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## Title of Paper or Presentation

A Review of Microbiologically Influenced Marine Corrosion Experiments: Artificial vs. Natural Seawater

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# Understanding marine biocorrosion: experiments with artificial and natural seawater

13

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## 13.1 Introduction

For many years there has been a debate over the preferred laboratory medium for studying marine corrosion, i.e., natural or artificial seawater (Dexter, 1988). It is generally recognized that seawater surrogates do not approximate the complexity of natural seawater. The dilemma is whether to start with a well-characterized controlled synthetic medium that may not replicate any natural environment or with a natural environment that cannot be thoroughly characterized, varies with collection location and changes over time. That debate has continued and has become more complicated in the study of microbiologically influenced marine corrosion (MIMC) and the consideration of nutrients for microorganisms. Recent MIMC investigations have included natural seawater with its endogenous microbial population (Lee *et al.*, 2004; Lee *et al.*, 2005; Jeffrey and Melchers, 2003), augmented natural seawater (Chang *et al.*, 2009; Dinh *et al.*, 2004), artificial seawater to which microorganisms have been added (Venzlaff *et al.*, 2013) and inoculated synthetic media (Zegeye *et al.*, 2007). Some investigators defined synthetic seawater as a certain salt concentration, e.g., 0.6M NaCl (Vargas-Avila *et al.*, 2009). However, in many cases the term synthetic seawater refers to commercially available carbonate-buffered salt solutions that mimic the composition of seawater. The following is review of MIMC experiments in natural and artificial seawaters that demonstrate the need to understand their differences.

Much of the chapter is focused on MIMC caused by sulphate-reducing bacteria (SRB), the organisms most closely identified with MIMC studies in both natural and artificial seawater. Natural seawater contains a minimum of  $2\text{ g L}^{-1}$  (21 mM) sulphate, independent of geographic location. SRB are a group of ubiquitous organisms that in the absence of oxygen use sulphate as the terminal electron acceptor, producing hydrogen sulphide ( $\text{H}_2\text{S}$ ). Although the oxygen content of seawater above the thermocline ranges from 5–8 parts-per-million (ppm), anaerobic microorganisms survive in anaerobic microniches until conditions are suitable for their proliferation. Hamilton (1985) demonstrated that SRB grow as communities or consortia within biofilms between 25 and  $100\text{ }\mu\text{m}$  thick. If the aerobic respiration rate within a biofilm is greater than the inward oxygen diffusion rate, the metal/biofilm interface can become anaerobic and provide a niche for sulphide production by SRB. The critical

biofilm thickness required to produce anaerobic conditions depends on availability of oxygen and the respiration rates of aerobic or facultative anaerobic organisms in the biofilm. SRB activity causes accumulation of metal sulphides near metal surfaces and corrosion of vulnerable metals and alloys, including carbon steel and copper.

## 13.2 Effect of nutrients and oxygen removal on biocorrosion

### 13.2.1 Nutrients

Dexter (1988) was among the first investigators to systematically examine differences between natural and artificial seawater. He made the following observations:

It is often stated in the literature that organic or biofilm effects or both were avoided by using NaCl solution. This is a misleading statement. Sodium chloride salts are not free of organic matter and no NaCl solution will remain free of bacteria for more than a few hours unless it is prepared and carefully maintained under sterile conditions. Organics are not absent in NaCl solutions, they are just different from those in natural seawater.

Another common misconception is that ‘... biofilms do not form under nutrient-poor conditions’ (Ismail *et al.*, 2002). In fact, the opposite is true. Inert surfaces are sites of nutrient enrichment. Zobell and Anderson (1936), Stark *et al.* (1938) and Heukelekian and Heller (1940) demonstrated that nutrients sorb to surfaces. Dawson *et al.* (1981) showed that cells deprived of nutrients became more adhesive. At low-nutrient concentrations, bacteria are associated with surfaces whereas, at high-nutrient concentrations typically encountered in laboratory experiments, cells tend to be uniformly distributed in the aqueous phase. Attachment of cells to surfaces is one of the most common strategies for bacterial survival in environments where the bulk water phase is nutrient limiting. Geesey (1987) stressed that many survival mechanisms, including adhesion to surfaces, may not be expressed in laboratory experiments.

Oxyanions, e.g., nitrates, phosphates and sulphates, routinely added to both culture media and to artificial seawater as nutrients, can act as corrosion inhibitors (Little, 2003). Webster and Newman (1994) examined the impact of media constituents on localized corrosion of a creviced stainless steel (Fe-15Cr-10Ni) and concluded that localized attack would not readily occur unless chloride was the predominant anion in an artificial medium for studying MIMC. They concluded that chloride must be present in a concentration at least comparable to that of all other anions combined; otherwise, corrosion was inhibited even at H<sub>2</sub>S concentrations up to 500 ppm. In their studies, reduction of the ratio of chloride to other inhibiting anions increased the time to initiation and decreased the rate of propagation of the corrosion. Other investigators have concluded that additional nutrients cannot be added to stimulate bacterial growth and MIMC if those nutrients inhibit corrosion by introducing too many non-chloride anions (Ringas and Robinson, 1988). It is conceivable that bacterial consumption and

fixation of nutrients, including sulphate, could render an initially inhibiting seawater solution aggressive by removing non-chloride ions.

In many artificial seawater media yeast extract is added to encourage microbial growth. Webster and Newman (1994) reported interferences in electrochemical measurements when yeast extract was included in the culture medium/electrolyte. The interferences were absent when the yeast extract was removed.

### 13.2.2 Oxygen removal

To further complicate interpretation and comparison of experimental data, many laboratory investigations with SRB rely on gas purging to remove oxygen and create an anaerobic environment in the laboratory solution of choice, which is often natural and/or artificial seawater. The pH of synthetic carbonate-buffered and natural seawater is controlled (buffered) by carbon dioxide ( $\text{CO}_2$ ). Gaseous carbon dioxide  $\text{CO}_2(\text{g})$  from the atmosphere dissolves into seawater forming aqueous carbon dioxide  $\text{CO}_2(\text{aq})$  (Equation (13.1)), reacts with water, and forms carbonic acid ( $\text{H}_2\text{CO}_3$ ) (Equation (13.2)). Proton dissociation from  $\text{H}_2\text{CO}_3$  proceeds with interdependence on pH. The first proton dissociation forms bicarbonate ( $\text{HCO}_3^-$ ) (Equation (13.3)), with carbonate ( $\text{CO}_3^{2-}$ ) formation from subsequent proton dissociation (Equation (13.4)).

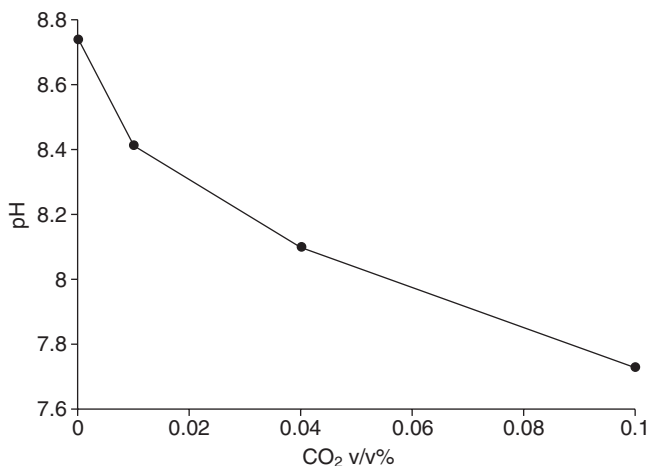


Increasing atmospheric ( $\text{CO}_2(\text{g})$ ) increases dissolved ( $\text{CO}_2(\text{aq})$ ), which elevates ( $\text{H}_2\text{CO}_3$ ) resulting in decreased seawater pH. If  $\text{CO}_2(\text{aq})$  is removed from seawater, pH increases.

For example, pH values were recorded in Key West, FL (KW) seawater purged with mixed gases of hydrogen ( $\text{H}_2$ ) (10 v/v%) and  $\text{CO}_2$  (0 to 0.1 v/v%), balanced with nitrogen ( $\text{N}_2$ ). Figure 13.1 shows pH values recorded in KW seawater as a function of  $\text{CO}_2$  v/v% (Lee *et al.*, 2010b). Increase in  $\text{CO}_2$  v/v% resulted in decreased pH values. The highest pH value (8.74) was recorded with the 0 v/v%  $\text{CO}_2$ , and the lowest pH (7.73) with the 0.1 v/v%  $\text{CO}_2$  gas mixture. In addition to the removal of oxygen, in some experiments an oxygen scavenger (e.g., 75  $\mu\text{M}$   $\text{Na}_2\text{S}$ ) is added to ‘shorten the lag phase’ (Enning *et al.*, 2012). The lag phase to which Enning *et al.* (2012) referred is the time between inoculation and the onset of corrosion.

## 13.3 Comparison of experiments in natural and artificial seawater

Dexter (1988) compared laboratory solutions and natural seawater for studying the corrosion of aluminum alloys and prepared a specific artificial medium that reproduced



**Figure 13.1** Key West seawater pH as a function of CO<sub>2</sub> v/v% in bubbled mixed gas containing 10 v/v% H<sub>2</sub> and balance N<sub>2</sub> (Lee *et al.*, 2010b).

the short-term behavior of aluminum alloys in natural seawater. However, none of the laboratory solutions were able to simulate the long-term corrosion behavior of aluminum alloys in natural seawater.

The relationship between oxygen and MIMC resulting from obligate anaerobic microbial processes is complex. Most reported cases of MIMC of carbon steel and copper alloys are caused by SRB with some dissolved oxygen in the bulk medium. Yet most laboratory experiments with SRB are conducted in the absolute absence of oxygen (notably in deaerated solutions). The presence of oxygen can determine the rate and extent of corrosion resulting from obligate anaerobic microbial processes. Furthermore the concentration of oxygen and the manner in which it is removed can affect experimental results. The specific coupled reactions for which oxygen is the final electron acceptor can vary. The role of oxygen in anaerobic MIMC in both field and laboratory studies is reviewed with an emphasis on SRB.

Syrett (1981) demonstrated that aggressive abiotic sulphide-induced corrosion of copper alloys required oxygen. Filtered Pacific Ocean water was used in the experiments. The seawater was deaerated by sparging N<sub>2</sub> then polluted with 0.2 g m<sup>-3</sup> sulphide. 90:10 copper:nickel was exposed to alternating polluted deaerated and unpolluted, aerated seawater. In the presence of oxygen, the possible corrosion reactions in a copper sulphide system are extremely complex because of the large number of stable copper sulphides, their differing electrical conductivities, and catalytic effects. Transformations between sulphides or of sulphides to oxides result in changes in volume that weaken the attachment scale and oxide subscale, leading to spalling. Bared areas repassivate forming cuprous and/or cupric oxides that are subject to further sulphide induced attack.

Similarly, Hardy and Bown (1984) investigated the effect of aeration on the corrosion of carbon steel by SRB. Their work was conducted in a lactate yeast extract

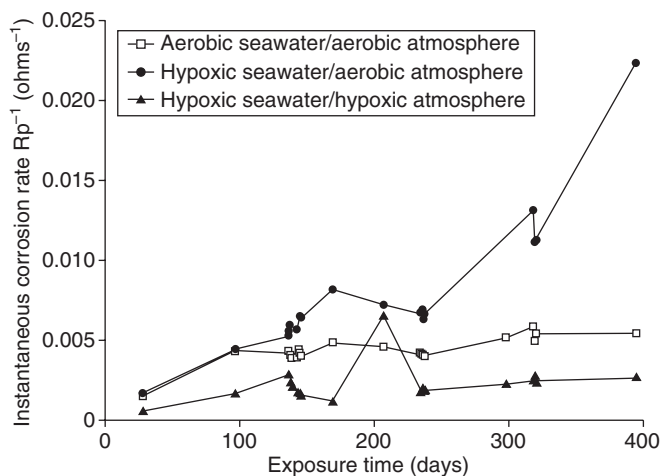
medium with synthetic seawater (undefined). The following were added in gL<sup>-1</sup>: NH<sub>4</sub>Cl, 1.0; KH<sub>2</sub>PO<sub>4</sub>, 0.1; Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, 0.1; Tris (tris-hydroxy methyl) amino methane 0.4; sodium DL lactate, 4.5; yeast extract, 0.5; and ascorbic acid, 1.0 in 750 ml synthetic seawater (undefined) and 250 mL distilled water. The pH was adjusted to 7.2 with 1 M NaOH. Vessels were sparged with N<sub>2</sub> with intermittent introduction of air. They used successive aeration-deaeration shifts to produce pitting. Without aeration, corrosion rates were low (1.45 mg dm<sup>-2</sup> d<sup>-1</sup>) and corrosion was uniform. In the presence of aeration, corrosion rates increased dramatically (129 mg dm<sup>-2</sup> d<sup>-1</sup>) and corrosion was localized. They reported; 'The similarity between the form of corrosion attack seen in these tests and the pitting corrosion often attributed to the SRB in the field, strongly suggests that exposing biogenic sulphide films on steel to air might account for their corrosivity' (Hardy and Bown, 1984). They did not establish the minimum quantity of air needed to initiate the pitting they observed. Lee *et al.* (1993a; 1993b) examined the effects of dissolved oxygen and ferrous concentration on SRB corrosion of mild steel in an artificial medium '1/10 strength artificial seawater' with the following in mg L<sup>-1</sup>: lactic acid, 200; yeast extract, 50; NH<sub>4</sub>Cl, 50 and Na<sub>2</sub>HPO<sub>4</sub>, 5. They demonstrated that the corrosion rate under totally anaerobic conditions was negligible compared to that under aerobic conditions. No direct correlation was observed between corrosion and sulphate reduction in the absence of ferrous iron.

Lee *et al.* (2004; 2005) designed field experiments to evaluate deoxygenation of natural seawater as a corrosion control measure for unprotected carbon steel seawater ballast tanks. Carbon steel exposed to cycles of hypoxic (<0.2 ppm dissolved oxygen) seawater and oxygenated atmosphere had higher corrosion rates than coupons exposed to either consistently aerobic or hypoxic conditions (Figure 13.2). Under anaerobic conditions sulphide corrosion products formed. The field experiments also demonstrated the difficulty of maintaining hypoxic seawater (<0.2 ppm dissolved oxygen). The oxygen could be removed to any level using a Venturi device, but the remaining oxygen was quickly consumed by aerobic respiration and corrosion of carbon steel.

Lee *et al.* (2010b) demonstrated that deaeration methods influenced the chemistry and microflora of two natural coastal seawaters, and the outcome of MIMC experiments. The authors evaluated the influence of deoxygenation techniques on corrosion. They maintained two coastal seawaters (KW and Mina Sulman, Bahrain, Persian Gulf [PG]) under three laboratory conditions:

1. open to air (aerobic)
2. deoxygenation with bubbled N<sub>2</sub> and
3. deoxygenation in an anaerobic hood.

KW and PG seawaters represent two different microbial and chemical environments. KW is typical of open-ocean oligotrophic water. In the Persian Gulf, total evaporation exceeds fresh water input, resulting in the concentration of dissolved species relative to those characteristic of typical seawater (Bashitalshaaer *et al.*, 2009). The anaerobic atmosphere was maintained using a gas mixture of 10 v/v% H<sub>2</sub>, 5 v/v% CO<sub>2</sub>, and bal. N<sub>2</sub>. Hydrogen was added to the anaerobic gas mixture to aid removal of oxygen. A

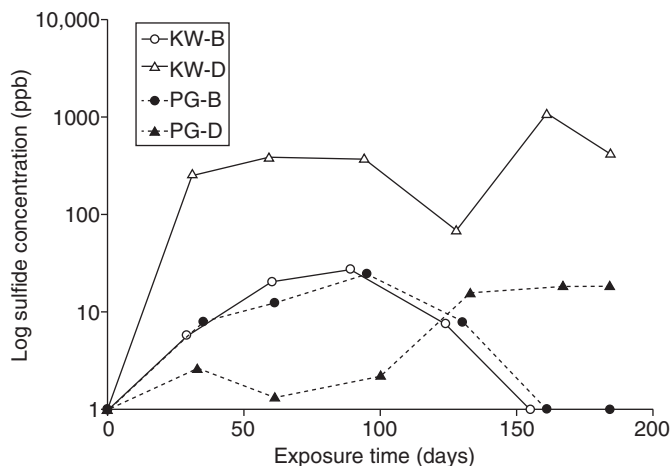


**Figure 13.2** Instantaneous corrosion rates from inverse polarization resistance ( $R_p^{-1}$  ( $\text{ohms}^{-1}$ )) of carbon steel exposed to Key West seawater side for 395 days under different seawater and atmospheric conditions: aerobic seawater = natural, hypoxic seawater = natural seawater treated with hypoxic gas mixture, aerobic atmosphere = air, hypoxic atmosphere = hypoxic gas mixture (Lee *et al.*, 2005).

palladium catalyst inside the anaerobic chamber served as an oxygen and  $\text{H}_2$  reaction site resulting in the formation of water and increased humidity. The latter was maintained between 20–30% using an alumina desiccant. Atmospheric oxygen concentration was continuously recorded as less than 1 ppm. In parallel experiments house  $\text{N}_2$  was bubbled into both seawaters. Bubbling was not vigorous with approximately 2 bubbles per second. No nutrients or other additions were made to any of the exposure conditions. In all experiments, pH values, dissolved sulphide concentrations and culturable planktonic microbial populations were monitored. In both seawaters, exposure to the mixed gas anaerobic environment caused a decrease in pH values to below 7.0. Bubbling  $\text{N}_2$  had the opposite effect, resulting in an increase in pH to above 9.0. Dissolved sulphide was monitored over time for all exposure conditions. Anaerobic stagnant seawaters maintained in a hood with mixed gases produced  $10^1$  to  $10^3$  parts-per-billion (ppb) dissolved sulphide. Seawaters maintained with bubbled  $\text{N}_2$  produced  $10^1$  ppb dissolved sulphide during the course of the experiment, however, at the conclusion of the experiment the concentration of sulphide was below the detection limit (Figure 13.3). Generally, KW seawater had higher numbers of planktonic bacteria than PG seawater in all exposure conditions. Bubbling  $\text{N}_2$  through the seawaters produced the lowest concentration of bacteria in comparison to seawaters which were either left open to air or tested in the mixed gas anaerobic chamber.

Aktas *et al.* (2010; 2013) and Lee *et al.* (2014) demonstrated another important consequence of using gas mixtures to remove oxygen. In a series of experiments





**Figure 13.3** Log sulfide concentration in parts per billion (ppb) as a function of exposure time (days) for Key West seawater (KW) and Persian Gulf seawater (PG) under nitrogen-bubbled (B) and anaerobic (D) conditions (Lee *et al.*, 2010b).

aimed to duplicate each other, researchers at the University of Oklahoma (OU), Norman, OK, USA, and the Naval Research Laboratory (NRL), Stennis Space Center, MS, USA, evaluated biodegradation of hydrocarbon and cellulosic fuels in the presence of KW natural seawater. Both laboratories used identical starting materials, i.e., the same fuels and the same seawaters. However, in the NRL experiment, the systems were allowed to become anaerobic naturally as a result of aerobic microbial respiration and corrosion reactions (carbon steel). In the OU experiments dissolved oxygen was replaced with a headspace gas  $N_2/CO_2$  (80:20 v/v%) by repetitive vacuum/gas purging cycles and sodium sulphide (0.16 mM) was added as an oxygen scavenger. Seawater pH in the OU experiment decreased from 8 to 6 while the NRL exposure maintained pH near 8. Similar results involving seawater pH were observed by Lee *et al.* (2010b) in a gas mixture containing 5 v/v%  $CO_2$ . According to the authors, sulphate was depleted after three months in the NRL exposure while there was no evidence of sulphate removal in the corresponding OU experiments. The authors attributed the differences in sulphate depletion to the presence of transient oxygen in the NRL experiment, compared to absolute absence of dissolved oxygen in the OU exposure. The authors surmised that the initial presence of oxygen in the NRL exposure provided microbially-mediated aerobic degradation pathways of hydrocarbons. The resulting degradation products were readily biodegradable by anaerobic microorganisms (including SRB) once the oxygen was depleted. Oxidation of the degradation products was coupled with sulphate reduction producing corrosive biotic sulphides. Supporting their hypothesis, diagnostic catechols, which are known aerobic degradation products of hydrocarbons, were detected in the NRL exposures but absent in the OU exposure. Lee *et al.* (2012) detected catechols during similar

exposure conditions with transient oxygen in mixtures of natural KW seawater and fatty acid methyl ester biodiesel.

Hamilton (2003) reviewed the data of others and offered a model for microbiologically influenced corrosion. He concluded that all mechanisms, both aerobic and anaerobic, involved a process of electron transfers from base metal to oxygen as the ultimate electron acceptor through a series of coupled reactions (Nielsen *et al.*, 1993). He hypothesized that in the case of SRB and carbon steel, sulphate, an intermediate electron acceptor, is reduced to sulphide that reacts with iron to form a corrosion product that ultimately transfers electrons to oxygen. In the model, sulphate is the terminal electron acceptor in anaerobic respiration, but oxygen is the terminal electron acceptor in the corrosion reaction. In his model, he hypothesized that 'microbial action has supplied products which are thermodynamically compatible with the potentials of the putative corrosion reactions of the relevant substratum and critically, has generated appropriate kinetically favoured pathways of electron flow from the metal anode to the universal electron acceptor, oxygen' (Hamilton, 2003). The examples presented herein support the Hamilton model.

Recently investigators have proposed an alternative electron transport mechanism for SRB influenced corrosion of ferrous alloys. Venzlaff *et al.* (2013) postulated direct electron uptake from iron by SRB. They used an artificial seawater, buffered by  $\text{CO}_2/\text{NaHCO}_3$  and provided with an anoxic headspace of  $\text{N}_2/\text{CO}_2$  (90:10 v/v). Artificial seawater contained 28 mM sulphate as an electron acceptor and no oxidizable substrates. Sodium sulphide (0.1 mM) was added as a reducing agent to remove traces of residual oxygen and 'shorten the lag-phase of cultures.' Using *D. corrodens* strain IS4 (tentative name) the authors demonstrated direct uptake of electrons from iron through a semiconductive ferrous sulphide corrosion crust. In their experiments there was no indication of enhanced catalytic proton reduction by sulphide corrosion products. Instead, the sulphides on the surface mediated electron flow from the metal to the cells.

Zegeye *et al.* (2007) demonstrated reduction of biogenic sulphated green rust ( $\text{GR2}(\text{SO}_4^{2-})$ ) using a pure culture of the SRB *Desulfovibrio alaskensis*. Lactate served as the electron donor and  $\text{GR2}(\text{SO}_4^{2-})$  as the acceptor at circumneutral pH in an unbuffered medium. *Desulfovibrio alaskensis*, a marine organism, was originally isolated by Feio *et al.* (2004) and maintained in marine Postgate C medium. The batch medium in the Zegeye *et al.* (2007) experiments contained the following in  $\text{g L}^{-1}$ : sodium lactate, 3.5;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 3.4;  $\text{NaCl}$ , 0.3;  $\text{K}_2\text{HPO}_4$ , 0.5;  $\text{CaCl}_2$ , 0.1. The medium was sterilized by autoclaving and purged with filter sterilized  $\text{N}_2$ . 80 ml of the medium was dispensed into sterile 100 mL flasks and crimp sealed with butyl rubber stoppers. Samples of  $0.402 \pm 0.002 \text{ g}$  of biologically produced  $\text{GR2}(\text{SO}_4^{2-})$  (containing  $\gamma\text{-FeOOH}$ ) were introduced into the flasks in the anaerobic chamber. The medium in which Zegeye *et al.* (2007) demonstrated that biogenic  $\text{GR2}(\text{SO}_4^{2-})$  was an electron acceptor for SRB was 1/100 the  $\text{NaCl}$  concentration of seawater. Yet the authors relate their work to corrosion in the marine environment. ' $\text{GR2}(\text{SO}_4^{2-})$  is formed during the marine corrosion of iron in aquatic environments ...' (Zegeye *et al.*, 2007).

### 13.4 Variability in the composition of natural seawater

The variability of experimental conditions and seawater-based media composition in laboratory experiments is undisputable. However, the potential variability in the chemistry and microflora of coastal seawaters has received little attention. Martin *et al.* (2007) measured open circuit potential (OCP) for a series of metals exposed in two US coastal locations: Delaware Bay, DE, USA and KW. Their experiments are particularly enlightening because they used identical materials and exposure devices in the two locations. The only variable was the exposure site. They demonstrated that for a particular material, the extent of ennoblement (increase in OCP) varied between the two locations and could not be used to predict an increased likelihood of localized corrosion. Presumably, site-specific mechanisms were responsible for the observed ennoblements. In earlier work Dexter (1988) concluded that natural coastal seawater from a variety of locations gave results that were internally consistent within a single batch of water, but were inconsistent from batch to batch at the same site and between sites.

Similarly, using polarization resistance measurements, Ray *et al.* (2005) demonstrated that the chemistry of seawater alone cannot be used to predict corrosivity under all conditions. KW and PG seawaters were used in their experiments with the chemical analyses for KW and PG presented in Table 13.1. PG seawater had higher values for all parameters, including salinity, indicating that the PG was likely to be more corrosive when compared to KW. In addition, freshly collected PG contained higher concentrations of SRB (as indicated by growth in liquid culture media), sulphate and total organic carbon than KW and therefore, a potentially higher capacity for sulphide production than KW. When corrosion experiments using carbon steel were conducted under aerobic conditions the PG seawater was more corrosive than its KW counterpart. However, when the experiments were conducted with seawaters maintained in an anaerobic atmosphere containing a mixed gas (5 v/v% CO<sub>2</sub>, 10 v/v% H<sub>2</sub>, bal. N<sub>2</sub>), the KW seawater was more corrosive due to higher concentrations of biotic sulphide. For both KW and PG seawaters maintained under aerobic conditions, bacterial numbers including general heterotrophic aerobes, general anaerobes, acid-producing bacteria and SRB decreased over time. Under anaerobic conditions, bacterial numbers decreased in PG seawater while KW seawater maintained steady or increased bacterial numbers compared to freshly collected KW seawater. Specifically, SRB numbers in KW seawater increased by five orders-of-magnitude

**Table 13.1 Seawater physical chemistries**

Seawaters	pH	Salinity (g L <sup>-1</sup> )	Total organic carbon (mg L <sup>-1</sup> )	Sulphate (mg L <sup>-1</sup> )
Key West	7.82	38	1.79	3864
Persian Gulf	7.98	44	1.94	4696

Source: Lee *et al.*, 2010b.

over the course of the 150-day exposure. Consistent results were observed by Lee *et al.* (2010b) using KW and PG seawaters with anaerobic conditions maintained using a gas mixture of 5 v/v% CO<sub>2</sub>, 10 v/v% H<sub>2</sub>, bal. N<sub>2</sub> where differences were observed in microbial sulphide production in the two natural coastal seawaters maintained in the laboratory. The authors hypothesized that the organics in PG were not as readily biodegradable under anaerobic conditions as the organics in KW. However, Lee *et al.* (2010a; 2012; 2014) subsequently demonstrated that addition of readily biodegradable fatty acid methyl ester biodiesel to both anaerobically maintained KW and PG seawaters still resulted in higher corrosivity and sulphide production in KW. Aktas *et al.* (2010) observed similar results of elevated sulphide production in KW seawater amended with biodiesel.

## 13.5 Conclusions

Seawater surrogates do not approximate the complexity of natural seawater. However, many of the key publications addressing SRB corrosion in the marine environment have been conducted in artificial media to which nutrients and microorganisms have been added and from which oxygen has been removed to decrease the bacterial lag phase. Few investigators have considered the differences that can be induced in natural and synthetic seawater maintained under different experimental conditions. Data reviewed in this chapter emphasize the importance of dissolved oxygen and the techniques for oxygen removal on the outcome of laboratory experiments involving SRB. Conclusions based on experiments carried out under laboratory conditions using artificial media should not be interpreted as applicable to a natural seawater environment. Indeed, many of the observations made with coastal natural seawater are specific to those environments and should not be more broadly interpreted.

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