high alloy stainless steels used in offshore applications is of major importance as it is one of the most deleterious forms of localized corrosion which may result in sudden marine corrosion failure. The resistance of UNS S31803 duplex stainless steel (DSS) to crevice corrosion in natural seawater was evaluated by immersion and electrochemical tests. Artificially creviced specimens were tested before, during and after immersion in natural seawater under stagnant conditions for up to eight weeks allowing indigenous marine microorganisms to adhere to the alloy surface and form a biofilm. The changes in biofilm community structure and the influence of biofilm on the crevice corrosion of DSS specimens in seawater were investigated at two different exposure times (4 and 8 weeks) using a combination of potentiodynamic and potentiostatic measurements, surface inspection and bacterial community profile analysis by 16S rRNA gene PCR–DGGE and DNA sequencing. Results indicate that our selection approach to evaluate crevice corrosion yields highly reproducible results. Crevice corrosion was observed only in electrochemically polarized specimens that had been exposed to natural seawater containing bacteria. The possible mechanisms involved in the biofilm enhanced crevice corrosion are discussed.

Key words: Crevice corrosion, duplex stainless steel, seawater, biofilms, PCR-DGGE, bacterial community structure.

INTRODUCTION

Highly alloyed stainless steels have been considered as important alternatives to conventional low grade steels in aggressive environments. For marine applications, duplex stainless steels (DSS) such as UNS S31803 are becoming important structural materials due to their combination of high strength, resistance to localized corrosion and competitive price when compared to austenitic stainless steels, ferritic stainless steels and nickel based alloys. The performance of alloys in chloride environments is

generally established under laboratory conditions where parameters such as critical temperatures and potentials for localized corrosion initiation are determined to predict their performance in an actual application.[1, 2] However, in real service conditions, these alloys may still be attacked due to unforeseen circumstances.[3,4] In addition, during the manufacturing and installation period, the interior of pressure equipment is exposed to water for hydrostatic pressure testing, ballast and preservation, and this water can contain some residual bacteria. If unexpected delays occur, this equipment can lay dormant for extended periods, still containing water, during which the activity of bacteria can increase, as the effectiveness of the preservation chemicals decays and the seepage of seawater mixes with the volumes of treated fluids. These bacteria will form biofilms on the alloy surface and will likely promote electrochemical reactions that lead to corrosion in a phenomenon known as microbiologically influenced corrosion (MIC). In addition, high resistant alloys such as stainless steel are an ideal substratum for microbial colonization due to the lack of corrosion products on the surfaces.[5] However, the main concern with highly alloyed steels in seawater is crevice corrosion, a form of localized attack that occurs within occluded regions or crevices such as those man-made by design (washers, pipe flanges, joints, etc) or those naturally occurring (deposits, biofouling, debris). Crevice corrosion has been recognized as one of the most detrimental forms of localized corrosion that takes place under less aggressive conditions when compared with other forms of corrosion.[6] In addition, service failures have mainly been reported as a result of crevice corrosion, possibly not only because of the complexity in experimentally simulating the type of crevices found in actual structures, but also because it is almost impossible to avoid crevices completely in practical operations. These findings highlight the importance of investigating the crevice corrosion mechanisms of highly alloyed stainless steels in seawater and the possible relationship between naturally occurring marine bacteria and the corrosion performance of creviced alloys in seawater, in order to minimize the risk of equipment failures in marine environments.

In this study we evaluated the crevice corrosion resistance of a DSS in stagnant seawater at 20°C by conducting immersion tests, potentiodynamic polarization scans and potentiostatic determination of the critical crevice temperature (CCT). In particular, we examined the influence of marine biofilms on the crevice corrosion initiation, propagation and repassivation of the DSS in seawater. The changes in community structure during biofilm growth on the alloy surface were studied using denaturing gradient gel electrophoresis (DGGE) and DNA sequencing to help establish any association between biofilm community structure and crevice corrosion of UNS S31803 as a function of exposure time. Tested surfaces were subsequently examined by optical microscopy.

EXPERIMENTAL PROCEDURE

Specimen preparation and test conditions

Artificially creviced coupons of UNS S31803 duplex stainless steel (45 x 45 x 5 mm) were suspended in natural seawater and maintained at 20°C under stagnant conditions in 10 L reaction vessels for up to eight weeks (Figure 1). The composition (in wt%) of the steel was: Cr 22.35, Ni 5.72, Mo 3.16, N 0.18, Mn 1.53, C 0.015. To avoid crevice corrosion in areas other than the crevice formers, samples were electrocoated using a protective epoxy at the surface area where the spot weld was made for electrical connection and further covered by epoxy resin. Prior to immersion, specimens were wet-ground to 600 grit finish, degreased with acetone and dried with nitrogen. Crevice assemblies were prepared based on the design of a Round Robin test[7] using PVDF crevice formers and an applied torque of 3 N.m. Specimens and all pieces for the crevice assembly were soaked in decontaminant solution for 3 hours and sterilized by immersion in 70% ethanol for 1 hour before exposure. Control experiments consisted of artificially creviced coupons immersed in sterile seawater (0.22 µm filter-sterilized natural seawater). Reaction vessels were maintained at 20°C using a refrigerated incubator.
Specimens were removed from the reaction vessel after 4 (t1) and 8 (t2) weeks exposure. Additionally, freshly ground creviced alloys were electrochemically tested in natural seawater at 20°C (t0). Electrochemical measurements, biofilm analysis and surface studies were conducted at each exposure

time. Before surface inspection, coupons were cleaned by following the standard procedure[8] and surface profile analysis was conducted using optical microscopy.

Electrochemical measurements

The corrosion potential, E_{corr}, of specimens was monitored daily throughout the immersion period. Crevice corrosion was evaluated in seawater at 20°C by measuring cyclic polarization scans at the times t0, t1 and t2 to determine the breakdown potential (E_b) and the repassivation potential (E_r) of the steel as a function of exposure time. Electrochemical tests were carried out in separate vessels using a three-electrode set up where the steels served as working electrodes, a platinum coated mesh was used as counter electrode and a Ag/AgCl electrode as reference electrode, RE.[9] Polarization curves were conducted using a forward and reverse scan rate of 0.167 mV/sec. The sweep direction was reversed when either a current density of 1.5 mA/cm^2 or a potential of 1.5 V vs. Ag/AgCl was reached. The initial and final point of the scan was set at a potential of -0.1 V vs. E_{corr}. The E_b was identified as the potential where the anodic current indicated the onset of transpassivity or stable crevice.[10] The E_r was identified as the potential at which the forward and reverse scans intersect, which is the potential at which crevice corrosion will cease to grow.[11] One sample per vessel was kept in open-circuit conditions to evaluate if crevice corrosion can occur with no external applied potential. In addition, potentiostatic evaluation of the critical crevice temperature (CCT) of specimens at t0, t1 and t2 was investigated using a critical temperature electrochemical cell following the standard recommendations.[12] To conduct the CCT test, specimens were retrieved from the reactions vessels at the predetermined exposure time and placed in the electrochemical cell which was filled with fresh natural seawater. CCT tests were conducted at an applied anodic potential of +700 mV vs. Ag/AgCl while the solution temperature was increased from 10°C until the CCT value was reached using a scan rate of 0.03˚C/min. Continuous stirring of the test solution was used to minimize the temperature lag between solution and specimen. The CCT test was set to stop when the current density exceeded 50 µA/cm^2 for 60 seconds indicating the onset of crevice corrosion. This value was chosen because it corresponded to a marked deviation from the passive current density of the alloy at the test temperature.

PCR-DGGE analysis of 16S rRNA gene fragments of biofilm cells

Detachment of biofilm cells was performed as previously described.[13] DNA was extracted using a commercial DNA isolation kit. The bulk 16S rRNA gene was amplified for denaturing gradient gel electrophoresis (DGGE) using nested PCR approach, which includes two consecutive PCR reactions with different primers. The primer pairs were 27F and 1492R, and BacV3f-GC and 907R, for the first and second PCR respectively (Table 1). These primers are general bacterial primers and will amplify most bacteria but not other microorganisms. DGGE was performed using the PCR-amplified 16S rRNA gene fragments to characterize the microbial community in biofilms developed on the steel coupons. The PCR products were mixed with DNA loading buffer and loaded onto 7% (w/v) polyacrylamide gel (40% acrylamide/bis solution, 37.5:1) with a denaturing gradient of 40-60% urea-formamide (100% denaturant: 7M urea, 40% deionised formamide) in 1x TAE buffer (2 M Tris base, 1 M glacial acetic acid, 50 mM, EDTA, pH 8.0). The DGGE was run at 150 V (60°C) for at least 5 hours. DNA bands were excised from each lane using sterile scalpel blades. The bands were resuspended in 40 µL of sterile RNase-free water overnight (4°C). Eluted DNA from individual bands was re-amplified by PCR using primers BacV3f no G-C clamp and 907R. Finally PCR products were visualised by electrophoresis and sequenced. DNA sequences were compared against reference sequences in the GenBank[(1)] database using a software tool[14] to identify the most similar species in the database.

RESULTS AND DISCUSSION

Electrochemical measurements

[(1)] Trade name.

Figure 2 shows the average E_{corr} versus time data of creviced UNS S31803 immersed in sterile seawater (Control) and in seawater containing bacteria (Test) for 8 weeks. It can be seen that test and control displayed similar corrosion potential versus time transients until approximately the 40th day of exposure. During these initial stages of exposure, E_{corr} of both test and control was relatively steady with only a few oscillating periods, which can be related to active–passive stages at the surface.[15] After the 40th day of exposure, E_{corr} of the alloy in sterile seawater remained steady until the end of the exposure whereas the E_{corr} of the alloy in seawater with bacteria showed a gradual shift in the negative direction until the completion of the immersion period. This drop in the corrosion potential toward negative values has been found to be related to passivity deterioration[16] and processes involved in crevice corrosion initiation.[7]

Electrochemical parameters and critical crevice temperatures (CCT) identified at the times t0, t1 and t2 are summarized in Table 2. It can be seen that CCT were rather similar regardless of the exposure time and the presence of microorganisms in the seawater. Initially, the CCT value was expected to be influenced by the presence of a biofilm through different mechanisms, e.g, release of aggressive bacterial metabolites inside the crevice, creation of a diffusion barrier for oxygen into the crevice and/or depletion of oxygen in the crevice solution due to microbial respiration. Any of these mechanisms could disrupt the stability of the passive film, decrease the likelihood of repassivation, facilitate depassivation processes and promote crevice corrosion initiation. However, our results show that the CCT of UNS S31803 remained effectively unchanged throughout the immersion time regardless of the presence of biological components in the seawater. The stable CCT values observed in different settings may be due to a number of factors. For instance, a limited abundance of microorganisms on the alloy surface may have contributed to the availability of oxygen to sustain passivation process on the alloy surfaces. In addition, nutrients required for bacterial growth may have been exhausted due to the closed experimental setup. Hence, electrochemical mechanisms are likely to dominate the corrosion processes and biological activity perhaps had only limited influence on the initiation time for crevice corrosion. Besides, material properties related to temperature resistance may also play a role in the conservative character of the CCT. Figure 3 shows some optical images of the surface of UNS S31803 after CCT determination at the completion of 8 weeks of exposure in seawater. Surface inspection at the crevice boundaries revealed the presence of small areas of concentrated attack and larger unaffected areas that exhibited only etching. This pattern of attack was found in all the surfaces after CCT testing regardless of the immersion time and the exposure to bacteria in seawater. Overall, the potentiostatic method used in this study to determine the CCT of UNS S31803 proved to be a very practical, simple and highly reproducible technique to evaluate crevice corrosion as a function of temperature.

Crevice corrosion was also evaluated by measuring the E_b and the E_r of UNS S31803 in seawater at the times t0, t1 and t2 by measuring cyclic polarization scans at 20°C (Table 2). In all cases, passivity breakdown occurred through transpassive dissolution rather than crevice corrosion[17] which means that passivity breakdown took place after the samples were anodically polarized to potentials above +800 mV. No differences were observed in the E_b regardless of the immersion time and the presence of microorganisms in the seawater. Similarly, the E_r was found to be very positive (~+900 mV) in all cases except for the specimens exposed for eight weeks to seawater containing bacteria (t2-test). These specimens displayed an average E_r of -227 mV.

The potential versus current density transients obtained by cyclic polarization scans are illustrated in Figure 4. In this figure, polarization curves at t1 (Figure 4 (a)) and t2 (Figure 4 (b)) are displayed against t0, which corresponds to freshly ground specimens evaluated in natural seawater at 20°C. It can be seen that there are no major differences in the scans of t0, and the test and control at t1, except for the slightly higher current densities found in both t1-test and t1-control as compared with t0. In addition, the passivation area in specimens tested at t1 was quite irregular and poorly formed as compared with t0 where a well-defined passive film is developed and stabilized during the forward scan (Figure 4 (a)). Similar results have previously been reported for crevice-free alloys in seawater.[13]

Figure 4 (b) shows the polarization curves of specimens after 8 weeks exposure. In this case, polarization curves of t2-control and t1-control were similar, indicating that the corrosion of the creviced

alloy was not further influenced by the exposure time in seawater under sterile conditions. On the other hand, specimens exposed for 8 weeks to seawater containing bacteria (t2-test) displayed a substantially different polarization scan unlike those observed on other specimens/conditions tested in this study. During the forward scan, the alloy displayed very high current densities until the E_b was reached at a very positive potential. However, during the reverse polarization, the transient tracked back along the forward scan for about 300 mV and then the current started to increase and remained fairly constant while the potential decreased. This resulted in the late formation of a hysteresis loop and very active metal dissolution. Finally, the repassivation potential was reached at a negative potential (-227 mV). The reason for this is unclear yet but a possible mechanism involving the formation of soluble transpassive corrosion product species that induce a gradual acidification of environment inside the crevice sufficient to initiate crevice corrosion may be proposed.[18] However, as this was not observed in our control experiment, the mechanism that took place was most likely the result of the interaction between the transpassive dissolution process and the marine biofilm developed on the creviced alloy during the 8 weeks of exposure to stagnant seawater. Since crevice corrosion is usually considered to happen if the corrosion potential of a metal in a given environment exceeds the repassivation potential,[19] the phenomenon observed here should be of major concern. Figure 5 shows some selected surface images of the electrochemically polarized UNS S31803 after eight weeks exposure to seawater with and without bacteria. Surface inspection confirmed the electrochemical findings with pronounced crevicing evident only in polarized surfaces exposed to seawater with bacteria for up to eight weeks.
Specimens immersed in the stagnant seawater at 20°C that were not electrochemically tested at the completion of the immersion time (no external polarization was applied) remained completely unaffected throughout the exposure period regardless of the formation of a biofilm on the surface as indicated by surface inspection conducted at the times t1 and t2. Figure 6 shows the surface image of UNS S31803 after exposure to seawater containing bacteria for up to 8 weeks and that was not electrochemically tested at the completion of exposure. Since the surface remained unaffected and the crevice boundaries were not evident, a ring was drawn on the surface to indicate the area where the crevice former was in contact with the surface during the exposure period (Figure 6(a)). Figure 6 (b) is an expanded, cross section image of the same surface at the crevice boundaries. Same results were observed in all specimens that were not electrochemically polarized regardless of the immersion time and the presence of biofilm.

Microbial community composition by PCR- DGGE and 16S rRNA gene sequencing

The free-living bacteria in the seawater used as test solution as well as the attached bacteria at two stages of biofilm growth (t1: 4weeks; t2: 8weeks) were examined by PCR-DGGE. DGGE profiles of 16S rRNA bacterial gene fragments amplified from DNA samples are shown in Figure 7. The number of individual bands obtained in each lane of the DGGE is related to the number of the major bacterial populations present in the sample. Each band was excised from the gel and sequenced. The sequence information is given in Table 3. It can be seen that most of the populations were commonly found in both the early and late stages of the biofilm growth. As shown in Figure 7, populations represented by bands 1 and 2 were unique to the free-living community in the seawater and were not found in the biofilm. In contrast, bands 3, 4, 5, 9 and 10 were only detected in the biofilm but not in the seawater. One reason for this could be that these populations were present in low numbers in the seawater at the beginning of the immersion period and their DNA failed to be recovered. However once they colonized the alloy surface the biomass increased in the biofilm thus enabling them to be detected in the DNA sample.
It can be seen that the population represented by band 10 existed in the biofilm of t1 but disappeared in the biofilm of t2. In contrast, the population represented by band 3 appeared only in the biofilm of t_2 and was not found in the biofilm of t1. The shift in the bacterial community structure as the biofilm developed on the creviced surface suggest that some surface changes could have taken place during the exposure period. A modified surface could have created a selective environment for the growth of some particular populations and the dormancy or decrease of others. It is well known that the mechanism of crevice corrosion involves several steps, including oxygen depletion inside the crevice, formation of dissolved metal ions with subsequent acidification of the crevice solution, and finally

electromigration of chloride ions into the crevice.[20] Therefore, it is very likely that surface changes in creviced alloys associated with prolonged exposure times in seawater may have some effect on the bacterial community structure of the biofilm. In addition, once the bacterial populations were established they could have enhanced the aggressiveness of the metal-solution interface progressively so that during the late stages of biofilm growth the passive film of creviced surfaces could become more weakened and easier to breakdown.

It is important to highlight that crevice corrosion was only observed in polarized specimens (max. 1.5 V vs. Ag/AgCl) after exposure to seawater containing bacteria for up to 8 weeks (t2-test) and was not detected in the control (t2-control). In addition, crevice corrosion was either not found in specimens exposed to seawater for up to 4 weeks, with or without bacteria, or in samples that were not electrochemically polarized at the completion of exposure regardless of the presence of microorganisms. Results from the electrochemical testing, immersion tests, surface inspection and the analysis of bacterial community structure in biofilms of artificially creviced UNS S31803 suggest a relationship between a transition from transpassive dissolution to crevice corrosion and the shift in the community structure in the biofilm from t1 to t2. In addition, our results suggest that crevice corrosion was exclusively triggered by the external potential applied. The effect of applied potential on biofilms developed on metallic surfaces has been noted previously [21, 22] but is not well understood. Studies on bacterial adhesion show that bacteria in aqueous suspensions are almost always negatively charged [23] so it is expected that bacteria are further attracted to anodically polarized electrodes. These electrostatic interactions between surfaces oppositely charged will favour irreversible adhesion. From our results, it appears that the applied potential we used altered the biofilm/passive film interactions in a way that induced crevice corrosion initiation, i. e., the applied potential could have disrupted the oxide layer on the steel surface sufficiently to permit the microorganisms already present in the overlying biofilm to gain access to the metal. Once in contact with the steel the microorganisms may have made repassivation more difficult. Alternatively, the biofilm on the specimens may have responded to that applied potential and acted as a catalyst for the initiation of crevice corrosion. On the other hand, the biofilm developed at t2 could have induced changes in the passive film structure thus creating a more active surface as indicated by the active corrosion potential and the passive current densities measured at the late stages of the biofilm growth. These surface changes could have assisted depassivation and crevice corrosion initiation in the presence of aggressive transpassive corrosion product species formed in the surface of specimens polarized at the potential of the transpassive state.

CONCLUSIONS

- The CCT of freshly ground UNS S31803 in seawater was found to be 38.22°C ±0.06 using a potentiostatic method. CCT values were not affected by exposure time and by the formation of a biofilm on the surface.
- Crevice corrosion occurred only in UNS S31803 specimens electrochemically polarized to 1.5 V after 8 weeks exposure to seawater at 20°C containing naturally occurring marine bacteria. These specimens displayed very positive E_b values and negative E_r values. Crevice corrosion did not take place in UNS S31803 specimens electrochemically polarized to 1.5 V (Ag/AgCl) that were expose only to sterile seawater at 20°C regardless of the immersion time.
- Crevice corrosion did not occur in specimens exposed to seawater at 20°C that were not electrochemically polarized at the completion of the immersion period regardless of the exposure time and the presence of marine biofilms.
- There was a shift in the bacterial community structure as the biofilm developed on the surface of creviced UNS S31803 in seawater. Modifications in the surface of creviced alloys as a function of immersion time may have induced changes in the bacterial composition in the biofilm.
- Results from electrochemical measurements, surface inspection and the biofilm community structure relationship between the transition from transpassive dissolution to crevice corrosion and the biofilm

developed at the late stages of exposure. An anodic potential was necessary to achieve the conditions to initiate crevice corrosion in the presence of biofilms so it seems that crevice corrosion was exclusively triggered by the external potential applied, i. e., the applied potential may have disrupted the oxide layer on the steel surface sufficiently to permit the microorganisms already present in the overlying biofilm to gain access to the metal and to make repassivation more difficult. Alternatively, the biofilm on the specimens may have responded to that potential and acted as a catalyst for the initiation of crevice corrosion. On the other hand, the biofilm may have induced changes in the passive film structure creating a more active surface that could have assisted depassivation and crevice corrosion initiation in the presence of aggressive transpassive corrosion product species formed in the surface of specimens polarized at the potential of the transpassive state.

- The electrochemical methods used in this study proved to be a very practical, simple and highly reproducible approach to evaluate crevice corrosion. PCR-DGGE and DNA sequencing analysis of the biofilm community structure proved to be a powerful method to show the selectivity of microbial populations in response to changes in the surrounding growth environment such as those induced by the presence of crevices at the surfaces of alloys in seawater.

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Table 1.
Primers used for PCR amplifications

Primer	Amplification target	Sequence
27F	Bacterial 16S rRNA gene	5'- GAG TTT GAT CCT GGC TCA G -3'
1492R[#]	Universal 16S rRNA gene	5'- ACG GIT ACC TTG TTA CGA CTT -3'
BacV3f*	Bacterial 16S rRNA gene	5'- CCT ACG GGA GGC AGC AG -3'
907R	Universal 16S rRNA gene	5'- CCG TCA ATT CMT TTG AGT TT -3'

[#]Mixed base codes: I = deoxyribose inosine modification (universal base).
*Primers BACV3f-GC had a GC rich clamp (5'- GCCCGCCGCGCGCGGCGGGCGGGGCGGG-3') on the 5'-end.

Table 2.
Electrochemical parameters investigated to evaluate crevice corrosion of UNS S31803 in seawater

Sample	Average CCT[*] (°C)	Average E_b[#] (mV)	Average E_r[Δ] (mV)	Surface reaction
t0	38.22 ±0.06	+889	+980	Transpassive dissolution
t1-test	37.79 ±1.09	+822	+904	Transpassive dissolution
t1-control	38.46 ±0.01	+826	+918	Transpassive dissolution
t2-test	37.54 ±0.62	+850	-227	Crevice corrosion
t2-control	37.85 ±0.49	+900	+930	Transpassive dissolution

*CCT: critical crevice temperature [#]E_b: Breakdown potential [Δ]E_r: Repassivation potential

Table 3.
Microorganisms in DGGE bands with the highest homology to the reference sequences in the GenBank database.

DGGE Band	Closest relative	% homology/accession number
1	*Uncultured marine bacterium*	80%/GU235273
2	*Uncultured marine bacterium*	85%/GU235197
3	-	(poor sequence quality)
4	*Bacillus clausii*	97%/FN397480
5	-	(poor sequence quality)
6	*Uncultured marine bacterium*	90%/FM211111
7	*Marinobacter adhaerens*	97%/CP001978
8	*Marinobacter articus*	93%/AF148811
9	*Pseudomonas pachastrellae*	95%/HQ425676
10	*Uncultured bacterium*	100%/GQ159187

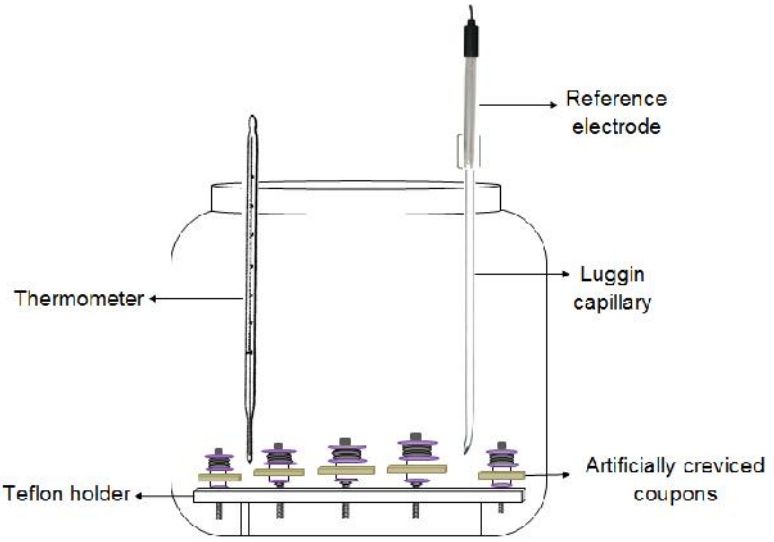

Figure 1: Schematic illustration of the reaction vessels used for experiments

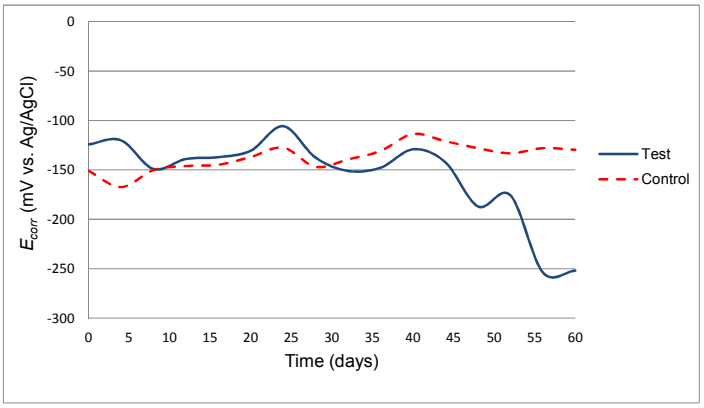

Figure 2: Average corrosion potential E_{corr} as a function of immersion time for creviced UNS S31803 in control and test seawater at 20˚C. (Control: Filter-sterelized seawater; Test: seawater containing bacteria).

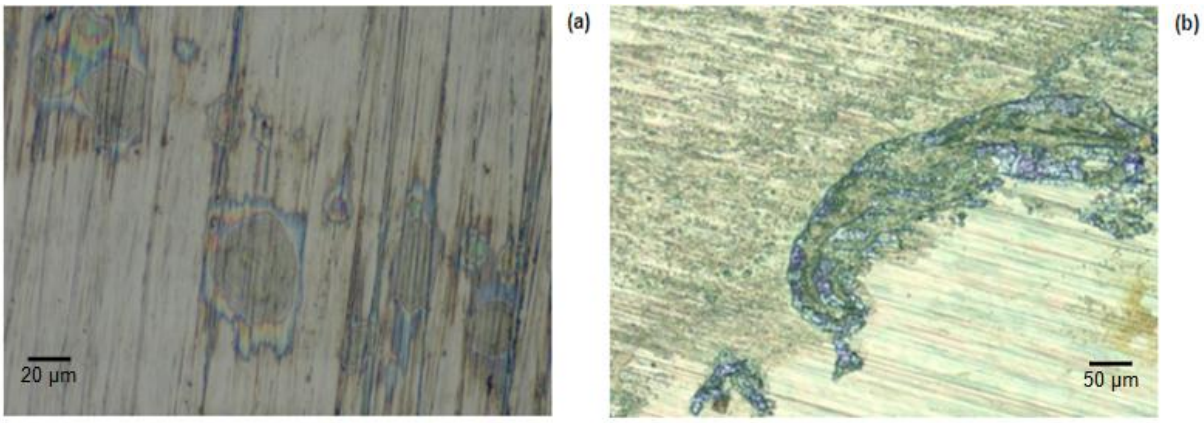

Figure 3: Optical images of UNS S31803 after potentiostatic evaluation of the CCT at 700 mV (Ag/AgCl) at the completion of 8 weeks exposure to seawater containing bacteria. (a) Unaffected areas at the crevice boundaries. (b) Area of concentrated crevice attack at the crevice boundaries. Same pattern was observed in controls without bacteria.

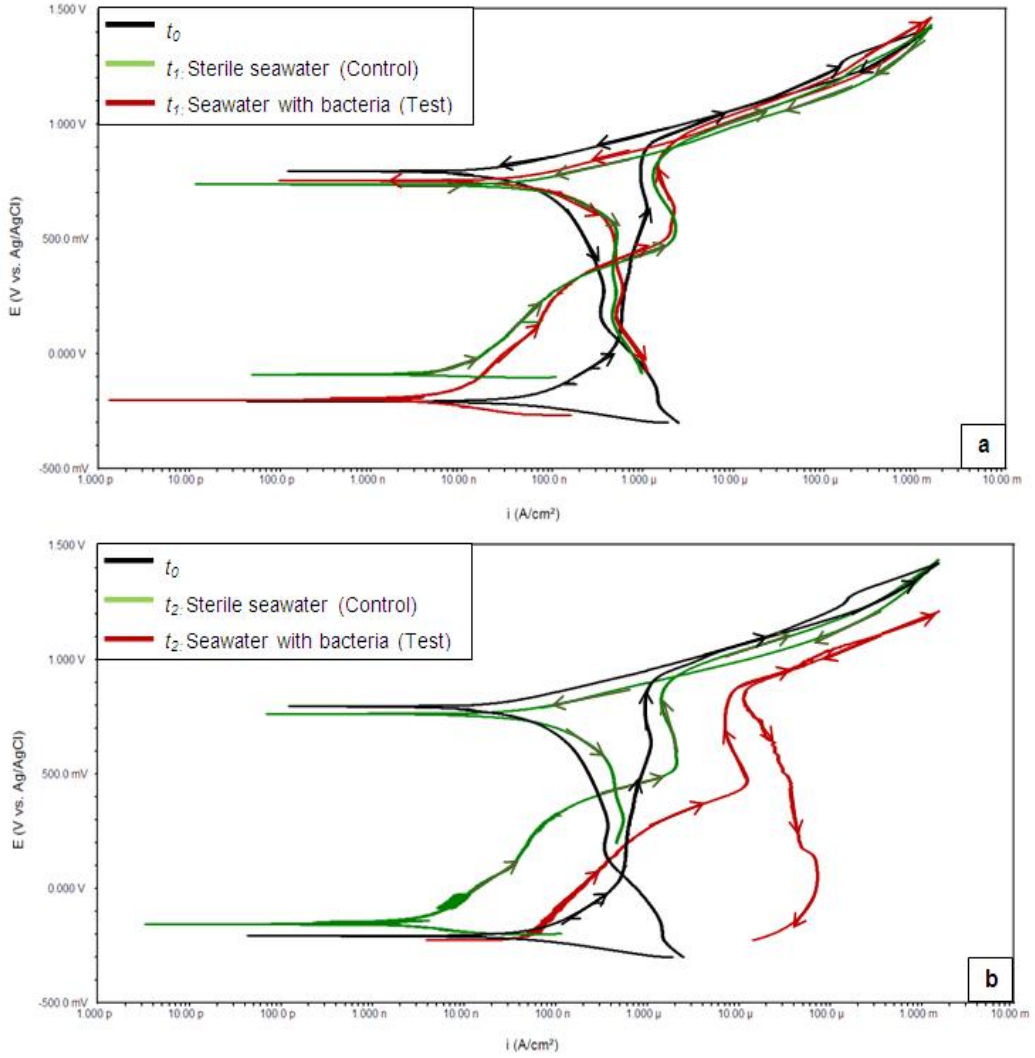

Figure 4: Cyclic polarization scans of UNS S31803 after exposure to natural seawater at 20°C. (a) t1: 4 weeks exposure; (b) t2: 8 weeks exposure. t0*:* freshly ground specimens.

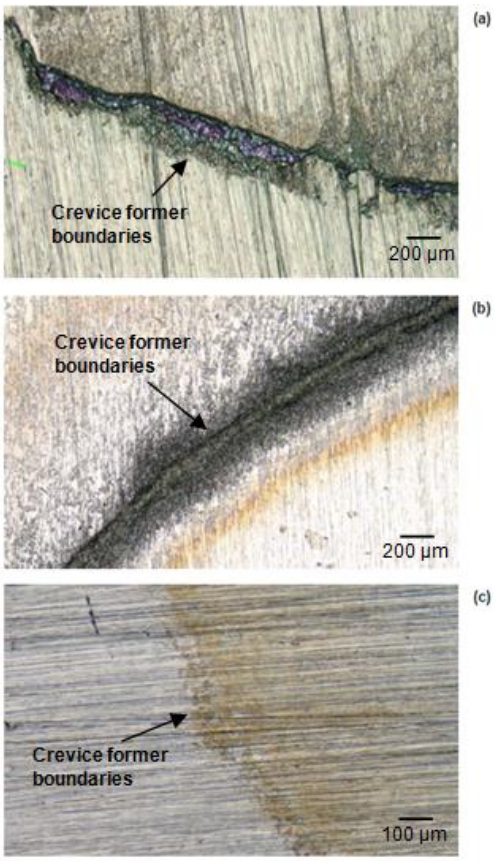

Figure 5: Surface images of electrochemically tested UNS S31803 after 8 weeks immersion in stagnant seawater at 20°C. (a) and (b) areas attacked at the crevice boundaries after exposure to seawater containing bacteria (Test); (c) unaffected areas at the crevice boundaries after exposure to sterile seawater (Control).

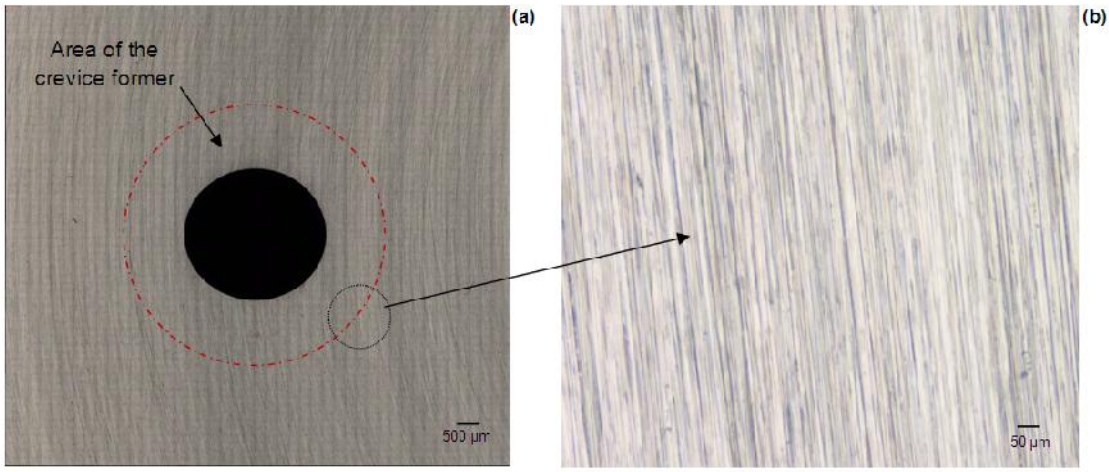

Figure 6: Surface images of UNS S31803 after exposure to seawater containing bacteria for up to 8 weeks. These specimens were not electrochemically polarized at the completion of exposure. (a) Unaffected surface; a ring was drawn to indicate the area where the crevice former was in contact with the surface during the exposure. (b) Expanded cross section image of the same surface at the crevice boundaries displaying no signs of crevice corrosion.

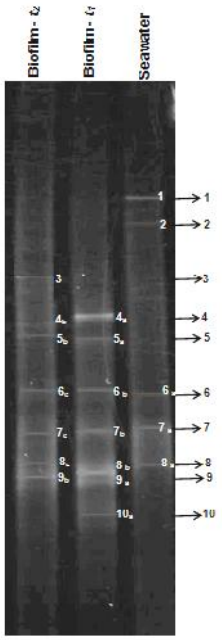

Figure 7: DGGE profiles of 16S rRNA bacterial gene fragments amplified from DNA samples from seawater and biofilms on UNS S31803. Biofilm community structure was assessed at 4 (Biofilm-t1) and 8 (Biofilm-t2) weeks of biofilm growth. Each band is numbered and its sequence information is given in Table 3.

Appendix 5

Original reprint of publication

Chapter 6

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Microbial Corrosion Resistance of Stainless Steels for Marine Energy Installations

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Abstract. A range of stainless steels has been investigated for resistance to microbiologically influenced corrosion in seawater. The corrosion potential was monitored for stainless steel coupons exposed to sterilized seawater and to microbiologically active seawater, which showed the effect of the growth of microorganisms. Cyclic potentiodynamic polarization scans confirmed that 13%Cr stainless steel is very susceptible to localized corrosion under these conditions. 316L stainless steel was also quite susceptible to localized corrosion, whereas 2205 duplex stainless displayed good resistance to localized corrosion. Naturally occurring microorganisms in the seawater were shown to exacerbate the localized corrosion.

Introduction

There is increasing interest in the construction of energy production facilities offshore. Oil and gas production is increasingly focused on offshore deposits [1, 2], while some renewable energy sources are necessarily marine (wave and tidal energy), and at the same time there is a growing trend for offshore wind power generation [3]. The marine environment is aggressive towards corrosion of most structural metals, so corrosion resistant alloys such as "stainless steels" are generally used for construction of facilities in these maritime locations. Stainless steels do still exhibit a certain susceptibility to corrosion, and this can be exacerbated in the presence of microorganisms [4] – so called "Microbiologically Influenced Corrosion", MIC.

In this paper we examine the corrosion resistance of a range of stainless steels in seawater, and in particular, we examine the influence of microbial growth on the corrosion performance. The corrosion potential can be monitored continuously to give an indication of the state of the steel surface, and its interaction with the microorganisms. Sterile seawater with all microorganisms removed by filtration is compared against natural seawater containing the indigenous population of microorganisms. At the end of an exposure, a more comprehensive evaluation of the state of the surface, particularly with reference to localized corrosion, can be made by cyclic potentiodynamic polarization and the surface can be subsequently examined under a microscope.

Experimental Procedure

Stainless steel specimens (square coupons 50x50x5mm) were ground to 600 grit finish with SiC grinding paper, degreased with acetone and dried with nitrogen. The composition (in wt %) of the steels is described in Table 1. Coupons were immersed in the test solution by hanging from a coated copper wire via a spot weld which also functioned as electrical connection for electrochemical measurements. Any exposed wire was covered with epoxy resin. Electrochemical tests were performed on fresh-ground coupons as well as during and after 4 weeks immersion in seawater. Samples in triplicate were immersed in 2 L vessel filled with 1.5 L of aerated natural seawater (test) containing indigenous microorganisms, or aerated sterile seawater (control without microorganisms). The electrochemical activity was monitored using a three-electrode set up where the steels served as working electrodes, a platinum coated mesh was used as counter electrode and a Ag/AgCl electrode

worked as reference electrode, RE [5]. A 150 rpm agitation rate was sustained via a magnetic stirrer throughout the immersion period. Materials were tested in seawater at two different temperatures: 20°C and 30°C. The corrosion potential of the steels was monitored periodically and cyclic potentiodynamic polarization scans were carried out at the end of the exposure time to evaluate the steels' localized corrosion resistance. The long-term potential monitoring was performed using a multichannel potentiostat (ACM Instruments Potential 20). All potendiodynamic scans were performed using Gamry Instruments DC-105 software (Gamry Instruments, Inc.). The cyclic polarization scans were conducted using a forward and reverse scan rate of 0.5 mV/sec. The sweep direction was reversed when either a current density of 1.5 mA/cm^2 or a potential of 1.5 V vs. RE was reached. The final point of the scan was set at a potential of -0.1 V vs. E_{corr}. At the completion of the tests, coupons were cleaned by following the standard procedure [6] and surface inspection was conducted to confirm electrochemical findings.

Table 1. Test materials and chemical composition

Stainless steel	C wt %	Mn wt %	Fe wt %	Cr wt %	Ni wt %	Mo wt %	N wt %
13%Cr Martensitic stainless steel 13%Cr SS	0.19	0.85	bal	13.4	0.42	-	-
316L Austenitic stainless steel 316L SS	0.022	1.76	bal	17.4	10	2.03	0.046
2205 Duplex stainless steel 2205 DSS	0.015	1.53	bal	22.35	5.72	3.16	0.18

Results and Discussion

Evolution of the corrosion potential

Results of average corrosion potential E_{corr} values of the steels immersed in seawater at 30˚C and 20˚C for up to 4 weeks are shown in Fig. 1 and Fig. 2 respectively. 13%Cr SS showed a rapid negative shift of E_{corr} between 1 and 5 days followed by a stable region. Similar patterns of E_{corr} evolution over time in seawater for 13%Cr SS at the two tested temperatures were observed regardless of the presence of microorganisms in the seawater. 316L SS immersed in seawater at 30˚C showed a very unsteady E_{corr} throughout the immersion period in both test and control. 2205 DSS exposed to natural seawater (test) at 30˚C showed a very stable E_{corr} with only some drops and fluctuations at the end of the immersion period which can be related to disturbances of the passive film due to biofilm changes. 2205 DSS exposed to natural seawater (test) at 20˚C, showed a slight increase in E_{corr} overtime at the initial stages of immersion but remained quite stable until the end of the test. 316L SS exposed to natural seawater (test) at 20˚C showed an increase in E_{corr} with time after the 10 day of immersion which was not observed on the control. Ennoblement of E_{corr} of stainless steels in natural seawater has been previously reported [7]. Studies of microbiologically influenced corrosion (MIC) show that biofilms will soon cover the material's surface within a few hours of exposure to natural seawater, and microbial populations in biofilms covering stainless steel surfaces have been previously characterised [8]. Previous studies have shown that this phenomenon can shift the open circuit potential in the noble direction [9]; The significance of this effect lies in its influence on the initiation and propagation of localized corrosion of steels [10,11]. None of the stainless steels immersed in sterile seawater (control) at the two tested temperatures showed significant changes in E_{corr} with time.

Cyclic potentiodynamic polarization scans

Fig. 3 shows the potentiodynamic polarization curves obtained from freshly ground stainless steels as well after 4 weeks immersion in sterile (FSW) and natural seawater (NSW) at 20ºC and 30ºC. From this potentiodynamic technique, several measured parameters such as the breakdown potential E_b, the repassivation potential E_r and the presence or absence of a hysteresis loop, are used to evaluate the steel's resistance to localized corrosion[12].

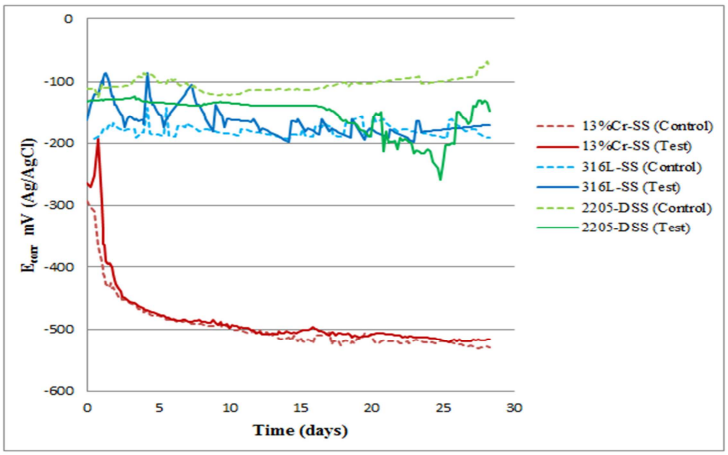

Fig. 1 Average corrosion potential E_{corr} values as a function of immersion time for stainless steels in control (sterile) and test (natural non-sterile) seawater at 30˚C

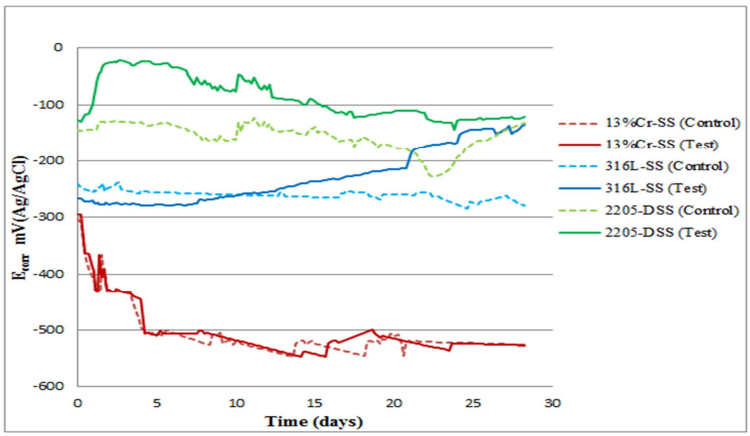

Fig. 2 Average corrosion potential E_{corr} values as a function of immersion time for stainless steels in control (sterile) and test (natural non-sterile) seawater at 20˚C

It was found that for 13%Cr SS the same scan pattern was obtained for the two tested temperatures and for the three different conditions (Fig. 3 a-b). It can be seen that 13%Cr SS is highly susceptible to localized corrosion in aerated seawater at the two temperatures evaluated. In addition, an increase in temperature from 20ºC and 30ºC as well as the exposure to seawater further enhanced the localized corrosion processes. Moreover, a detrimental effect due to the presence of microorganisms in seawater was also observed. For 316L SS immersed in seawater at 30ºC (Fig. 3 c) more active breakdown and repassivation potentials were observed in specimens that were immersed in seawater for 4 weeks indicating that localized corrosion took place to a higher extent than in freshly ground specimens. In addition, the presence of microorganisms in the seawater at 30ºC resulted in an increase in the localized corrosion of 316L SS. 316L SS exposed to seawater at 20ºC (Fig. 3 d) proved to be very susceptible to localized corrosion at all three conditions tested but still showed a better performance as compared to specimens exposed to seawater at 30ºC. Additionally, localized corrosion of 316L SS in seawater at 20ºC was intensified with increasing exposure time to seawater. In this case, the presence of microorganisms in the seawater made no appreciable difference to the corrosion pattern observed in the control. For the 2205 DSS (Fig. 3 e-f) it was found that this material is resistant to seawater at the two tested temperatures, both in the presence and absence of microorganisms in the seawater. All electrochemical findings were confirmed by surface inspection using optical microscopy (see later).

These results have revealed that localized corrosion of stainless steels is influenced by a number of critical factors such as material composition, seawater composition and temperature. Exceeding a critical temperature is not only a requirement for the steel to achieve its critical condition to undergo passivity breakdown, but temperature is also a major factor in the microbial colonization process,

biofilm formation and activities of microorganisms as well as in their ability to interact with electrochemically active surfaces. It is likely that the corrosion reactions taking place at the steel surfaces in seawater involve the interactions of several factors in which microorganisms play a key role.

Surface analysis

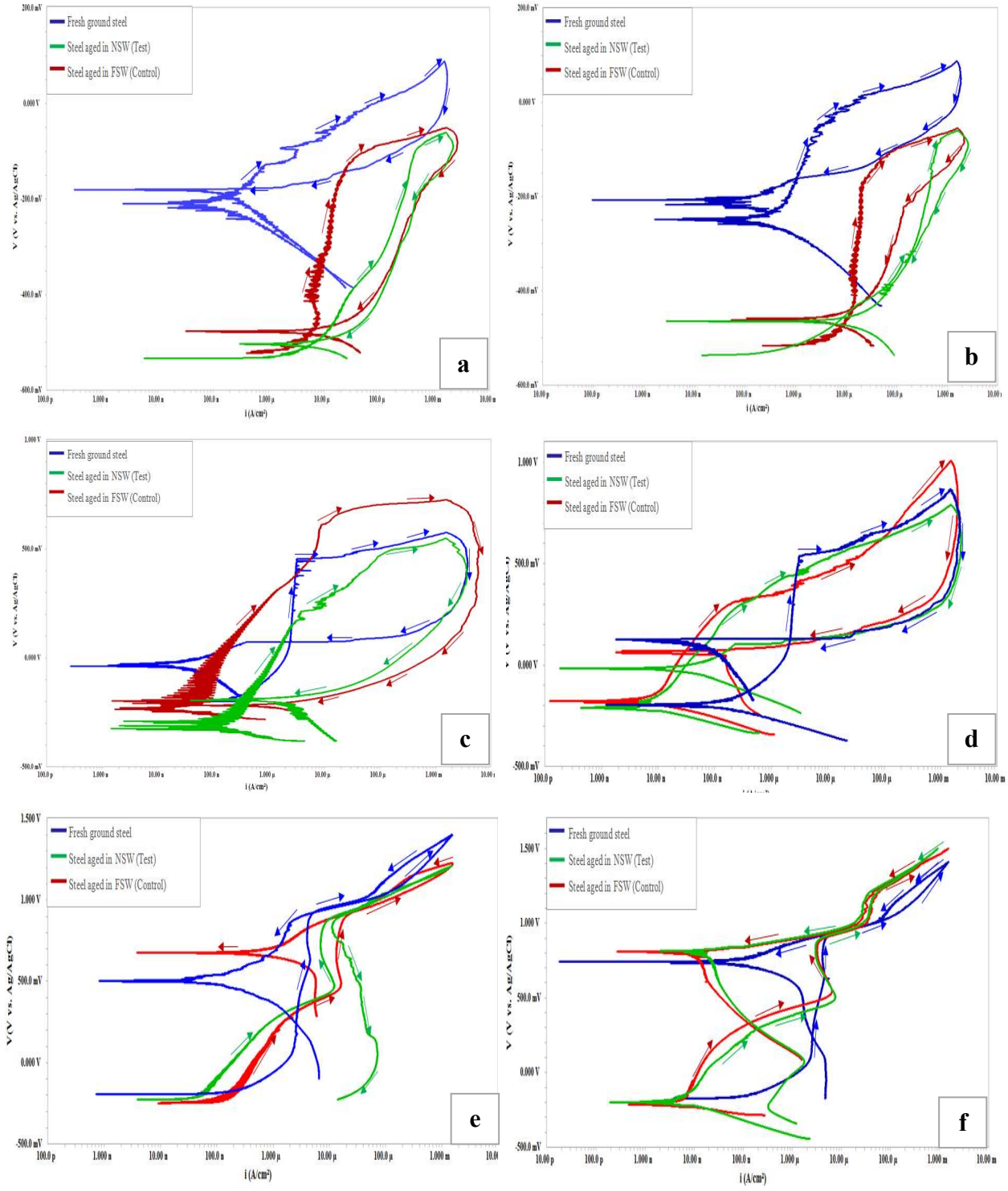

Fig. 3 Cyclic polarization scans of: 13%Cr SS in seawater at 30˚C (a) and 20˚C (b); 316L SS in seawater at 30˚C (c) (© NACE International 2011 [8], with permission) and 20˚C (d) and 2205 DSS in seawater at 30˚C (e) and 20˚C (f). NSW or "Test" = natural seawater with indigenous microorganisms; FSW or "Control" = sterile seawater without microorganisms. Arrows indicate the direction of the scan.

Fig. 4 shows some surface images of the steel surfaces after 4 weeks immersion in natural seawater and after conducting potentiodynamic polarization scans. The occurrence of crevices on the 13%Cr SS, pits on the 316L SS and no severe localized corrosion on the 2205 DSS support the electrochemical measurements. The superior performance of 2205 DSS is confirmed by these observations.

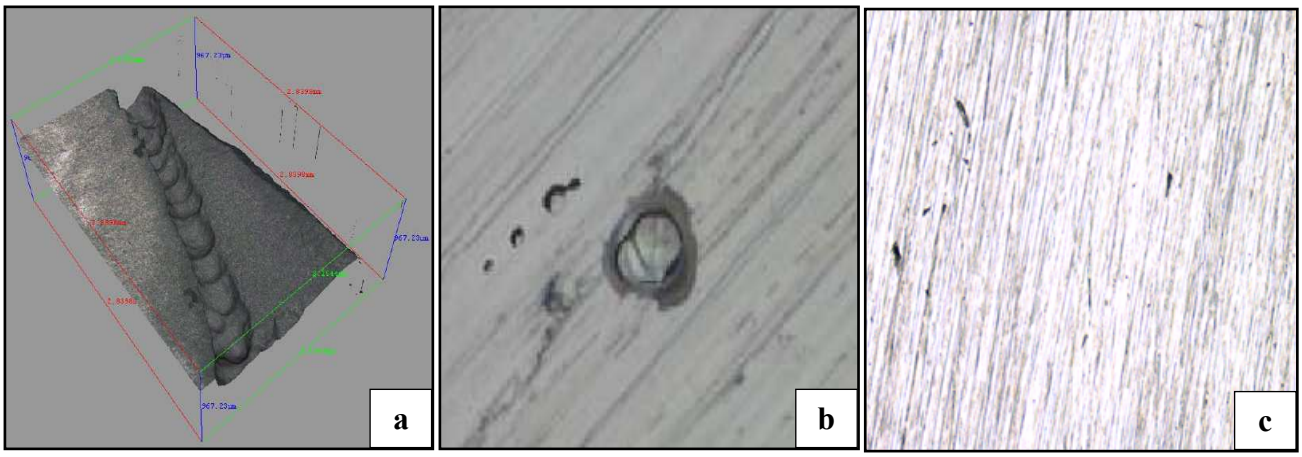

Fig. 4, Optical images of steel surfaces after 4 weeks immersion in seawater at 30˚C after electrochemical tests had been conducted. (a) crevices in the surface of 13%Cr SS; (b) pits in the surface of 316L SS (© NACE International 2011 [8], with permission) (c) pit-free surface of the 2205 DSS .

Conclusions

Accelerated electrochemical corrosion analysis indicates that:

- 13%Cr stainless steel and 316L stainless steel are very susceptible to seawater corrosion at temperatures of 20ºC and 30ºC. Localized corrosion of these steels is exacerbated by prolonged exposure to seawater and by the increase in the seawater temperature from 20ºC to 30ºC. In addition, the presence of microorganisms in seawater further accelerates the localized corrosion processes.
- 2205 duplex stainless steel exhibited good resistance to localized corrosion in seawater at 20ºC to 30ºC regardless of the exposure time and of the presence of microorganisms in the seawater.
- The stainless steels are ranked in terms of their resistance to localized corrosion and MIC in natural seawater at 20ºC and 30ºC : 2205 DSS > 316L SS > 13%Cr SS.

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Authors: **L. L. Machuca**, S.I. Bailey, R. Gubner, E. Watkin, A. Kaksonen

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Best wishes,
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Appendix 7

Proof of full paper peer reviewing for the conference publications included in this thesis; provided by ***NACE International*** (**Chapter 3 and Chapter 5**) and ***The Australasian Corrosion Association Inc.*** (**Chapters 4**)

NACE INTERNATIONAL

THE CORROSION SOCIETY

October 9, 2012

Laura L. Machuca-Suarez
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Corrosion Centre for Education, Research & Technology (Corr-CERT)
Faculty of Science and Engineering | School of Chemical and Petroleum
Engineering
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Dear Laura,

This letter is to provide you with some information on the peer-review process for papers presented at NACE's annual CORROSION conference.

The technical committee symposia are overseen by the NACE Annual Conference Program Committee (ACPC), which consists of a chair, vice chair, five Program Coordinators, the vice chair of the Technical Coordination Committee (TCC—the governing committee for all technical committees), and the vice chair of the NACE Research Committee. There are also five Associate Program Coordinators, who are learning the Program Coordinator role prior to moving into that role.

The symposia are organized by NACE technical committees, and a chair and vice chair are appointed for each symposium by the responsible technical committee chair.

The technical committee sponsoring each symposium also appoints at least one reviewer for the papers, depending on the number of papers in the symposium. The reviewers are assigned to specific papers to review, and the chair acts as the second reviewer. Only the chair may approve a draft or final paper.

Authors first must submit an abstract to a particular symposium using an online system. After the abstract submission deadline,

the symposium chairs access the system and accept or reject abstracts based on a grading system. Authors are notified of acceptance or rejection.

Accepted authors are sent a Technical Program Manual outlining the steps to be followed in the preparation of their papers, including formatting rules, deadline dates, etc. Reviewers and chairs also receive instructions.

The reviewers perform the reviews of the draft papers, and the chair may elect to make the reviewer's comments available to the author, or may transmit the review in his/her own way. The chair is the approval authority. If the author is asked to make changes, he or she must make the changes and resubmit the draft until the chair is satisfied and approves the draft. During this time, NACE staff also reviews the papers for formatting and commercialism and contacts the author if changes need to be made.

After the draft is approved, the author converts the paper to PDF and submits the final paper.

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I hope that I have provided a full explanation of the process we follow to ensure quality papers that are then published by NACE International on CD and online.

Sincerely,

Linda Goldberg

Linda Goldberg
Director, Technical Activities

Wednesday, 5 September 2012

To whom it may Concern,

This is to advise that each manuscript published in the proceedings of the 18th International Corrosion Congress has been peer reviewed by industry experts selected from ACA's review panel.

If you have any queries, please do not hesitate to contact me on kwebber@corrosion.com.au or 03 9890 4833.

Yours faithfully

Katherine Webber
18th ICC Secretariat
kwebber@corrosion.com.au

Appendix 8

Written statements of the co-authors of the publications forming the chapters of this thesis

To Whom It May Concern,

I, Laura Lizeth Machuca Suárez, as first author of the publication entitled ***"Systematic study of the corrosion properties of high-resistance alloys in natural seawater"***, declare that this work was primarily designed, experimentally executed, interpreted, and written by the first author of this manuscript.

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I, Laura Lizeth Machuca Suárez, as first author of the publication entitled ***"Crevice corrosion of duplex stainless steels in the presence of natural marine biofilms"***, declare that this work was primarily designed, experimentally executed, interpreted, and written by the first author of this manuscript.

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