EIS study of MIC in three different zones derived from ship ballast tank model system

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ABSTRACT

Microbiologically influenced corrosion (MIC) is a well known phenomenon in aquatic environments[1]. The presence of a biofilm on metal surface often establishes new electrochemical reaction pathways, or promotes reactions which are not normally favored in the absence of microorganisms, resulting in increased corrosion rates and on the performance of the material.

A new approach was made by using a new developed lab scale model system containing working electrodes in different height levels to simulate ground, mid and splash zone of a ship ballast tank with the help of a three dimensional shaking plate. By studying a defined mix culture of aerobic and anaerobic bacteria and a natural sample community it was possible to model the corrosion behavior of low carbon steel exposed to bacteria in different zones.

Electrochemical impedance data (EIS) provide the contingency to distinguish between general corrosion and MIC impact in a more detailed way. Fluorescent staining in liquid allowed monitoring biofilm development and activity on steel coupons supporting EIS data. By additional study of the electrode surfaces by SEM-EDX the impact of MIC could be shown.

Key words: MIC, EIS, ballast tank, Microscopy, SEM-EDX

INTRODUCTION

As intensively discussed in literature, corrosion in ship tank environments is shown to be a serious problem for decades. The study of Microbiologically Influenced Corrosion (MIC) is gaining increasing interest. However, until today no clear explanation and identification of the corrosion process influenced by micro-organisms is found. By coupling electrochemical analysis with microbiological tools, new insights in the corrosion process by attached microorganisms in the form of biofilms was gained. Electrochemical interaction between the biofilm consortia and the metal surface is based on a closed loop of metabolic activities within these micro-environments. The experiments were performed in lab scale ballast tanks designed for electrochemical measurements in combination with MIC causing micro-organisms. Electrochemical impedance measurements (EIS) are used to model the attachment of bacterial species on a corroding metal surface. In this new set up, 3 different zones of a ballast tank were taken into account. The zones are shown in Figure 1 and are derived from the Accelerated Low Water Corrosion (ALWC) model described by Little *et al* [2].

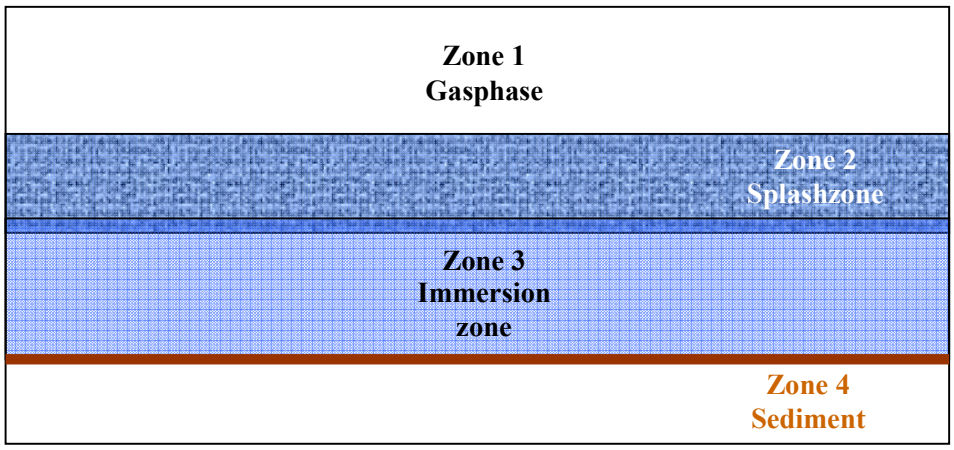


Figure 1: Different zones in ballast tank and corresponding zones
Zone 1 Gas phase aerobic
Zone 2 Splash zone aerobic-anaerobic
Zone 3 Immersion zone (aerobic)-anaerobic
Zone 4 Sediment anaerobic

Based on this model it was developed to study the impact of bacterial communities in the different tank levels. It is well known from literature that bacterial activity is different in the various levels indicated by strong changes in corrosion rates [3,4]. By the use of electrochemical techniques as Linear Polarization Resistance (LPR) and EIS it was possible to investigate the formation of corrosion products on the working electrode surface and relate equivalent circuits with biofilm formation processes [5,6].

EXPERIMENTAL PROCEDURE

Coupon preparation

316 stainless steel and carbon steel coupons (size 3x2 cm) were used to monitor bacterial attachment. The rectangular coupons with a dimension of 30x20x1 mm were ground sequentially up to grid 2400 grit SiC paper to obtain a smooth surface. The polished coupons were rinsed with deionised water twice, followed by degreasing with acetone, then sterilized by immersion in 70% ethanol for eight hours, and dried aseptically in a laminar flow. For biofilm imaging, the coupons were transferred into QuadriPerm [(1)] plates in deionized water and stained with DAPI for 1h in the dark.

Medium and cultivation

Pseudomonas Fluorescens DSM 4358 and *Desulfovibrio indonesiensis* DSM 15121 were obtained from Deutsche Sammlung von Mikrooganismen und Zellkulturen (DSMZ, Braunschweig, Germany) [(2)]. Cultures were maintained continuously in recommended medium (SRB DSM15121, Pseudomonas DSM220) of DSMZ. Electrochemical measurements were performed using low nutrient loaded artificial seawater (ASW) to simulate natural seawater conditions. The modified medium consists of: Part A: NaCl 23.93 g; Na_2SO_4 4.01 g, KCl 0.68 g, $NAHCO_3$ 0.197 g, KBr 0.099 g, H_3BO_3 0.03 g, NaF 0.01 g dissolved in 750 ml deionized water. Part B: 1.0M $MgCl_2.6H_2O$ 53.27 ml, 1.0M $CaCl_2.2H_2O$ 10.33 ml, 1.0M $SrCl_2.6H_2O$ 0.90 ml. Part A and B are combined to 1 litre. Modification was made by additional nutrient supply of 4.2 ml lactate and 0.01 g yeast. The medium was sterilized by autoclaving for 20 min at 121^oC at 1.5bar. The pH of the medium was 6.2±0.3 for *Pseudomonas spp.* in the experimental set up. For sulfate-reducing-bacteria a supplementary solution containing $FeSO_4*H_2O$ 0.004 g, $Na_3C_6H_5O_7*2H_2O$ 0.30 g, $C_6H_8O_6$ 0.10 g dissolved in 10 ml distilled water was added as to have efficient e^--acceptor for metabolic activity.

Electrochemical cells

The working electrode position was in vertical position to avoid precipitation of corrosion products directly on the electrode surface. All measurements were performed in a model set up made to simulate the corrosion processes on uncoated low carbon steel type ASTM A131 grade EH36 steel typically used for ballast tank construction in artificial sea water (ASW). Acryl measuring cells were house made with a size of 10.5 cm diameter and 8 cm height with a volume of 500 ml (Figure 2A). Electrodes were mounted in epoxy resin and the surface was ground using a series of grid 120, 500, 1000, 2400 SiC-paper (Struers) [(3)] to obtain a smooth surface (Figure 2B). The polished electrodes with an exposed surface area of 1 cm^2 were used as working electrodes. The top cover was configured with openings for three Ag/AgCl reference electrodes (Ref 201 Red rod, Radiometer Analytical) [(4)] in combination with platinum mesh counter electrode. Counter and reference electrode were made air tight using rubber stopper. Prior to immersion the working electrodes were rinsed with distilled water, degreased with acetone, sterilized in 70 % ethanol for 30 min and maintained under sterile conditions before mounting the electrodes inside the cell under sterile clean bench conditions.

(1) QuadriPerm Greiner Bio-One B.V. 2408 A/R Alphen a/d Rijn, The Netherlands.
(2) DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. Inhoffenstraße 7 B, 38124 Braunschweig, Germany.
(3) Struers GmbH Nederland 3144 CB Maassluis, The Netherlands.
(4) Radiometer analytical HACH LANGE, Laan van Westroijen 2a 4003 AZ Tiel, The Netherlands.

Liquid bacterial cultures of *P.fluorescence* (DSM 4358), and the sulphate reducer, *Desulfovibrio indonesiensis* (DSM 15121) were used as inoculum to monitor the corrosion impact of these bacterial species (Fig. 2a). For a more realistic environment of the tank, sterilized sea mud was used as sediment supplement as can be seen in (Fig. 2b). The working electrodes were fixed in different height levels in the set up (Fig. 2b, 2c (orange circle)). The blank cell set-up was treated equal and to avoid any kind of bacterial growth 0.01% of sodium azide was added.

2a

2b

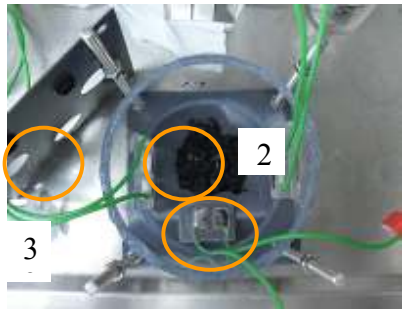
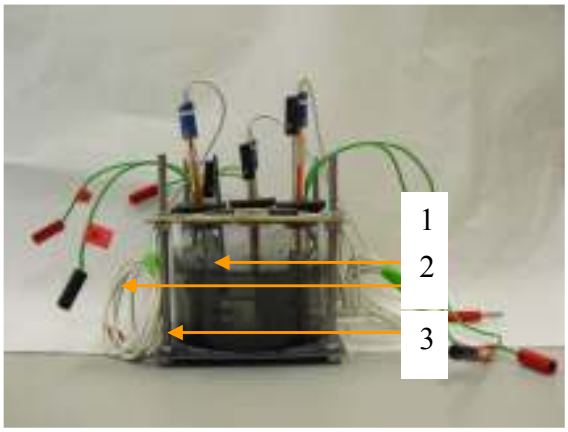


Figure 2: Impedance set-up for bacterial experiments

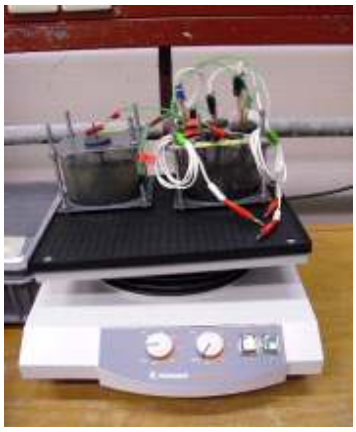
2a: Bacterial cultures used as inoculums at mid exponential growth phase indicated by black precipitation (SRB) and dense white culture (*Pseudomonas*); 2b: Cell set-up with sterile sea mud and fixed working electrodes on the side walls and bottom (orange circle, 1: Splashzone, 2: Immersion zone, 3: Ground)

2c



2c EIS lab scale set-up for different zones (1: Splashzone, 2: Immersion zone, 3: Ground)

2d



2d: 3D shaker to simulate ship rolling

Due to the fact that not a complete anaerobic set-up was the intention, hence a complete sealing was avoided. This will allow semi-anaerobic condition over the course of experimental time. The cells were placed on a three dimensional shaker to imitate the rolling of a ship (Fig. 2d). The movement of the shaker (15rpm) will allow simulating the splash zone of a ballast tank with continuous aerobic/anaerobic boundaries or conditions.

Electrochemical measurements

Liner polarization resistance (LPR)

LPR technique is widely employed and the relevant theoretical relationships are well known. Generally, the LPR technique is considered suitable to predict corrosion rates in the laboratory within a factor of 2. For these measurements, a potentiodynamic method was used to obtain the potential-currrent (E-I) ratio, applying a ± 10 mV overpotential, with respect to the free corrosion potential E_{corr}.

Electrochemical Impedance Spectroscopy (EIS)

EIS is a powerful, electrochemical technique for the characterization of electrochemical reactions at the metal/biofilm interface and the formation of corrosion products and biofilms in MIC [7]. EIS measurements were at the open circuit potential using a 10 mV amplitude sinusoidal signal over frequencies ranging from 10.000 to 0.01 Hz.

Bacterial colonization study with EFM

Bacterial colonization of the working electrode surface was analyzed after 21d of exposure to microorganisms. Electrode surfaces were gently rinsed twice with sterile oxygen-free deionized water. Light microscopy pictures were taken with a 20X magnification. Fluorescent staining was performed in liquid, for this a rubber ring fixed on the epoxy body of the working electrodes allowed to cover the working electrodes surface constantly with deionized water (flushed with N_2). By doing so, staining could be performed under liquid conditions to avoid structural disturbance of the 3 dimensional biofilm. For bacterial colonization studies, the nucleic acid dyes DAPI (25 µM) was used. Bacterial biofilms were imaged with 20X magnification using an Olympus BX51 fluorescent microscope (Olympus America Inc., NY) [(5)]

Interface analysis morphology and composition

For a better understanding of the evolution of the surface films of corrosion products with time of exposure and for evaluation of the influence of *Pseudomonas* and *Desulfovibrio* bacteria on the formation of surface films on carbon steel, EDX studies were undertaken. No surface cleaning of the electrodes was performed and the samples were just dried under nitrogen atmosphere to determine the corrosion layers. The dried working electrodes were stored in a glove box (Interflow) under nitrogen atmosphere until EDX study.

SEM analysis was performed with a Joel JSM-5800LV [(6)] scanning electron microscope and additional EDX techniques were performed at the working electrode surface after 21d of exposure to bacterial cultures and blank samples only exposed to medium. EDX spectra of the corrosion products and surface films were identified with software NSS Noran, (Thermo Scientific) [(7)].

[(5)] Olympus Nederland B.V. Industrieweg 44, 2382 NW Zoeterwoude, The Netherlands.
[(6)] JEOL (Europe) B.V. Lireweg 4, 2153 PH Nieuw-Vennep, The Netherlands.
[(7)] Thermo Fisher Scientific Inc. Janssen Pharmaceuticalaan 3A 2440 Geel, B-2440 Belgium.

The following parameters were measured over the period of 21 days.

Diagram 1: Experimental parameters measured over period of time.

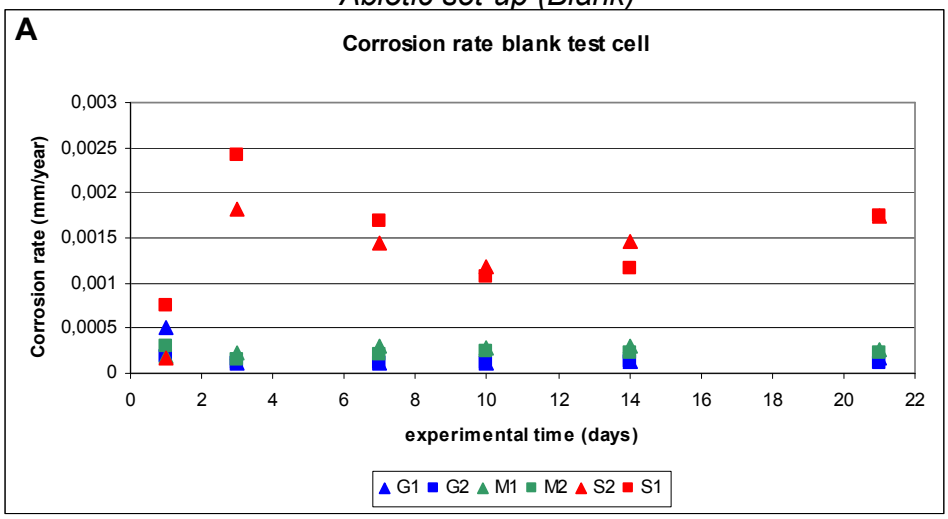
Microbiology	Electrochemistry
pH, T	OCP
planktonic/sessile bacterial cell count	LPR
fluorescent staining for growth monitoring	EIS

RESULTS & DISCUSSION

Corrosion rates and pH, T diagrams of 3 different zones

The following figures of corrosion rate, pH and temperature for the blank are given in Figure 3 A&B. Corrosion rates based on LPR measurements show a clear difference in the tested zones. Figure 3A gives an overview of the corrosion rates in the diverse zones over the period of time indicating that the highest corrosion rate can be found in the splash zone (red/orange dots) were a constant change in wet and dry conditions is predominant. For the immersion zone (green dots) and the ground (blue dots) were less oxygen is available the corrosion rates are low. In Figure 3B the pH values are in the range of 7,2 - 8,2 (blue dots) and the temperature varies from 21 – 25,5°C (pink dots). For these environmental parameters we can say that the changes not drastically and they have in this case not a significant impact on the corrosion rates.

Abiotic set-up (Blank)



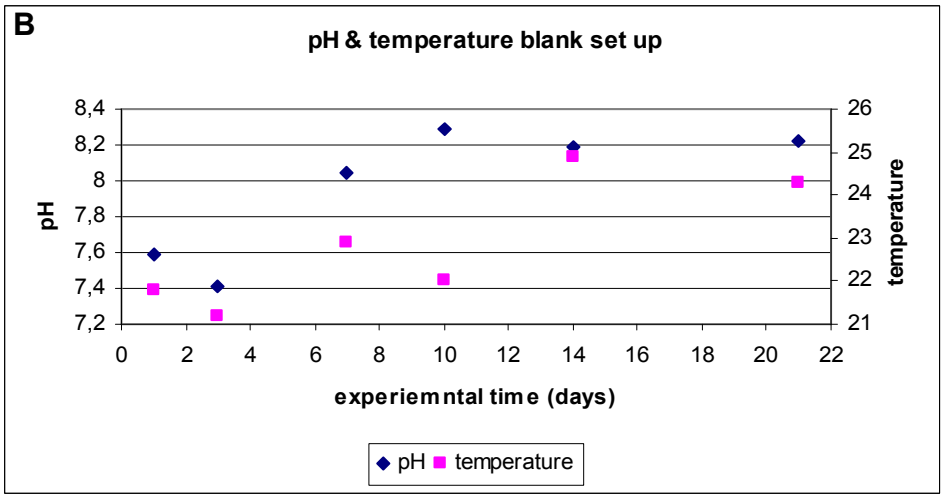


Figure 3: Corrosion rates vs. experimental time in days of the blank (no bacteria).

A: Corrosion rates (mm/year) vs time blank
Blue dots: Ground zone
Green dots Immersion zone
Red/orange dots: Splash zone

B: pH & Temperature trend vs time of the blank
Blue dots : pH
Pink dots : temperature

Biotic set-up (natural bacterial community)

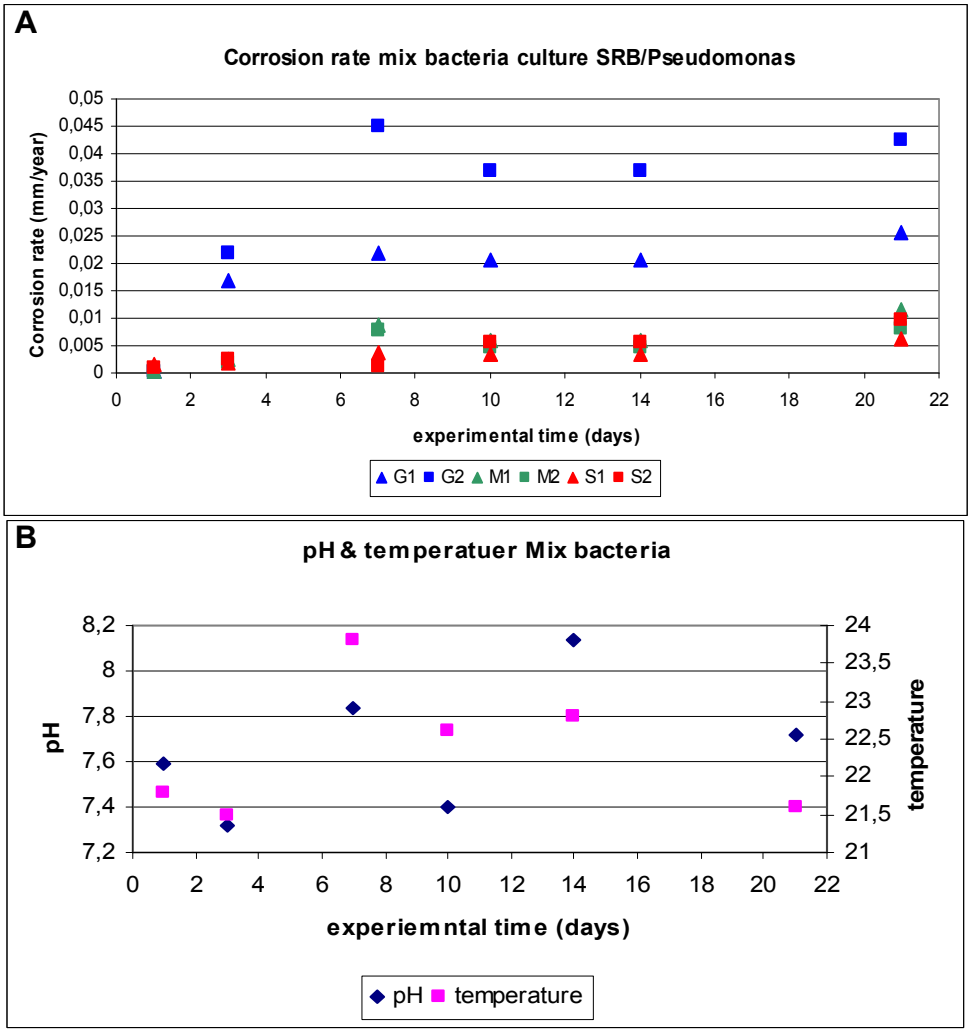


Figure 4: Corrosion rates vs. experimental time in days of bacteria (bacteria set up).

A: Corrosion rates (mm/year) vs time of bacteria
Blue dots: Ground zone
Green dots Immersion zone
Red/orange dots: Splash zone

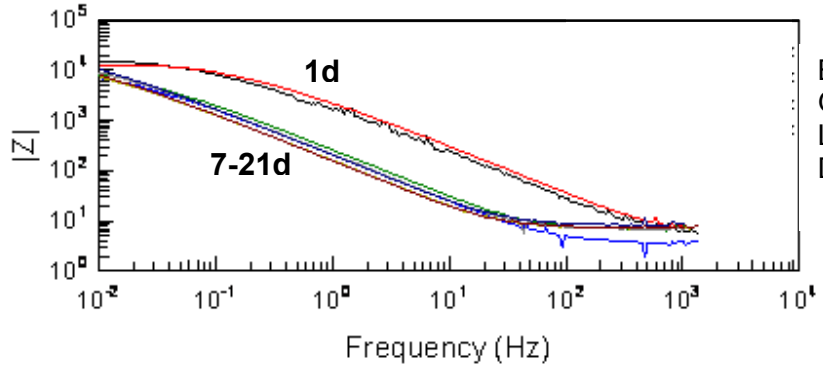
B: pH & Temperature trend vs time of bacteria
Blue dots : pH
Pink dots : temperature

Figure 4A represents the biotic set-up for which a clear difference to the blank for the corrosion rate can be found. For bacteria the ground zone seems to be more favourable for growth and in combination with growth a higher corrosion rate is reached (blue dots). For the immersion (green dots) and the splash zone (red/orange dots) a lower corrosion rate was monitored leading to the assumption that anaerobic zones are favourable for bacterial activity and growth. In Figure 4B the trends in pH and temperature are given showing the same trend as the blank in values of 7,2 - 8,2 for pH and a temperature between 21,5 - 24°C. From that we can conclude that bacterial impact on the pH in the bulk phase is not significant. Bacterial growth within the cell was monitored by coupons which were removed intermediate and stained with the fluorescent dye DAPI to monitor bacterial growth.

EIS measurements for abiotic and biotic set-up

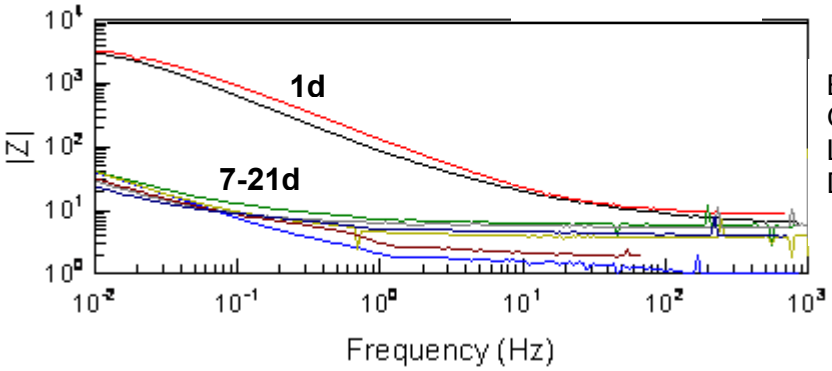
Impedance spectra are analyzed using equivalent circuits, taking into account the contribution of each phenomenon, such as film formation and adsorption of corrosion products on the metal surface. The respective Bode modulus plots obtained for biotic and abiotic set-up of the ground level (in duplicate) are shown in Figure 5. In the abiotic environment i.e. blank, the electrode impedance is relative constant over the period of time (Figure 5A). Experimental period (days) are indicated with numbers used in coloured lines (1d black & red line, 7d blue & green line, 14d green & grey line, 21d dark blue & brown). The small change in impendance (ıZı) after 7 days, indicates the formation and breakdown of corrosion product film on the surface. On the other hand, in the biotic conditions, a larger phase shift is noticed immediately after 1 day period of immersion. Not much large differences are observed in the impedance among 7d to 21d (Figure 5B), however decrease in ıZı after 1 day indicate an increase in the corrosion process due the growth of mixed bacterial biofilms compared to blank. This signifies the continuous strengthening of the corrosive biofilm in the presence of bacteria.

A



A Bode plots blank set up

B



B Bode plots mixed biofilm set up

Figure 5: Bode plots of impedance measurements (abiotic & biotic set up, ground level) Numbers indicating experimental time in days.

Fluorescent staining work

Fluorescence microscopy, a widely recognized tool to study bacterial attachment, was used to observe bacterial colonization on the working electrode surface. It has been reported that microorganisms tend to settle preferentially on steel in the form of colonies or at least in a patchy manner to reduce the total substrate surface area. The results obtained in this study confirmed the previous conclusions that patchy biofilms are formed on steel surfaces and acts as a good template for the proliferation of microorganisms and biofilm formation [8]. The images presented in Figure 6 give a clear overview over the different colonization steps of bacteria on carbon steel surfaces.

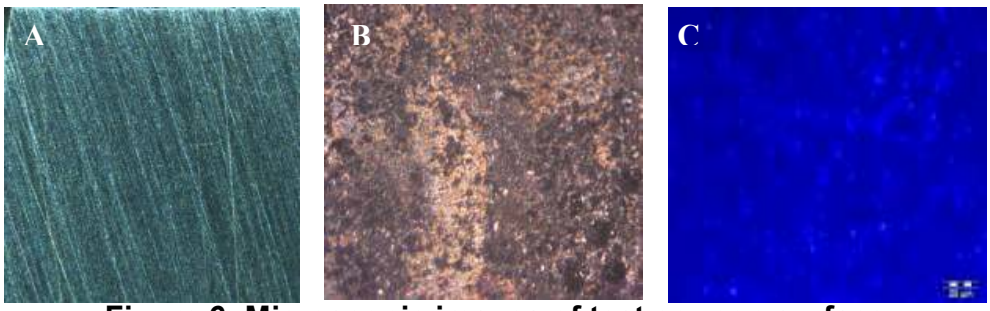


Figure 6: Microscopic images of test coupon surface before/after experiment of 21d.

Depending on immersion time in bacterial cultures, more and more cells attach to the surface until a 3 dimensional biofilm is formed after 21 days. In Figure 6A, the blank metal surface is shown before immersion in the medium without bacteria. Figure 6B shows the blank abiotic surface after immersion for 21d in ASW without bacteria. The apparent surface roughness indicates the formation of corrosion products on the surface. Figure 6C shows the metal surface with attached bacteria stained with DAPI.

Surface topography analysis with SEM and EDX

In the following section, different surfaces of low carbon steel exposed in abiotic and biotic conditions over 21 days are presented. In Figure 7 two regions are presented, the grey region direct on the surface (blue frame) indicated by a high iron content but also some chloride compounds are found, and the bright region can be seen as an outer layer on the surface (orange frame) as precipitated compounds of the medium. The presence of sulfur based compounds can be negligible in the abiotic system see Figure 7B, the identified S peak is just a result of medium compounds such as sulfate from the ASW.

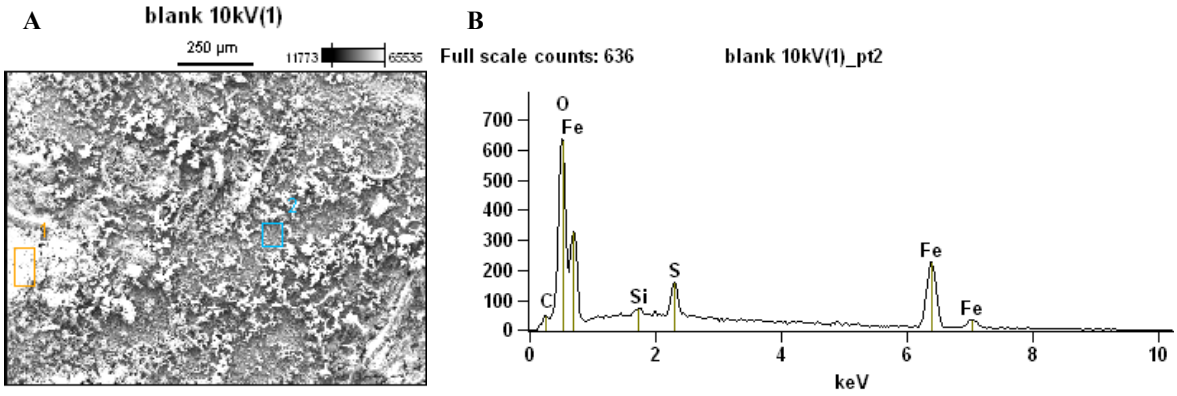


Figure 7: SEM and EDX analysis for the abiotic system (A) SEM image of carbon steel exposed to sterile artificial seawater (supplemented with nutrients) at 100x. Two regions can be observed over the metallic surface, a bright region and a darker region which can be each identified separately based on their identical composition of identical components and salts. (B) EDX analysis corresponding to the darker region (blue framed).

SEM and EDX analysis display the morphological and characteristics of the layer grown on carbon steel exposed to the mixed biofilm over 21d, as shown in Figure 8A and 8B respectively. In the presence of sulfate reducing bacteria, there is accumulation of sulfur-based compounds (Figure 8B2). A difference in the surface structure can be identified where biofilm structures appeared white on the surface (blue frame) a clear increase in the sulfur content (Figure 8 B1 and B2) of the same spot indicates bacterial attachment on the surface.

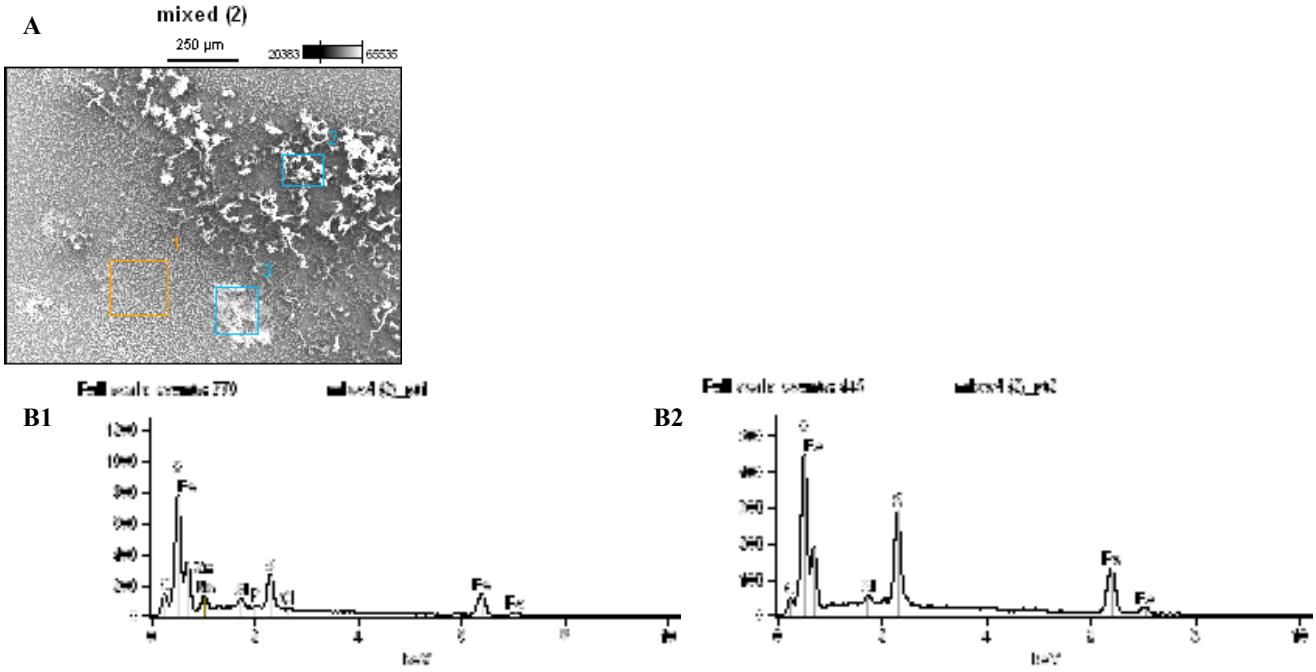


Figure 8: SEM and EDX analysis for the system under mixed biofilm *Pseudomonas-SRB* biofilm influence. (A) SEM image of carbon steel exposed to sterile artificial seawater (supplemented with nutrients) and with Pseudomonas-SRB, at a 100X magnification. EDX analysis corresponding to the SEM smooth region A (orange frame) and the rough biofilm structure B (blue frame) as shown in Figure 8A.

In summary, biotic and abiotic systems showed differences in distribution and composition of the corrosion products formed. In the presence of sulphate reducing bacteria, there is accumulation of sulfur-based compounds (Figure 8B2). In the biotic interphase (Figure 8A) substantial amount of products growing upwards are observed, and the interface does not have a rigid appearance, most likely due to biofilm matrix, whose nature is polysaccharidic and viscoelastic.

Industrial applications and valorisation of the results

Electrochemical impedance measurements are the first choice in analyzing corrosion processes at aquatic interphase and will lead to a better understanding of the whole processes of bacterial community interactions on the steel surface by using equivalent circuits. The different zone set up will allow studying the corrosivity in different parts of ballast tanks for better maintenance programs. By that a more detailed investigation of local corrosion interactions are possible. The knowledge generated in this project will also contribute to the development of advanced MIC detection techniques. Major benefits resulting from the project outcome include: reduction of down-time, increased safety, reduced pollution risk and better MIC prevention technologies. By combining these techniques from different scientific fields with a reliable set up, the fundamental understanding of the MIC process can be promoted which is the final goal of this research project.

CONCLUSIONS

Interactions of diverse bacteria are highly important. From that we can derive the following assumptions: the bacterial set up showed a 10 times higher corrosion rate than the corresponding blank set up without bacteria. The lab scale set up can just simulate the natural environment in perspective of corrosion rates the natural rates cannot be reached due to the fact that bacterial interactions need time to develop. By combination with LPR measurements and continuous monitoring of the growth of the biofilms on the coupons, it is possible to get better indications for bacterial metal interactions.

We could underline the importance of diverse bacterial strains which can have a significant impact on the corrosion behaviour. In natural environments this impact can even be stronger and more complex. The different zone set up will allow studying the corrosivity in different sections of ballast tanks for better maintenance programs.

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REFERENCES

1. Zinkevich, I. Hanjansit, R. Gubner, R. Avci, “The effect of *Pseudomonas* NCIMB 2021 biofilm on AISI 316 stainless steel,” Biofouling 15 (2000), p. 3-12.

2. B.J. Little and J.S. Lee, Microbiologically influenced Corrosion,
In Wily-Interscience Wiley & Sons, (Inc., Hoboken, New Jersey, 2007), chapter 2.

3. Suzuki, S., Muraoka, R., Obinata, T., Endo, S., Horita, T., Omata, K.,
Steel products for shipbuilding, JFE Technical Report No.2 (2004).

4. M.N.Tamburri, k. Wasson, M. Matsuda, “Ballast water deoxygenation can prevent aquatic introductions while reducing ship corrosion,” Biological Conversation 103 (2002), pp. 331-341.

5. J. Duan, S. Wu, X. Zhang, G. Huang, M. Du, B. Hou, “Corrosion of carbon steel influenced by anaerobic biofilm in natural seawater,” Electrochimica Acta 54 (2008): p. 22-28.

6. E.J. Pérez, R. Cabrera-Sierra, I. Gonzalez, F. Ramírez-Vives, “Influence of *Desulfovibrio* sp. biofilm on SAE 1018 carbon steel corrosion in synthetic marine medium,” Corrosion Science 49 (2007):
p. 3580-3597.

7. G. Gunasekaran, S.T. Chongdar, P. Kumar, “Influence of bacteria on film formation inhibiting corrosion,” Corrosion. Science 46 (2004): p.1953-1967.

8. S. Chongdar, G. Gunasekaran, P. Kumar, “Corrosion inhibition of mild steel by aerobic biofilm,” Electrochemica Acta 50 (2005): p. 4655-4665.