FIELD *VERSUS* LABORATORY-BASED EXPERIMENTS: DIFFERENCES ON MARINE BIOFILM FORMATION AND ELECTROCHEMICAL BEHAVIOR OF UNS S31600 STAINLESS STEEL COUPONS

Messano, L. V. R. de*; Ignacio, B. L.; Neves, M. H. C. B.; Coutinho, R.
Divisão de Biotecnologia Marinha, IEAPM, Marinha do Brasil
*corresponding author: lvicentebm@gmail.com

ABSTRACT

Effects of marine biofilms on UNS31600 stainless steel (SS) corrosion were evaluated under field and laboratory-based experiments in order to investigate the differences in biofilm formation, corrosion potential (E_{corr}) and breakdown potential (E_b). Laboratory experiments used seawater pumped from the same location of the field experiment. Coupons were immersed for 18 days and recovered for analysis at 2, 10, 14 and 18 days for both experiments. Laboratory experiment also included a biofilm control (sterile conditions with no biofilm formation). E_{corr} was daily measured, while E_b and microscopic analysis of the biofilm were performed after retrievals. Biofilms from both experiments comprised the same groups (bacteria, diatoms and cyanobacteria) and were dominated by the same diatoms genera (Navicula, Nitzchia and Licmophora). However, bacteria and diatoms cell densities were much higher in field conditions, as well as diatoms and cyanobacteria richness. E_{corr} and E_b showed different trends between experimental designs. Our data highlighted the importance of performing field tests, and how they are important to validate laboratory results, since erroneous assumptions could be made if only the results of laboratory tests were taken into consideration.

INTRODUCTION

Biofilms are a synergistic complex community that attach to the immersed surfaces, after the accumulation of organic molecules, embedded in a sticky matrix formed by extracellular polymeric substances (Beech and Gaylarde, 1999). Marine biofilms are typically composed by aerobic and anaerobic bacteria and microalgae - mainly Bacillariophyceae (diatoms), Cyanophyceae (cyanobacteria) and also Dinophyceae (dinoflagellates) (Marzalek et al.., 1979). The effects of marine biofilms on corrosion processes are well studied, particularly in relation to stainless steels (SS) deterioration (Videla, 1994), given that important surface damages and alterations on electrochemical parameters behaviour were already reported to SS in the presence of microorganisms (Little and Lee, 2007).

Most of microbiologically influenced corrosion (MIC) tests are carried out in laboratory conditions. In the case of marine biocorrosion studies, tests are performed pumping seawater to tanks and/or chambers or other kinds of apparatus. Field experimental designs for marine MIC investigations are scarce, probably due to methodological issues (e.g. limitations in the use of electrochemical techniques). Despite methodological difficulties and/or restrictions, in several circumstances, results from field based-experiments might be closer to operational conditions than laboratory results. Misinterpretation of this tests may underestimate the useful life of structures and cause financial losses.

The present paper compared the results from field and laboratory-based experiments (using seawater pumped from the same location of field experiment) in order to investigate the influence of the experimental designs in biofilm communities, corrosion potential (E_{corr}) and breakdown potential (E_b) of UNS31600 SS coupons.

MATERIALS AND METHODS

Field and laboratory-based experiments were carried out at Praia dos Anjos, Arraial do Cabo, located on the coast of the state of Rio de Janeiro, Brazil (22°57' to 23°00'S, 41°59' to 42°01'W). Praia dos Anjos is a enclosed beach influenced by anthropogenic activities, including a presence of a small harbor and domestic-sewage discharges. Field experiment was performed using floating rafts moored at harbor area. This area is one kilometer distant from where seawater was pumped to large tanks and then to 250 liter tanks with flow system to laboratory experiment.

Coupons of UNS31600 (70x50x2mm) with electric contacts were immersed for 18 days and recovered for analysis at 2, 10, 14 and 18 days for both experiments. E_{corr} was daily measured *in situ*, while (E_b) and analysis of the biofilm were performed after retrievals. Laboratory experiment also included a biofilm control (sterile conditions formation) to compare with E_{corr} and E_b variations. Biofilm communities were analyzed under optical and epifluorescence microscopy.

Microorganisms were identified and counted using based on three randomly chosen fields. Cellular density was expressed in 10^4cells.cm^{-2}. E_{corr} was measured with a multimeter and a Ag/AgCl electrode, while anodic polarization tests were carried out using a Saturated Calomel Electrode (SCE) as reference electrode and a PalmSens® equipment to register the breakdown potential (E_b).

RESULTS AND DISCUSSION

We recorded the same groups in field and in laboratory-based experiments: diatoms, bacteria and cyanobacteria. Diatoms and bacteria were dominant in the biofilms, as already reported in literature about microfouling ecology (Mitchell and Kirchman, 1984; Cooksey and Wigglesworth-Cooksey, 1995; Callow and Callow, 2002). Biofilms from both experiments were similar in terms of relative abundance of diatoms. *Navicula*, *Nitzchia (*mainly *N. longuissima*) and *Licmophora* corresponded to more than 90% of total diatoms cell density, together with the genera *Grammatophora* (at field conditions) and *Cocconeis* (at laboratory) (Fig. 1).

On the other hand, richness and cell density were completely different contrasting the results of field *vs*. laboratory-based experiments. Diatoms richness were much higher in field than in laboratory conditions. Twenty two other genera were recorded than the four dominants, while only four genera were recorded at laboratory experiment. The same was true to cyanobacteria - five *taxa* were recorded in field and only one in laboratory conditions. Cell densities values of all groups were also much higher in field conditions, particularly for diatoms. In this case cell density values was two orders of magnitude higher in biofilms formed in field conditions (Fig. 1C).

Biological composition, richness and cell densities showed temporal variability. Biofilm in field conditions showed a directional succession and no clear trend was registered in the biofilm at laboratory. Succession in field conditions was characterized by the higher cell densities of bacteria and cyanobacteria on day 2 followed by the highest diversity of cyanobacteria and diatoms on day 10 and the higher cell density of diatoms on days 14 and 18 (Figures 1A to 1C).

As can be seen in figure 2, E_{corr} and E_b showed different variations between field and laboratory-based experiments. In field conditions, E_{corr} values showed a transient increase on 5th day and after that E_{corr} decreased and fluctuated around negative values. In laboratory conditions, E_{corr} values were more positive, a small extent E_{corr} ennoblement was registered on the 9th day and then E_{corr} remained stable in higher values until the end of experiment. At sterile conditions E_{corr} values were intermediate if compared to the experimental conditions. These results not only indicated that biofilms actually altered corrosion potential behavior, but also confirmed the entirely different trends of E_{corr} behavior. E_b values were also very distinct among experimental conditions. In laboratory, values decreased with the time of immersion, in field conditions increased to more noble values, and remained stable in sterile conditions.

As could be observed, the results obtained in our study were strongly influenced by experimental design. If comparisons of E_{corr} data from different investigations about SS biocorrosion are already complicated because of methodological issues (e.g. sample size, flow rate and temperature), as pointed out by Little et al. (2008), our results indicated that field *vs.* laboratory experimental designs should be also a issue to consider. For example, Acuña et al. (2006) studied biofilm effects on SS UNS 31603 electrochemical behavior in field conditions in a tropical area. Compared to our results, E_{corr} behavior in field conditions was similar to our laboratory behavior. This apparent divergence in results probably occur due to a methodological difference: in the current study, corrosion potential was obtained *in situ*, and in the former, coupons were transported to laboratory for measurements.

In relation to biofilm colonization, although relative abundances were similar, laboratory-based experiment did not reproduce biofilms ecological dynamics. In field-based experiment microorganisms showed higher richness and diversity, higher cell densities and also a clear trend of successional process. In our opinion, field experimental designs should be improved and modern techniques adjusted in order to provide precise and accurate results of electrochemical behavior as well as microbial ecology. Microbiologically induced corrosion on stainless steels is well studied particularly regarding the role of bacteria on the process. Other microorganisms participation, like diatoms, have been less studied as also the effect of ecological interactions, mainly bacteria-diatoms (Landoulsi et al., 2011). Probably field experiments advances would fill these and other gaps in the understanding about biofilms and the complex mechanism involved in the interaction with stainless steels.

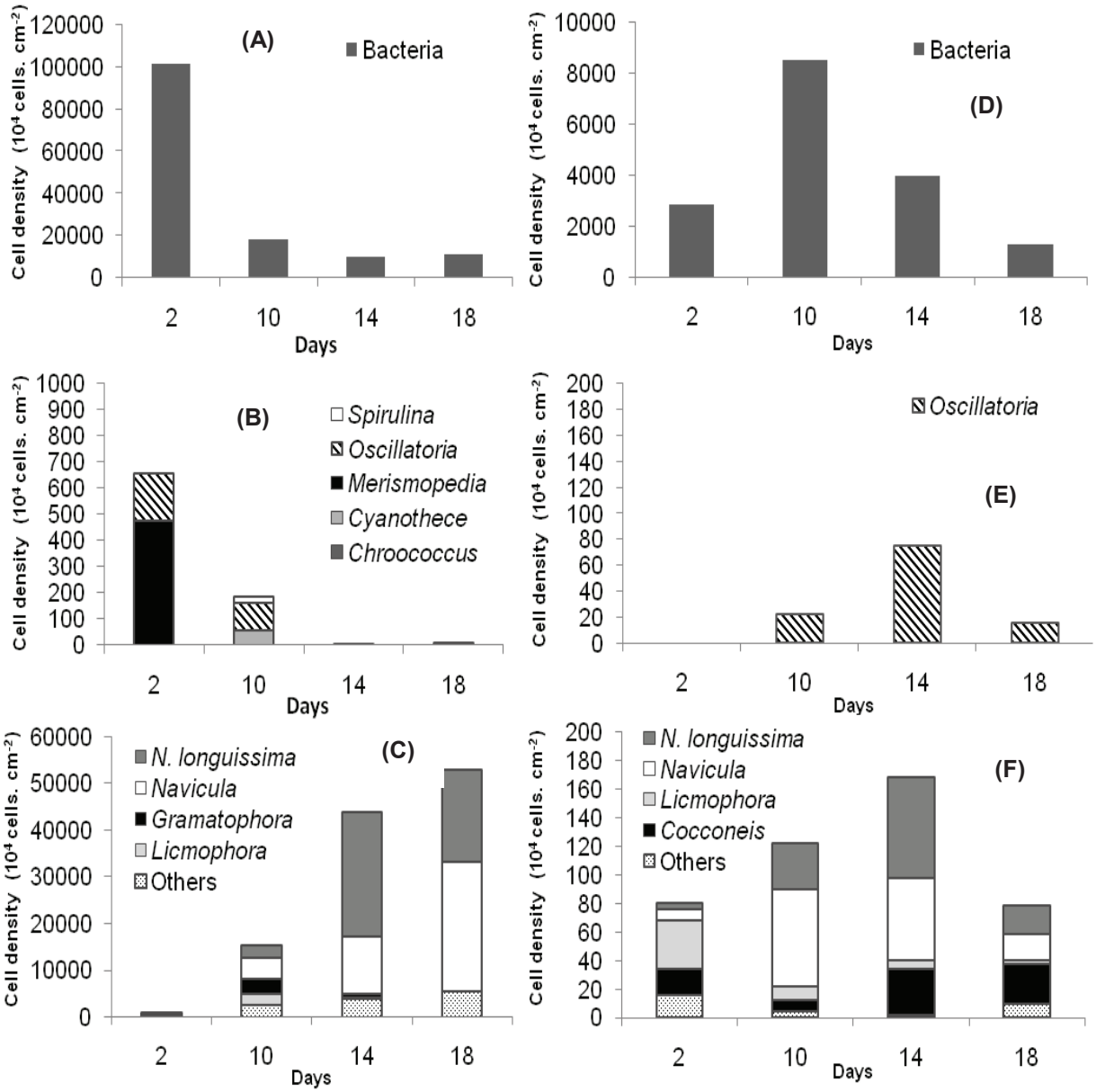

Figure 1 - Cell densities of bacteria, cyanobacteria and diatoms in biofilm communities on UNS S31600 stainless-steel coupons at field conditions (A, B, C) and laboratory conditions (D, E, F) and the time of immersion of the coupons (2nd, 10th , 14 h and 18th days).

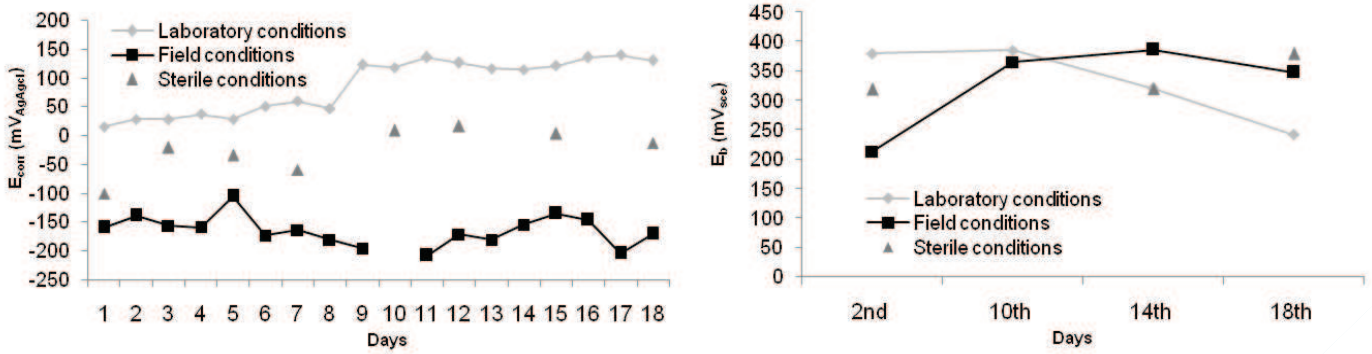

Figure 3 – Corrosion potential (E_{corr}) and Breakdown potential (E_b) *vs.* time (days) of UNS S31600 stainless steel coupons under both conditions (field and laboratory) and under sterile conditions.

CONCLUSIONS

Concerning marine biocorrosion studies, our data highlighted the importance of performing field experiments given that not only biofilm community but also electrochemical behavior of SS were influenced by the experimental design. Field tests should be perform at least to validate laboratory results, since erroneous assumptions could be made if only the results of laboratory tests were taken into consideration.

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