Identification of key factors in Accelerated Low Water Corrosion through experimental simulation of tidal conditions: influence of stimulated indigenous microbiota

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Biotic and abiotic factors favoring Accelerated Low Water Corrosion (ALWC) on harbor steel structures remain unclear warranting their study under controlled experimental tidal conditions. Initial stimulation of marine microbial consortia by a pulse of organic matter resulted in localized corrosion and the highest corrosion rates (up to 12-times higher than non-stimulated conditions) in the low water zone, persisting after nine months exposure to natural seawater. Correlations between corrosion severity and the abundance and composition of metabolically active sulfate-reducing bacteria (SRB) indicated the importance and persistence of specific bacterial populations in accelerated corrosion. One phylotype related to the electrogenic SRB Desulfopila corrodens appeared as the major causative agent of the accelerated corrosion. The similarity of bacterial populations related to sulfur and iron cycles, mineral and tuberculation with those identified in ALWC support the relevance of experimental simulation of tidal conditions in the management of steel corrosion exposed to harbor environments.

Keywords: ALWC; bacterial communities; electrogenic SRB; MIC; simulated tidal conditions; 16S rRNA/dsrB genes and transcripts

Introduction

The global cost of marine corrosion is estimated to total 50–80 billion $ yr⁻¹ (NACE 2013). A particularly aggressive and localized corrosion phenomenon, Accelerated Low Water Corrosion (ALWC), is encountered in several harbors worldwide. It is associated with strong tubercle formation and typical corrosion rates of >0.5 mm yr⁻¹ (PIANC 2005). Despite considerable research efforts consensus about its microbiological nature (Gehrke & Sand 2003; Beech & Campbell 2008; Pineau et al. 2008; Jeffrey & Melchers 2010; Melchers & Jeffrey 2012), the mechanisms of corrosion remain unclear and therefore, not predictable. The high corrosion rates observed have often been attributed to the activity of sulfate-reducing bacteria (SRB), and the production of iron sulfide. However, SRB are known to differ in corrosiveness (eg Beech et al. 1994; Sunny Cheung et al. 1994). Recently, detailed bacterial community studies of deposits from European harbors affected by ALWC showed that metabolically active bacteria involved in the sulfur cycle with unique metabolic capabilities, from specific taxonomic lineages and associated with specific minerals are characteristic of deposits with ALWC features (Païssé et al. 2013; Marty et al. 2014). A combination of macroscopic and microscopic characteristics of ALWC deposits suggested higher rates of electron transfer from the steel and acidification driven by biological sulfur and iron cycling as main mechanisms of ALWC (Marty et al. 2014). The peculiarities of inferred bacterial ecophysiology suggested that steel, organic pollution, and macroorganisms (eg algae) within harbors may significantly contribute to microbial activity, and to the introduction of local and distant cathodes, resulting in higher thickness loss of anodic areas with corrosion rates exceeding 0.3 mm yr⁻¹. This agreed with the results of previous observations whereby harbors with corrosion rates ≥0.5 mm yr⁻¹ in the low water zone (LWZ) hosted significantly more algae, higher total organic carbon (TOC), and lower redox as compared to

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non-affected harbors with corrosion rates of $\leq 0.5$ mm yr$^{-1}$ (Gubner & Beech 1999).

Screening of several physico-chemical parameters in Australian harbors concomitant with in situ corrosion rate measurements of carbon steel strips during one to three years also showed that nitrogen concentrations (a limiting nutrient) and temperature were linearly correlated with corrosion rates in the LWZ (Melchers & Jeffrey 2012). Although the correlation between organic/nutrient pollution, microbial stimulation and corrosion is known, related quantitative and characterization studies are scarce (eg Gubner 1998; Kjellerup et al. 2009; Melchers 2013) and therefore, mechanisms not well understood. One important reason for the lack of mechanistic understanding of a complex phenomenon such as ALWC lies in the difficulty in conducting in situ experiments and site comparisons, since logistics, confidentiality, costs, and uncontrolled environmental conditions are major obstacles.

As an alternative, laboratory set-ups are proposed, although these are criticized for their incapacity to simulate realistic ALWC conditions (Melchers & Jeffrey 2012). This is mainly because these studies were performed in batch mode, with artificially rich media followed by waste compound accumulation and/or defined bacterial consortia (Gubner 1998; Beech & Campbell 2008). The inhibitory effects of batch mode on Microbiologically Inflated Corrosion (MIC) and electro-activity have been observed in several studies (eg Taheri et al. 2005; Erable et al. 2010), which more likely explain the conflicting results of laboratory experiments (Little et al. 2008). Although these conditions may be representative in certain cases (eg stagnant and enclosed water system such as ballast tanks) (Lee et al. 2004; Machuca et al. 2013), the dynamic nature of waterfront conditions is totally ignored. Research on bacteria–surface interactions (ie biofilms) should preferentially be conducted in flow-through systems since eg continuous nutrient supply and product removal (ie continuous mode) and hydrodynamics determine biochemical reaction kinetics in biofilms, responsible for growth, and strength of adherence of biofilms to surfaces (van Loosdrecht et al. 1995). In waterfront corrosion, the influence of tide on the physico-chemical and biological parameters of seawater and the macro-galvanic cells established on steel structures is critical and has to be considered in the design of relevant test systems. The few studies that reported the development and testing of such devices indicated results comparable to in situ conditions (Baorong & Bin 2003; Xiaodong et al. 2011). These results were however limited to a comparison of the composition of corrosion products and corrosion rates in different exposures zones.

Here, the authors report the use of tidal microcosm units developed to simulate structural steel exposed to marine tidal conditions. These were applied to investigate the relative contribution and synergies between parameters susceptible to influencing marine corrosion and low water corrosion in particular. The authors studied the impact of transient stimulation of planktonic seawater and sessile steel consortia by temporary supply of carbon/nutrient, on the abundance and diversity of the total and metabolically active fraction of bacterial communities, and on the mineralogy of corrosion products and the consequences on steel corrosion.

**Experimental procedure**

**Reactor set-up**

Each reactor was composed of control and test vessels made of polypropylene specially built to study the effect of various parameters (metallurgical, hydro dynamical, physico-chemical, electrochemical, and biological factors) on the corrosion of steel exposed to tidal conditions in a controlled way (Appendix 1 in Supplementary information). [Supplementary information is available via a multimedia link on the online article webpage.]). Seawater was supplied to each reactor by a common separate tank constantly fed with natural seawater pumped in the port of Flamands, Cherbourg-Octeville (49°38′33″N 1°37′31″W). A semi-diurnal tidal regime (fixed to 12 h 30 min) was set with new seawater introduced after each tide except during the recycling mode where water was pumped back and forth between the control and test vessels. The fluctuations in tidal amplitude according to the lunar/sun/Earth cycles were not reproduced. Hence, the air/water interface (±10 cm) at low tide was considered as the LWZ. The heights of immersion (32 cm) and tidal (40 cm) exposure zones were set to similar values. The tide amplitude was controlled by pump speed, timer, and levels of the inlet and outlet pipes in the test vessel equipped with a liquid level switch (Mobrey Mini Squing, Emerson) for overflow protection. Automatic filling of the control vessel set to 15 min was ensured by electrovalve. The maximum seawater volume at high tide was 150 l.

**Carbon steel specimens**

Carbon steel specimens from S430GP steel sheet piling products of dimensions $950 \times 100 \times 7.8$ mm$^3$ were used as delivered, ie without prior sand blasting and polishing. To prevent border effects, the peripheries of the steel specimens were protected by paint (monolayer acrylic polyurethane anticorrosive paint (Manufacturer: Peintures Robin s.a. This paint complies with norms ISO12944-2 and AFNOR NF T 36-005. Famille 1 Classe 6a/7b1) filled with zinc pigments [120 μm]). Two specimens, designated ‘pre-corroded steel’, had
been previously exposed in the same units for nine months with natural seawater from the same harbor and at the same tidal regime. They were introduced as the natural inoculum of steel-adapted sessile microorganisms, designated ‘steel-adapted inoculum’ in the next section.

**Experimental design**

To study the impact of planktonic populations originating from seawater and populations adapted to sessile life on steel (‘steel adapted inoculum’), three reactors (called units 1, 2, and 3) were run in three steps whereby phases 1 and 2 aimed at enriching the indigenous populations (Figure 1). Unit 1 (U1) was not subject to microbial enrichment and served as the experimental control.

The tests first started by two weeks of batch operation (i.e. phases 1 and 2) with tidal effect. In the second week, the steel coupons were added to the units. After two weeks, the units were run in flow-through mode (phase 3).

Phase 1: Unit 3 (U3) was supplied with the two ‘pre-corroded steel’ specimens used as the ‘steel-adapted inoculum’. Yeast extract was added to units 2 (U2) and 3 (U3) at a final concentration of 1 g l⁻¹ to stimulate planktonic populations from seawater in U2 and both the planktonic and ‘steel-adapted inoculum’ populations in U3. All reactors were run in recycling mode for one week.

Phase 2: Two pristine steel specimens were immersed in each test vessel and the units were run for another week in recycling mode without seawater renewal or organic enrichment.

Phase 3 or continuous phase: The units were run in continuous mode (renewal of natural seawater at each tide) during nine months from July 2010 to April 2011.

**Physico-chemical and bacteriological analysis of seawater**

The pH, temperature, dissolved O₂ (dO₂), salinity, and redox potential were monitored continuously via a data

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Figure 1. Schematic overview of the experimental design. During phases 1 and 2 of one-week duration each, no renewal of natural seawater occurred (‘recycling mode’). During phase 3, new natural seawater was introduced for each tidal cycle (‘continuous mode’). Note that yeast extract was only added to natural seawater during phase 1. Full and dashed rectangles correspond respectively to new and ‘pre-corroded’ steel bars.
logger linked to portable multi-meters connected to the \( \text{dO}_2 \) probe with salinity and temperature compensation and \( \text{pH} \) with temperature compensation (Neotek-Ponsel, Odeon). Total iron concentration was determined by atomic absorption spectroscopy in flame mode. Total cell counts were performed by epifluorescence microscopy with acridine orange. The number of SRB was assessed by the Most Probable Number method [AFNOR standard, NF T 90-413, Essais des eaux, 1985] based on Starkey culture medium (Starkey 1938).

**Steel corrosion potential measurements**

The free corrosion potential (Ecorr) of steel specimens exposed in each unit was monitored continuously with respect to a zinc anode. Data were acquired every 10 min via multiplexer and its adapted module (34970A-Agilent).

**Corrosion deposit sampling and thickness loss measurements**

Corrosion deposits from the steel specimens exposed to the immersion zone (IZ) and the LWZ (ie tubercle area) were sampled aseptically at high tide to limit oxygen exposure and were immediately transferred into sterile containers and kept frozen in dry ice or in a \(-80^\circ\text{C}\) freezer until further analysis. The removal of residual corrosion products from steel surface specimens was performed by a chemical cleaning procedure (C.3.5. from ASTM G1-03 2011). For each specimen, a transversal section 1 cm in height was cut at 330 mm from the extremity of the constantly immersed part of the specimen. This section corresponded to the zone of tubercle formation. Each section was further cut transversally into three equal subsections, each mounted in resin, ground and polished with an alumina suspension (about 1 \( \mu \text{m} \) particle size) for observation. Residual thickness measurements were performed with an optical metallographic microscope using a calibrated focusing knob on cross-sections of the steel specimen from the low water zone. The corrosion rates were calculated from residual thickness measurements.

**Microbial community analysis**

DNA and RNA extracts were obtained according to preliminary optimized extraction procedures (Marty et al. 2012) based on commercial kits (PowerSoil kits from MoBio Laboratories Inc. for DNA and RNA isolation). cDNA was obtained from DNase-treated RNA using the high capacity cDNA reverse transcription kit (Applied Biosystems) with random hexamers primers. PCR assays for DGGE and quantitative PCR were performed as previously reported (Marty et al. 2012; Païssé et al. 2013) as well as statistical treatment of DGGE gels, band extraction, and sequencing. The taxonomic marker 16S rRNA was applied in qPCR and in PCR-DGGE analysis whereas the functional marker (dsrB coding for the \( \beta \)-subunit of the dissimilatory sulfite reductase) was only used for qPCR. The quality of the sequences obtained from the DGGE was first checked and the sequence extremities trimmed with the CodonCode Aligner software. Subsequently, the program Bellerophon version 3.0 (Huber et al. 2004) was used to check for chimera sequences. Thereafter the sequences were aligned to sequences of near affiliates as determined by BLAST or SILVA (Pruess et al. 2012) using ClustalX2 (Larkin et al. 2007). Finally, the multiple sequence alignment generated was used to construct a phylogenetic neighbor joining (NJ) tree with bootstrap values (from 1,000 replicates) using the software program MEGA version 5.1 (Tamura et al. 2011). The robustness of branches within sub-trees corresponding to phylum/class was also verified by comparing results obtained with NJ algorithms and maximum parsimony/likelihood methods. The sequences have been deposited in Genbank under accession numbers KF049712 – KF049776.

**Elemental and mineralogical analysis**

Before elemental and mineralogical analysis, each sample was ground and dried (using filter paper) in the frozen state to prevent product oxidation and stored frozen for future analysis. The Bruker S4 Pioneer model (Wavelength Dispersive X-ray Fluorescence with an excitation power up to 4 kW) was used to determine the elemental composition of each sample. Due to the low quantity of material available per sample (around 0.1 g of dry powder sample\(^{-1} \)), the samples were deposited on substrata of boric acid. The minimum irradiation area (8 mm diameter) was irradiated on each sample. The software SPECTRAPlus was used to interpret the spectra obtained. The mineral composition of each sample was determined by X-ray diffraction (XRD). XRD patterns were obtained with a Bruker diffractometer (D8 Advance) with a graphite monochromator and CuK\(\alpha\) radiation (\( \lambda = 1.54 \text{ Å} \)). The 2\( \theta \) angle ranged from 5\(^\circ\) to 75\(^\circ\) with a step of 0.04\(^\circ\) and a counting time of 5 s, the total time scan being of 2 h and 26 min per diffractogram. EVA software and the associated JCPDF database were used for the analysis of crystalline phases.

**Statistical analysis**

All statistical univariate and bivariate analyses were performed using the software program SPSS 19. The Spearman’s rank pairwise correlation was used for
bivariate analysis. To compare the microbial community structure between deposits, hierarchical agglomerative clustering of Pearson similarities from normalized DGGE fingerprints (band positions and intensities) was performed using the software program GelCompar-II. Simpson community diversity index and Euclidian distances of standardized elemental and mineralogical contents from samples were determined with the software program PRIMERv5, and the hypothesis of no relation between multivariable elemental and mineral patterns was tested (Mantel test type).

Results

Monitoring of seawater parameters

Microbial enrichment phase (phases 1 and 2)

Addition of yeast extract to Units 2 and 3 resulted in significantly decreased values for seawater parameters such as redox potential, dissolved oxygen (dO2), and pH during the two week start-up period (Table 1). Anoxic (0.2 mg l\(^{-1}\) dO2 on average the first week and not detectable in unit 2 the second week after steel addition), reduced (average \(-68 \pm 175\) mV the first week down to \(-202\) mV), and slightly acidic (pH 6.5 on average during the first week) conditions were experienced in both units. In contrast, the seawater in control unit 1 showed a positive redox (469 mV), oxygen (4.4 mg l\(^{-1}\)), and a pH of 8.2 (Table 1). In parallel, the planktonic cells increased at least two logs and the sulfidogens reached the order of 10\(^4\) and 10\(^3\) MPN cells ml\(^{-1}\) in U2 and U3, respectively, while they were not detected in the control unit U1. Due to the presence of extra steel bars (ie two ‘pre-corroded steel’ specimens) in U3, the concentration of dissolved iron was at least 10-times higher in this unit than in the other units at the end of phase 1 (Table 1). After the addition of unexposed steel in all units in phase 2, a tenfold decrease in the SRB/total cell ratios at the end of this phase was observed in U2 and U3 with unchanged or higher total cell concentrations. This also agreed with the striking differences in cell morphologies between planktonic populations from U2 and U3 (Figure 2).

Continuous phase (phase 3)

With continuous seawater renewal hydraulic retention time of one day, the dO2, redox potential, and pH increased within five days to reach similar mean values between units, alleviating any further influence of these parameters between units. The somewhat lower seawater redox potential values in U3 (322 ± 107 mV) than in the

| Table 1. Summary values of physico–chemical and microbial parameters in each unit during the three test phases (mean ± standard deviation values are given for physico–chemical parameters). |
|---------------------------------------------------------------|
| **Test phases** | **Unit 1 (U1) (Control)** | **Unit 2 (U2)** | **Unit 3 (U3) (+Pre-corroded steel)** |
| Phase (1) (n = 645) | | | |
| Temperature | 20.4 ± 0.5 | 20.5 ± 0.5 | 20.3 ± 0.6 |
| Salinity % | 31.72 ± 0.48 | NP | 31.78 ± 0.10 |
| dO2 (mg l\(^{-1}\)) | 4.4 ± 0.8 | 0.2 ± 1.2 | 0.2 ± 0.8 |
| pH | 8.21 ± 0.07 | 6.50 | 6.57 ± 0.53 |
| Redox (mV SHE\(^{-1}\)) | 469 ± 3 | NP | \(-68 \pm 175\) |
| Total Fe (mg l\(^{-1}\)) | <0.04 | <0.04–1.2 | 1.96–10.79 |
| MPN SRB ml\(^{-1}\) | <1 | 4.30E + 04 | 2.30E + 03 |
| TCC ml\(^{-1}\) | 8.60E + 05 | 2.30E + 07 | 1.30E + 07 |
| Phase (2) (n = 662) | | | |
| Temperature | 20.7 ± 0.6 | 20.7 ± 0.6 | 20.6 ± 0.7 |
| Salinity % | 32.39 ± 0.09 | 32.05 ± 0.35 | 33.27 ± 0.17 |
| dO2 (mg l\(^{-1}\)) | 3.6 ± 1.0 | 0.0 ± 0.0 | 0.2 ± 0.9 |
| pH | 7.76 ± 0.49 | 7.01 ± 0.29 | 7.28 ± 0.51 |
| Redox (mV SHE\(^{-1}\)) | 411 ± 56 | \(-202 \pm 30\) | \(-202 \pm 42\) |
| Total Fe (mg l\(^{-1}\)) | 0.49 | 1.09 | 0.70 |
| MPN SRB ml\(^{-1}\) | <1 | 2.30E + 03 | 3.00E + 02 |
| TCC ml\(^{-1}\) | 6.60E + 05 | 2.90E + 07 | 2.60E + 07 |
| Phase (3) (n = 22,573) | | | |
| Temperature | 12.6 ± 4.7 | 11.8 ± 5.1 | 11.5 ± 4.7 |
| Salinity % | 30.15 ± 2.90 | 30.08 ± 2.43 | 29.37 ± 3.25 |
| dO2 (mg l\(^{-1}\)) (CV) | 5.8 ± 2.6 (45) | 4.2 ± 2.9 (69) | 5.6 ± 2.7 (48) |
| pH | 7.55 ± 0.42 | 7.77 ± 0.40 | 7.81 ± 0.33 |
| Redox (mV SHE\(^{-1}\)) | 465 ± 50 | 395 ± 119 | 322 ± 107 |

Notes: NP: not performed; TCC: total cell count; MPN SRB: most probable number sulfidogens; CV: Coefficient of Variation; SHE: Standard Hydrogen Electrode.

\(^a\)Beginning and end-period values.

\(^b\)End-period value.
other units (395 ± 119 in U2 and 465 ± 50 mV in U1) may be linked to a periodically higher concentration of dissolved iron in U3 due to the presence of the extra steel compared to the other units. The dynamic trends of all measured parameters were similar in all units following the semi-diurnal regime: a pH decrease and a small dO2/redox potential increase was occurring at low tide, while the opposite was true on average towards high tide (results not shown).

**Progression of steel corrosion potential (Ecorr), corrosion rates, and corrosion topography**

*Microbial enrichment phase (phases 1 and 2)*

In unit 3 with ‘pre-corroded steel’, a positive shift (within 8 h) of Ecorr was observed (from −650 to −560 mV). Ecorr remained stable (−574 ± 11 mV) over the two weeks’ recycling (Table 2 and Figure 3). Addition of unexposed steel to units 2 and 3 resulted in a rapid stabilization of their Ecorr around −552 ± 26 and −557 ± 57 mV in U2 and U3, respectively. The Ecorr of the steel specimens introduced in unit 1 stabilized at much lower values (−682 ± 32 mV).

*Continuous phase (phase 3)*

With continuous seawater renewal, the Ecorr values of steel specimens exposed in U1 remained at −683 ± 23 mV during the entire test period (Table 2 and Figure 3). In contrast, the Ecorr values of steel specimens in U2 and U3 decreased compared to the values recorded during the enrichment phase, and stabilized within one month to values still more noble than in U1 (−628 ± 22 and −604 ± 10 mV, respectively). Macroscopically, no differences in the topography of corrosion deposits formed after six and nine months were observed between steel specimens immersed in the different units (Figure 4). Typical tubercle-like corrosion deposits developed on all steel specimens specifically at the LWZ line. On average, these products were 10 times thicker than

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**Figure 2.** Epifluorescence microscope photographs of planktonic cells observed after phase (1) in each Unit. Note the differences in cell morphologies between units 2 and 3 (1,000× magnification). Scale bars = 5 µm.

**Table 2. Summary of values of free corrosion potentials and maximum corrosion rates of immersed steel per unit and test phases.**

| Phases                  | Ecorr (mV SCE−1) | Max. corrosion ratea (mm yr−1) |
|-------------------------|-----------------|-------------------------------|
|                         | (1)            | (2)                          | +6 months LWZ | +9 months |
| Unit 1 (control)        | N/A            | −682 ± 32                    | 1.04          | 0.75      | 0.23      |
| Unit 2                  | N/A            | −552 ± 26                    | 3.85          | 3.77      | 0.23      |
| Unit 3                  | N/A            | −557 ± 53                    | 2.09          | 1.42      | 0.17      |
| Unit 3 (pre-corroded)   | 574 ± 11       | −574 ± 46                    | NP            | NP        | NP        |

Notes: NP: not performed; N/A: not applicable; LWZ: low water zone; IZ: immersion zone.
aDetermined from mean difference between measurements of initial steel thickness and minimum residual steel thickness.
the products formed in the other exposure zones. Only the tubercle deposits of the pre-corroded steel immersed in U3 remained very compact and less thick than the other tubercles analyzed in this study. Removal of
corrosion deposits showed that the most affected steel areas were the ones previously covered by tubercles in all units.

The steel immersed in U2 had clear shallow wide and elliptical pits after nine months exposure at the LWZ while damage of the steel in U1 and U3 was less visible, and was characterized by large and uniformly corroded areas underneath tubercles (Figure 5). In agreement with the corrosion damages observed, steel from U2 had the highest maximum corrosion rates (3.85 mm yr\(^{-1}\)) after six months exposure, followed by steel from U3 (2.09 mm yr\(^{-1}\)). Steel from the non-enriched U1 had significantly lower corrosion rates (1.06 mm yr\(^{-1}\)) (Table 2). After nine months, the corrosion rates in U3 and U1 significantly decreased (34 and 28% less than after six months, respectively), while the rates in U2 remained similar. Steel from U2 also presented the highest acceleration of corrosion rates (16-times) relative to the IZ, with LWZ rates in U1 and U3 only three and eight times higher. The lowest maximum corrosion rates were measured in the IZ of U3 after nine months (0.17 mm yr\(^{-1}\) compared to 0.23 mm yr\(^{-1}\) in U1/2-IZ).

Mineral composition of the corrosion products

Comparison of element and mineralogy distribution between the different types of corrosion products analyzed (ie tubercles from LWZ and products from IZ) showed high congruence between both types of analysis \((r_s = 0.85, p = 0.017)\).

The tubercles developed on steel after nine months exposure in each unit were all characterized by a majority of Fe and O elements from compounds identified as iron oxyhydroxides, eg lepidocrocite \((\gamma-\text{FeOOH})\) and goethite \((\alpha-\text{FeOOH})\) (Figure 6). While goethite and lepidocrocite were identified in similar amounts in tubercles formed in U2 and U3, respectively (45 and 55%), the tubercles from U1 were composed mainly of lepidocrocite (80%).

Figure 5. Macroscopic pictures of cross-sections of steel bars taken at the low water line. The three steel surfaces shown for each unit correspond to sub-sections of the bar cross-section cut at the same low water line position. The scale bars correspond to 4.8 mm. The sum of the three sub-sections length equals 10 cm.

Figure 6. Distribution of minerals within corrosion deposits.
Corrosion products from IZs had a very different elemental composition and mineralogy with a majority of Ca and S elements, related to the presence of aragonite, sulfated and/or carbonated green rusts identified in the deposits.

Products from the U1-IZ and U3-IZ had a similar mineral distribution with sulfated green rust (GR2) being the dominant product (62 and 72%, respectively). Despite a significantly higher S% in U2-IZ (results not shown), GR2, which was the sole S-based compound identified, represented only 20% of the detected compounds, indicating that other S-based minerals, such as amorphous or nanocrystalline iron sulfides, were present and not detected by XRD. In contrast, aragonite (40%) and carbonated green rust (GR1; 25%) were more abundant in U2-IZ than in U1-IZ and U3-IZ.

Microbial community analysis

Quantification of total (16S rDNA/dsrB) and metabolically active (16S rRNA/dsrB transcripts) bacteria and SRB within the corrosion deposits retrieved from LWZ (during high tide) after nine months showed that despite abundant sessile bacterial populations in all units, the level of active bacteria was only significant in the most corroded LWZ (ie U2-LWZ; Table 3). This was in agreement with the detection of active SRB only in U2-LWZ. Although of low number compared to the rest of the community members, the proportion of S-based microbes within the total bacterial community was only significant in the most corroded LWZ (Table 3). This was in contrast to the active bacteria detected within all IZ deposits. However, the bacterial activity was highest in U2-IZ related to the total highest 16S rDNA/16S rRNA ratios in U2-LWZ and U2-IZ, reflecting also the measured corrosion rates in U2-LWZ.

Table 3. Quantitative bacterial community data.

| Simpson index | 16S rDNA copies g⁻¹ (mean ± SD) | 16S rRNA copies g⁻¹ (mean ± SD) | dsrB copies g⁻¹ (mean ± SD) | SRB/total bacteria % | 16S rRNA/16S rDNA | dsrB mRNA copies g⁻¹ (mean ± SD) | 16S rDNA |
|---------------|---------------------------------|---------------------------------|----------------------------|----------------------|---------------------|---------------------------------|---------|
| U1-LWZ        | 0.77                            | 6.12E+06 ± 6.88E+05             | 1.50E+04 ± 7.12E+03        | 1                    | ND                  | ND                              | 0.000   |
| U1-IZ         | 0.90                            | 7.34E+06 ± 7.07E+03             | 9.06E+05 ± 3.84E+04        | 44                   | 5.49E+07 ± 9.63E+06 | 3.66E+03 ± 4.17E+02            | 7.487   |
| U2-LWZ        | 0.90                            | 2.35E+06 ± 2.76E+05             | 8.30E+04 ± 1.15E+04        | 13                   | 3.79E+07 ± 7.94E+06 | 2.65E+03 ± 1.07E+03            | 16.103  |
| U2-IZ         | 0.93                            | 1.54E+07 ± 1.85E+06             | 1.25E+06 ± 1.30E+04        | 29                   | 9.97E+08 ± 9.26E+07 | 2.32E+05 ± 6.61E+03            | 64.684  |
| U3-LWZ        | 0.90                            | 1.01E+07 ± 3.18E+06             | 4.53E+04 ± 8.46E+03        | 2                    | ND                  | ND                              | 0.000   |
| U3-IZ         | 0.86                            | 1.57E+07 ± 1.09E+06             | 9.53E+05 ± 3.58E+04        | 22                   | 4.42E+04 ± 2.49E+03 | ND                              | 0.003   |

*3.6 copies of 16S rDNA/bacterial cell were considered (for the calculation of SRB/total bacteria ratio) (Klappenbach et al. 2001; Leloup et al. 2006). 0 ≤ Simpson index (1−λ) ≤ 1; ND<1E + 03 copies g⁻¹.

Diversity of bacterial communities

Comparative analysis of DGGE fingerprints and band-related sequences revealed corrosion-specific bacterial diversity patterns inhabiting the steel deposits of the LWZ (tubercle areas). Clear structural differences were observed between the IZs and LWZ units, with higher bacterial diversity in the corrosion deposits of the LWZ.

Biofouling
between LWZ communities from units 2 and 3 (U2-LWZ and U3-LWZ) and unit 1 (U1-LWZ) were observed at DNA and RNA analysis level (Figure 7A). While the diversity level of active (RNA) and total (DNA) populations from U3-LWZ remained similar (Simpson diversity index (DI) of 0.90 vs 0.87), the active populations from U2-LWZ were much less diversified than the total populations (DI: 0.30 vs 0.90) (Table 3). Such differences were not observed for U1-LWZ. The total and metabolically active bacterial communities within deposits from IZs were of less dissimilar structures than within deposits from LWZ (Figure 7A).

### Phylogenetic characterization of bacterial populations

Phylogenetic analysis of the sequences from DGGE bands (Figure 7, Appendices 2 and 3 in Supplementary material) showed that the structural differences observed between the total and metabolically active bacteria within all deposits were due to changes in their relative...
abundance rather than changes in their composition. Different exposure zones and treatment conditions hosted populations of different composition and abundance, functionally related to specific putative metabolic capabilities (such as sulfate-reducing, sulfur-oxidizing, and/or iron-oxidizing bacteria). Active populations from U2-LWZ were almost exclusively composed of one phylotype affiliated to Desulfopila corrodens (previously named Desulfobacterium corrodens, (Dinh et al. 2004)) (94% relative abundance) (Figure 7B). Although the level of active populations from U3-LWZ was insignificant during the time of sampling, PCR-DGGE revealed that the active community was dominated by Chromatiales (50%) from the Gammaproteobacteria related to an uncultured bacterium from crude oil contaminated saline soil (EU328006) or Ectothiorhodospiraceae (~25%), this later encompassing sulfide-oxidizing photo-/chemoauto-
photrophs and chemoheterotrophs (Imhoff 2005). Members affiliated to SRB from Deltaproteobacteria (~30% relative abundance) were also identified related to D. corrodens (15%), Desulfovibrio aespoeensis, and Desulfovibrio algidum, previously designated as strain JHA1 (Figure 7B). The active populations of U1-LWZ were dominated by SRB affiliated to D. algidum accounting for 52% of the active community, with autotrophic capabilities (Könneke et al. 2013). The other members were related to putative iron-oxidizing bacteria from the Zetaproteobacteria (Maripseudomonaceae) and Bacteriovorax from the Deltaproteobacteria, a bacterial predator of some Gram-negative bacteria (Chen et al. 2011). One member affiliated to Rhodobacteraceae (Sulfobacter), heterotrophic thiosulfate/sulfite-oxidizing bacteria from Alphaproteobacteria also represented 8% of the active populations of U1-LWZ. Among the minor active populations (~1% relative abundance) of U1-LWZ and U2-LWZ, members of the Betaproteobacteria (Gillianellales), putative iron-oxidizing bacteria were identified.

The active community of U2-IZ was dominated by a phylotype related to magnetic magnetite containing vibrio MV-1 (DeLong et al. 1993) from the Alphaproteobacteria (84%), also known for autotrophic sulfur oxidation (Bazyliński et al. 2004) and a minor fraction of phylotypes related to SRB (Desulfovibrio sp. and D. aespoeensis). One phylotype affiliated to Lutibacter sp. from the Bacteroidetes dominated the active community of U3-IZ (35% relative abundance) together with SRB consortium composed of D. corrodens, Desulfobact-
ula phenolica, and phylotypes related to uncultured Chromatiales (Figure 7B and Appendix 3).

The active community from U1-IZ was dominated by phylotypes related to the Chromatiales (50%) with uncultured members of the Ectothiorhodospiraceae (15%), unclassified bacteria from enrichments on dimethylsulfide (DQ660937) and Nitrospinaeaceae, these latter encompassing nitrite oxidizers (Garrity et al. 2005).

**Relationships between corrosion rates and biotic and abiotic parameters**

Under all exposure conditions (ie U1, U2, and U3) and independently of the level of bacterial activity detected, higher dissimilarity was observed between the total and metabolically active bacterial communities in deposits from LWZ than from IZ (Figure 7). Furthermore, clear differences were observed between LWZ and IZ, with distinctive microbial populations and inferred metabolic functions, and reducing conditions, (as indicated by the different iron valence of the corrosion products). One phylotype closely related to D. corrodens was solely identified among the active SRB populations from LWZ communities of U2 and U3; which experienced transient stimulation of indigenous microflora, while not detected in U1. The relative proportion of this member among the active LWZ bacterial communities of each unit was in perfect correlation with the associated corrosion rates ($r_s = 1.0$, $p < 0.01$). In IZ, active populations related to eg D. corrodens were negatively correlated with corrosion rates together with uncultured members from Alphaproteobacteria and Chromatiales ($r_s = -1.00$; $p < 0.01$), indicative of opposite impact on corrosion depending on environmental conditions.

Minerals were also significantly correlated with specific populations such as lepidocrocite and Gallionell-
aceae at DNA level ($r_s = 0.84$, $p < 0.01$), indicating bacteriological influenced mineralization.

Aragonite and carbonated green rust detected in IZ were strongly correlated with D. aespoeensis ($r_s = 1.00$, $p < 0.01$).

**Discussion**

**Caustative bio-agents of simulated ALWC**

Yeast extract, with an approximate C: N: P ratio of 95:17:1 (DeGermann et al. 2012), is close to the Redfield ratio of dissolved organic matter (Redfield 1958) and was used as organic substrate to stimulate microbial growth of natural planktonic seawater bacteria (U2), and both planktonic bacteria and the ‘steel-adapted inoculum’ (U3).

The establishment of anaerobic conditions in these two units (dO$_2$ ~0.2 mg l$^{-1}$ and redox potential of ~69 mV) was correlated with a significant increase in planktonic (10$^2$-fold), including sulfidogenic (10$^3$–10$^4$ fold) populations compared to U1 (Table 1). Experiments to demonstrate MIC are traditionally performed in inoculated and sterile controls without consideration of substrate limitations over short time periods (ie from days to two months) with for example, SRB concentrations
10^3–10^4-times higher than temporary (phase 1 and 2 only) promoted in this study (eg Kuang et al. 2007; Castaneda & Benetton 2008; Wan et al. 2010). It is surprising in the present case that a transient substrate pulse (although 10^1–10^3 more concentrated than under natural conditions) led to corrosion rates of 3- (U3) to 12- (U2) times higher than in the control U1 over six to nine months with major differences in bacterial activity and composition within the respective corrosion deposits. A few representative studies solely (ie applying experimental conditions as close as possible to real conditions) have shown the influence of nutrients on the stimulation of indigenous flora leading to higher steel corrosion (Gubner 1998; Kjellerup et al. 2009; Melchers 2013). The similar trends and range of physico–chemical values measured in the three microcosms over more than eight months allowed the authors to discard the possible influence of bulk environmental conditions on the corrosion thermodynamics and kinetics. Rather, these results confirm that ALWC can be driven by the activity of specific SRB.

Significant bacterial and SRB metabolic activities (as assessed by the number of 16S rDNA and dsrB transcripts g^-1) were only detected in corrosion deposits with the highest corrosion rates and localized corrosion (U2), while similar 16S rDNA and dsrB gene copies were quantified independently of the corrosion rates. Nevertheless, the proportion of SRB/total bacteria was only significant (>10%) in LWZ associated with the highest corrosion rates (Table 3). The absence of a significant activity level detected by qPCR also paralleled the lesser dissimilarity measured between the total and active microbial communities, suggesting lesser dynamics and/or higher competition within communities (Figure 7). Despite reinforcing the importance of bacterial activity over numbers as an indicator of accelerated corrosion, these observations indicate the relevance of population ratios and highlight the importance of molecular studies both focused on RNA and DNA still scarcely considered in MIC studies.

Furthermore, although Deltaproteobacteria were identified within the total and metabolically active community of LWZ deposits, a different community structure and composition were observed depending on the exposure conditions (Figure 7A), which is in line with other studies (eg Païssé et al. 2013; Duncan et al. 2013). The unique detection of a phylogenotype closely related to *D. corrodens* among the active LWZ-U2/U3 communities where corrosion rates were the highest with clear evidence of localized corrosion and perfect correlation between phylogenotype concentration and corrosion rates (r_s = 1, p < 0.01) clearly support the causative role of this bacterium in the corrosion process observed.

It is usually accepted that SRB consortia are more corrosive than each one individually (Beech et al. 2005). However, this striking result suggests that despite coexisting SRB, the activity of one phylogenotype only had a direct impact on corrosion rates. One obvious reason for its dominance here certainly lies in the recent proof of the direct electron transfer (DET) abilities from steel of the type species, newly classified as *D. corrodens* within the Desulfovibulbaceae (Gittel et al. 2010), unraveling a conceptually new mechanism of SRB corrosiveness enabling higher oxidation kinetics than ‘conventional’ SRB (Enning et al. 2012; Venzlaff et al. 2013). Nonetheless, the significance of this eco-physiological property is largely unexplored in marine corrosion and has been limited so far to the sediment or buried zone, where anoxic conditions are encountered (Dinh et al. 2004; Enning et al. 2012). This is the first confirmation of the ecological significance of bacteria closely related to *D. corrodens* on corrosion of waterfront steel structures. The close proximity of the collection point of natural seawater from the sediment bed (30 cm), certainly underlines the sedimentary origin of this bacterium, whose presence in seawater was likely due to sediment resuspension by local water turbulences (eg tides and boat wakes). Overall, it is questioned whether the known anodic nature of LWZ relative to the cathodic tidal and IZs (Elbeik et al. 1986; Benedetti et al. 2007), could be significantly exploited by members of Desulfovibulbaceae (considering their capability to oxidize sulfide via DET to a solid anode (Holmes et al. 2004; Holmes, Bond, O’Neil, et al. 2004; Sun et al. 2009; Cheng et al. 2010)) resulting in constantly accelerated sulfur cycling within the LWZ, despite anoxic phases (ie high tide). Their ability to disproportionate sulfide intermediates may also give them a selective advantage in Fe (III)-rich habitats.

**Influence of inoculum, bacterial diversity, and exposure zone on corrosion mechanisms**

Although corrosion rates were higher for steel exposed in U3 than in U1, pointing to the promoting effect of bacteria on low water corrosion, the rates were not the highest as expected from the initial enrichment phase with stimulation of both the ‘steel-adapted inoculum’ and seawater consortia. Active microbial communities observed in U3-LWZ were both associated with SRB and putative SOB, two groups incriminated in ALWC, as recently confirmed on natural long-term corrosion deposits (Marty et al. 2014). Yet, qPCR indicated that the absolute activity of bacteria including SRB was negligible at the time of sampling in U3-LWZ.

Several hypotheses derived from these results can be proposed reasonably excluding stochastic effects or methodological bias, due to the consistency of the present results with *in situ* observations (discussed below):
The putative SOB member identified was related to Ectothiorhodospiraceae with a different metabolism (ie anoxygenic photosynthesis) than the chemotrophic type commonly incriminated, although some members can grow aerobically or microaerobically in the dark. Contrary to the other members from the Chromatiales, elemental sulfur (S₈) resulting from sulfide oxidation is deposited outside the cell among the Ectothiorhodospiraceae (Prange et al. 2002). S₈ is known to cause severe damage in pipelines and corrosion rates of 1 mm yr⁻¹ have been reported (Fang et al. 2008). Nevertheless, the presence of this SOB among the active community was not linked with accelerated corrosion in the present conditions. However, little is known about the corrosiveness of biologically formed S₈ and corrosion decrease has been reported in particular cases (Johnston & Voordouw 2012). Possibly, the low light energy received in each unit strongly limited SOB activity or favored instead chemoorganotrophic growth.

The 10-times higher iron concentrations in U3 at the end of the enrichment phase 1 due to the presence of ‘pre-corroded steel’ concomitant with a 10-fold lower SRB concentration than in U2 followed by a 10-fold decrease in the SRB/total cell ratios in all units after addition of unexposed steel in phase 2, suggested that other populations than SRB may have been stimulated earlier in U3. The high concentration of dissolved iron encountered in U3 during phase 1 (up to 10.79 mg l⁻¹) likely favored the coexistence with SRB of other anaerobic bacteria (eg iron cycle bacteria) as primary steel colonizers. Although no direct affiliations with known iron reducers were identified, a wide range of heterotrophic bacteria can reduce iron, such as fermenters (eg Clostridium or members of the Bacteroidetes) (Lin et al. 2007) or SRB (Lovley et al. 2004). This may also explain the higher diversity of active bacteria and coexisting SRB in U3-LWZ than in U2-LWZ (Figure 7). Likely, the higher ratio of SRB among the planktonic population and higher total SRB biomass stimulated by the organic pulse in U2-LWZ in the absence of high concentration of dissolved iron, increased the probability of SRB cells (ie conventional and electrogenic SRB, both benefiting from primary fermentation products released from aerobic/anaerobic biodegradation of organic matter) colonizing and persisting on steel surfaces. These conditions followed by nine months of substrate-limited conditions and diffusion limitation within the inner layers of deposits under build-up concomitant with organic consumption mainly confined to outer layers, likely contributed to the selective enrichment of electrogenic (and hydrogenotrophic) SRB. These latter were then the only ones able to use the local energy/electron resources therein (ie Fe⁹ and H₂ from steel) in presence of sulfate.

Besides, since no macroscopic differences in deposit thickness and mineral composition were observed between LWZs, O₂ diffusion limitation due to the build-up of a partially protective mineral layer over time which would be specific to U3-LWZ cannot solely explain the higher decrease in corrosion rates in U3-LWZ between six and nine months. This supports the direct influence of microbial communities on the corrosion rates observed. The specific decrease in corrosion rates in U3-LWZ might also reflect the competition between electrogenic and conventional SRB associated with a different fluxed colonizing and persisting of electrons (8e⁻ from Fe⁹/mol SO₄²⁻ vs 2e⁻ from Fe⁹/mol SO₄²⁻).

Finally, the striking differences between bacterial communities, mineralogy, and corrosion rates obtained between LWZ and IZ (Figures 6 and 7, Appendix 2), reflected the different exposure conditions. LWZ was characterized by higher dynamics and steeper oxygen gradients compared to IZ. The identification of oxy-hydroxide minerals (indicative of highly oxidized conditions) and dynamic communities (as suggested by the higher differences in diversity between the total and active LWZ populations) were encountered in LWZ. Conversely, the IZs were dominated by mixed valence iron products (GR1, GR2) and calcium-deposits (aragonite) (indicative of anoxic-micro-oxic and alkaline conditions) (Pineau et al. 2008; Dupraz et al. 2009) and more stabilized communities with a higher proportion of SRB/total bacteria in IZ and less dissimilarity between the diversity of total and active communities (Table 3 and Figure 7). Biologically influenced alkalinization leading to carbonate precipitation has already been reported in biofilms due to SRB activity (Reid et al. 2000; Dupraz et al. 2009; Almahamedh 2013). Concomitantly, heterotrophic growth, especially stimulated in the constantly immersed zone (IZ) in the presence of yeast extract may have also contributed to the higher pH in the biofilm. The strong correlation between minerals identified in IZ with the relative abundance of D. aespoeensis also supports this scenario. Nonetheless, SRB activity in IZ was not a significantly (p > 0.05) correlated with corrosion rates and negative correlation with D. corrodens was even observed as opposed to LWZ. This suggests the absence of a significant biotic influence in IZ corrosion compared to LWZ and the importance of environmental conditions (eg oxygen intrusion as previously reported by for instance Kjellerup et al. 2009) in triggering the highly corrosive behavior of specific SRB, such as D. corrodens.

**Comparison of simulated ALWC with in situ experiments and natural deposits**

In all units, typical tuberculation usually observed with corrosion deposits associated with ALWC (Little & Lee 2006), appeared with increasing thickness over time.
Localized corrosion was however only observed in U2 and U3 indicating that tubercle formation was primarily favored by environmental conditions (fluctuating O₂ level generating differential aeration cell at the air–water interface) (Moulin et al. 2001). Ray et al. (2011) comparing tubercles from diverse steel structures, similarly observed that tuberculation was not necessarily linked to localized corrosion or MIC. According to their results and recent analysis performed on 20-year old natural deposits (Marty et al. 2014), goethite and lepidocrocite constituted major products of tubercles. Tubercles from U2 and U3 had similar to higher ratios of goethite and lepidocrocite, while the ones from U1 had a much higher fraction of lepidocrocite (Figure 6). This latter positively correlated with putative iron oxidizing bacteria affiliated to Gallionellaceae. Among the factors favoring lepidocrocite over goethite formation, high kinetics of Fe(II) oxidation, such as catalyzed by enzymatic reactions over chemical oxidation in micro-oxic conditions supports the biological origin of the mineral pattern observed in U1 (Cornell & Schwertmann 2006). This underlines the significant microbial influence on mineral formation during the corrosion process. Usual products previously observed in core layers of tubercles such as sulfated green rust, magnetite, and iron sulfide in long-term corrosion (>1 yr) (Génin et al. 1994; Pineau et al. 2008) were not detected despite probable anoxic inner zones therein, indicated by the sufficient thickness of corrosion products (5 mm) and detection of SRB activity. However, XRD analysis is only suitable for the identification of minerals in crystalline phase, implying that minerals likely in amorphous phase such as iron sulfide could not be detected with this method. The heterogeneity of the deposits combined with the small quantity of deposit available for analysis could also have resulted in the failed detection of less abundant and/or localized compounds.

Similar to the mineral products, the bacterial communities identified were previously observed in short-term and long-term immersed steel corrosion, such as Proteobacteria and Bacteroidetes dominated corrosion deposits. In U1, members of the Zetaproteobacteria (Mariprseudaceae), Deltaproteobacteria (Desulfoconvexum), and Gallionellaceae were also detected on corroded immersed steel surfaces. Zetaproteobacteria have been detected among the early colonizers of steel in Qingdao harbor coastal waters (Dang et al. 2011) and associated with steel corrosion (McBeth et al. 2011). SRB closely affiliated to D. phenolica, D. alginum and D. aestoeoensis were identified among the dominant SRB within natural corrosion products from long-term ALWC in European harbors (Païssé et al. 2013; Marty et al. 2014). A member affiliated to Sideroxydans lithoautotrophicus from the Gallionellaceae was frequently recovered from products of accelerated corrosion occurring at the air–water interface of a non-tidal freshwater harbor structure (Hicks 2007). The identification of such bacteria not common in marine waters in the present study is probably linked to its halotolerance (McBeth et al. 2013) and the periodic freshwater supply (via the river estuaries and/or snow melting) received inside the harbor. D. corrodens unique to U2 and U3 has previously been identified in long-term ALWC deposits (Marty et al. 2014). Yet, the populations of Alpha- and Gammaproteobacteria differed in relative abundance and composition previously observed, likely due to the absence of stimulation of anaerobic consortia as primary colonizers in U1. Alphaproteobacteria from Rhodobacteraeae (Sulfitobacter sp.) observed in several studies were only identified in U1. Gammaproteobacteria have been reported during short-term incubation (maximum 1 month) (Dang et al. 2011) including chemotrophic SOB (eg Thiorella, Thiomicrospira) while members affiliated to Chromatiales were marginal (Dang et al. 2011).

Relevance of simulated tidal conditions in management of marine harbor corrosion

Taking into account Melchers’ phenomenological corrosion loss model (Melchers 2009; Melchers & Jeffrey 2012), the phases 3–4 described as driven by anaerobic activity would be achieved after 6 months in all units in this study. Initial promotion of the anaerobic inoculum as pioneer steel colonizers followed by non-saturated dissolved oxygen concentration within seawater (6–8 mg L⁻¹) and the absence of turbulences (waves) were certainly determining factors for shortening phases 1 and 2 of Melchers’ model dependent on oxygen diffusion rates. This implied that simulated tidal conditions may advance the calibration of current corrosion models as well as the understanding of multi-parametric effects on short-term and long-term accelerated corrosion.

Due to the uncontrolled abiotic and biotic parameters in situ, prohibitive investment efforts (eg monitoring, logistics) would be necessary to gain a mechanistic understanding of the multi-parametric effect on short-term and long-term accelerated corrosion rates.

Overall, these results support the view that controlled laboratory and pilot-scale tidal simulation setups constitute valuable tools to assist in the development of predictive models and diagnosis and standardized tests of material resistance to marine port environments. Still, parameters such as local hydrodynamics, light, macroorganisms, and sediments may need to be considered and consequently system dimensions adjusted to better reflect natural conditions. Cross-validation with in situ experiments and natural corrosion products remain necessary.
Conclusions

The experimental tidal simulation unit used in this study led in a relatively short time (nine months) to a corroded steel surface in the LWZ, similar to *in situ* steel surfaces after several years’ exposure. The similarities applied to both macroscopic aspects and microbial/mineral composition of corrosion products. This highlights that systems such as the one reported in this study can clearly foster the understanding of multi-parametric effects on short-term and long-term accelerated corrosion in port environments and hence, parameterization of predictive models, diagnosis improvement and material resistance validation therein.

The low water and IZs were characterized by mineralogical patterns and microbial communities reflecting the environmental conditions, with higher propensity for MIC in fluctuating (redox/O₂) environments such as the LWZ, preventing the formation of protective layers (e.g., aragonite), and likely promoting acid regenerating oxidative reactions (e.g., sulfur and iron oxidation coupled to O₂ reduction).

Transient stimulation of indigenous microbial populations from harbor seawater by a pulse of organic matter led to a typical signature of ALWC (mineralogy/bacterial communities/localized corrosion), pointing to the causal effect of periodic pollution common to the harbor environment (e.g., nutrient/organic supply through fishing activities/aquaculture, sediment resuspension, algal proliferation and oil spillage). Temporary anoxic conditions and an increase in SRB populations (including conventional and electrogenic ones) within seawater where waterfront steel structures are immersed select for electrogenic SRB which can significantly increase corrosion.

RNA-based quantification, community characterization, and population/activity ratios are likely cues of accelerated corrosion in LWZ, contrary to DNA-based quantification.

Direct correlation of corrosion rates with active populations related to the electrogenic SRB *D. corrodens* also observed *in situ* reveals their potential role and ecological significance in accelerated corrosion of waterfront steel structures. Further research to determine the significance of electrogenic SRB and to adapt analytical methods to their detection is required.

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