INTERCORR 2014 ABRACO
Hotel Praia Centro – Fortaleza/CE
19 a 23 de Maio

Technological Development of Anti-fouling Paints with EPS as a Natural Biocide

Rodrigo de S. Melo[a], Simone L. D. C. Brazil[b], Aricelso M. Limaverde Filho[c], Ladimir J. De Carvalho[b] e Odara R. Baptista Melo[d]

Abstract

The exopolysaccharides (EPS) are a class of renewable natural polymers, which present anti-fouling property. Therefore, it may be used as an alternative to conventional additives currently used in anti-corrosive paints. The copper oxide is an additivecommonlyused due to itsanticorrosive andanti-fouling properties. However, this metal oxide presents toxicity to some marine organisms and it can promotesenvironmental problems. The incorporation of EPS in paints based on copper oxide can reduce its contents, since both present anti-fouling characteristics. Another possibility is to incorporate this polymer to a paint that shows anti-corrosive characteristics, such as niobium oxide based paint.This work presents the steps of obtaining the EPS, extracted from cyanobacterium *Phormidium sp*., and its incorporation to the composition of paintsbased on copper and niobium oxides. The main objective of this research is to produce alternative paints to the anticorrosive and anti-fouling coatingsused nowadays.

Keywords: corrosion, coating, antifouling, copper, niobium.

Introduction

Microorganisms have a strong tendency to populate surfaces, giving rise to a complex and strongly adhering microbial community, termed ‘‘biofilm”. Biofilms are detrimental to the underlying substrates, causing physical degradation or biodeterioration of metal surfaces.This phenomenon has been widely recognized as biocorrosion or microbiologically influenced corrosion (MIC) (Stowe *et al*., 2011). Biocorrosion is a serious problem for aquatic and maritime industries. Among the various microorganisms that induce corrosion or degradation of metallic materials, sulfate-reducing bacteria (SRB) are the most problematic and the principal cause of failure in many steel-based structures.Therefore, is very important to inhibit biocorrosion caused by SRB in order toenlarge the service life of maritime structures and equipment.A variety of toxic materials (eg copper, lead, mercury,arsenic) were used to control fouling organisms untilorganotins, such as tributyltin (TBT) were introducedin the 1960s. Organotins were the most effectiveantifouling (AF) agents known, but also among themost toxic biocides ever introduced because they arenot readily degraded in the natural environment andbecause they act on both target and non-targetorganisms. This led the International Maritime Organization(IMO) to prohibit their application to ships,effective from17 September 2008.Currently, the most commonly used protective systems are based on copolymers with copper oxide (Cu2O) or zinc oxide (ZnO) and organic biocides. However,

[a] Mestre-Doutorando, Escola de Química – UFRJ.
[b] Doutor(a), Professor(a), Escola de Química – UFRJ.
[c] Doutor-Professor, Universidade Federal do Rio de Janeiro – Campus Macaé.
[d] Mestranda-Bióloga, Universidade Federal do Rio de Janeiro.

these coatings do not provide greater protection than 12-18 months due to theconstant erosion during its lifetime. There is a great concern for biocides used in antifouling protection systems business due to the high concentration of metals, such as copper and zinc, found in coastal areas and the potential damage they can cause to marine organisms (Yebra, 2004). The replacement of these biocides for environmentally friendly substances is a matter of great interest for the entire industrial sector. Accordingly, the use of natural products which inhibit the settlement of fouling organisms is a key - factor in the antifouling coating technology. From this point of view, the exopolysaccharides have been proposed as a promising solution.

The marine environments are recognized as very corrosive to carbon steel alloys. For reasons of economy, such steels are also preferred materials for use in structures on and off - shore. Statistical data show that 90% of failures in ships' hulls are attributed to corrosion. For oil tank and bulk carriers, there were a number of shipwrecks and environmental disasters attributed to lack of maintenance highly corroded hulls. To protect steel structures, coatings and cathodic protection are the main methods employed. With a good maintenance of the structure and the proper application of cathodic protection, corrosion should not be a concern (Johnston &Voordouw, 2012). However, maintenance procedures are not always sufficient, and there are some areas of ships, platforms, ports, etc, that can not be protected.

Paints reduce its efficiency of protection against fouling and corrosion over time. Water, for example, can penetrate the paint decreasing its resistivity, and corrosive species, such as Cl^-, can diffuse through the holes and cracks reaching the steel surface, causing corrosion. Mucilaginous layer are common around the marine cyanobacteria that live in hypersaline environments and hence most of these organisms can produce EPS (Golubic & Campbell, 1985). Some species of unicellular cyanobacteria producing EPS were isolated from hypersaline environments. Furthermore, Cyanothece EPS, for example, contains uronic acid of six to eight monosaccharides with one or two sugar acids. Other chemical groups, such as acetyl, pyruvyl and/or sulphate are also detected. Exopolysaccharides cyanobacteria isolated from saline environments containing various sugars such as glucuronic acid, galacturonic acid, galactose, glucose, mannose, xylose and fucose. According to Chi *et al*. (2007), a new type of homoglucana α has been detected in isolates of saline lakes in China.In laboratory culture Phormidium sp. had a yield of approximately 400 (±15.38) $mg.L^{-1}$EPS culture with extraction yield of 32 (± 0.8) %.

Previous work has demonstrated the great biotechnological application related to the EPS of cyanobacteria, such as Cyanospira capsulata (Cesàro et al., 1990) and Aphanothece halophytica (Morris et al., 2001). The cells of most cyanobacteria are surrounded by an outer layer consisting essentially of mucilaginouspolysaccharide material. In atolls of French Polynesia, the algal mats developed in marine lagoons exposed to a great variety of salinity with high solar irradiation (Richert et al., 2005). The cyanobacterium Phormidium sp. isolated from samples-microbial mats was evaluated using a magnifying glass and microscope for the purpose of determining the layer is located where the cyanobacteria of interest with a view to their removal after completion of isolation.

In the present work, exopolysaccharide of cyanobacteriumPhormidium sp.has been incorporated as a biocide in paints based on niobium (Nb) and copper (Cu)oxides, applied to carbon steel samples.Subsequently, the samples will be tested in natural marine environment to evaluate the performance of the coating modified by the incorporation of EPS against marine biofouling.

Metodology

The cyanobacterium, Phormidium sp. was collected and acclimatized for laboratorial conditions through agradient of decreasing salinity, to be cultured in axenic culturein BG-11. The culture médium (BG-11) liquid was prepared by dissolving all the componentes listed in Table 01 in the sea water pre-treated by filtration and then sterilized at 120°C for 20 min. After cooling, the pH value was 7.0 and salinity 50 (5.0%).

Table 01. Composition of Medium BG-11

Components	Concentration (g/L)
$NaNO_3$	1,5
NaCl	15,0
K_2HPO_4	0,04
$MgSO_4.7H_2O$	0,075
$CaCl_2.2H_2O$	0,036
Na_2CO_3	0,02
$C_6H_8O_7$	0,006
$C_6H_8FeNO_7$	0,006
EDTA	0,001
Micronutrients (*)	1 mL / L

* SolutionMicronutrients: H_3BO_3: 2,86 g/L; $MnCl_2$. $4H_2O$: 1,81 g/L; $ZnSO_4$. $7H_2O$: 0,222 g/L; Na_2MoO_4 . $2H_2O$: 0,39 g/L; $CuSO_4$. $5H_2O$: 0,079 g/L; $Co(NO_3)_2$. $6H_2O$: 0,0494 g/L.

During the extraction of EPS produced after 20 days of culture (Figura 01), the biomass has been subjected to extraction by soxhlet 70 0C / 2 h using a 1:1 mixture of chloroform and methanol to remove lipids (Figure 02). Subsequently, the EPS contained in the culture and the biomass has been extracted using sodium hydroxide and precipitated by absolute ethanol for 24 h at -20 C^0.

Figure 01 - Culture of *Phormidium sp.* – production of exopolysacharides.

For the extracted EPS purification, chromatography was performed with DEAE-cellulose (+) as eluent buffered system, which subsequently was carried out in agarose gel electrophoresis. The purified material was determined using spectroscopic techniques in Fourier transform infrared (FTIR). Purified EPS has been incorporated into two different paints: (1) niobium oxide based and (2) copperoxide based. During this research, electrochemical impedance spectroscopy will be used in order to evaluate the coatings performance.

Figure 02. Extraction the lipid fraction.

Preparation of Test Samples

AISI 1020 carbon steel (CP) coupons, with 2,0 cm^2, were previously embedded in epoxy resin in order to keep only one side exposed. Thus, only one side was painted and this procedure aims to prepare the coupon to further electrochemical tests. The coupons were polished with 100 and 600 sandpaper, degreased by immersion in acetone and dried with hot air jets and welded to copper wires in order to get electrical connections needed to the electrochemical tests (Figure 3).

EPS Incorporation and Coupon Painting

In order toincorporatetheEPS into the paints based on niobium oxide and copper oxide, the exopolysaccharide was dried using a heating under reduced pressure. Subsequently, the EPS was added into the matrix of the paint sand the components have been mixed through mechanicalagitation. Two coats of paints have been appliedwith abrushandthe surface roughnessand the paint layerthicknesshave been analyzed usingan appropriate instrument (Ecometer).

Figure 03. Coupons with niobium oxide based paint (white) and copper oxide based paint (red).

Results and Dsicussion

To obtain axenic culture of Phormidium sp., samples have been inoculated in test tubes containing 10 mL of liquid medium. After monitoring the growth by optical microscopy, the cells have beenprecipitated by centrifugation at 5000 x for 10 minutes and resuspended in test tubes containing 10 mL of liquid medium plus a respiratory chain inhibitor (sodium azide to a final concentration of 5 mM) and an inhibitor of glycolysis (sodium fluoride at a final concentration of 50 mM). Then, only the photoautotrophic growth was allowed. After seven days exposed to inhibitors, the cyanobacteria was subcultured in liquid medium in the absence of inhibitors, thus obtaining unialgais and axenic cultures, kept at room temperature under constant illumination (20 µmols of photons.m^{-2}.s^{-1}) (Melo *et al*., 2011).

The profile of neutral sugars (mannose, rhamnose, glucose, galactose, arabinose and xylose) varies according to the specific species and stage of growth (Tazi and Allard, 1993). By comparison, microalgae tend to accumulate starch as cyanobacteria accumulate primarily glycogen (Madhavi - Shekharam *et al*., 1987). The functional groups of EPS were identified by infrared analysis. Figure 04 show the FTIR spectrum of EPS Phormidium sp. obtained from the alkaline extraction with the different transmittance peaks representing the major functional groups. Table 2 show thethe values corresponding toIR spectrum.

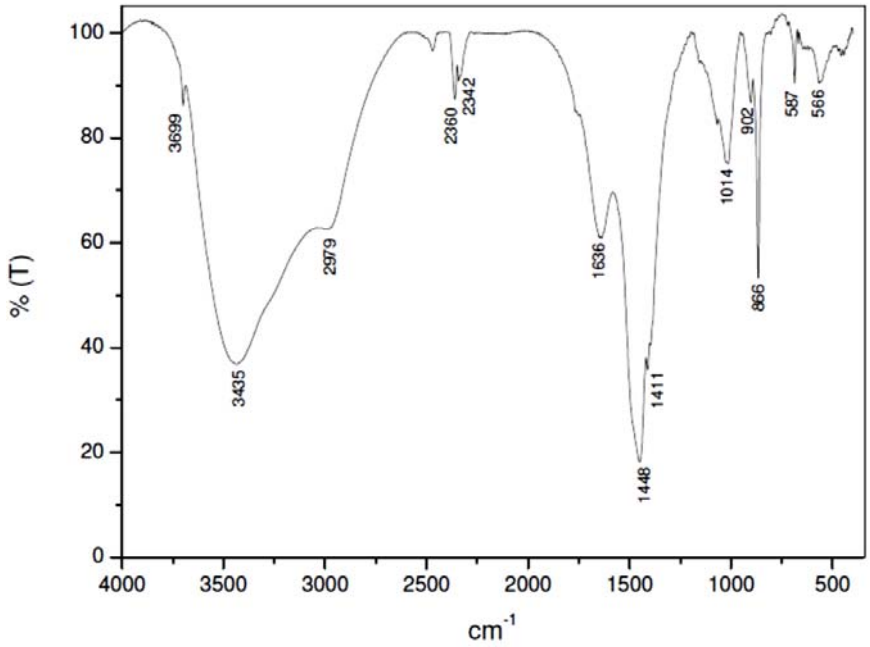

Figure 04 - FTIR spectrum of EPS after alkaline extraction and purification with chloroform.

Table 02. Correlation values and functional groups on the IR spectrum of EPS Phormidium sp.

cm^{-1}	Major Funcional Groups
3699	NH axial direction
3435	OH bond
2979	CH bond
2360 and 2342	C = O bond of CO_2
1636	C = C and C = O protein
1448	NH protein
1411	link symmetric C = O group COO
1014	CS group
866	PO

Table 3 presents the measurements of the paint layer thickness, applied on the coupon surface. It can be observed that the paints with EPS present a thicker layer than those without the polymer. The coupons prepared with paints without EPS show thickness that correspond to the value indicated in standards.

Table 3. Measuerements of the Paint Layer Thickness

Paint	Thickness
Cu_2O based	65 µm (± 3,0 µm)
EPS + Cu_2O based	240 µm (± 13 µm)
Nb_2O_5 based	800 µm (± 30,0 µm)
EPS + Nb_2O_5 based	950 µm (± 47,0 µm)

Conclusions

- It was possible to isolate and cultivate the Cyanobacteria using the methods presented in this work.
- Paints based on niobium and copper oxides have been produced with EPS incorporated. Steel coupons have been painted with these paint systems.
- Considering previous studies, the EPS Phormidium sp has a great potential to be used as a natural biocide in anticorrosive paints.

Reference Bibliography

(1) CESÁRO, A., LIUT, G., BERTOCCHI, C., NAVARINI, L., URBANI, R. Physicochemical properties of the exocellular polysaccharide from Cyanospira capsulata. **Int. J. Biol. Macromol.,** v.12, p. 79 – 84, 1990.

(2) JOHNSTON, S. L., VOORDUW, G. Sulfate-Reducing Bacteria Lower Sulfur-Mediated Pitting Corrosion under Conditions of Oxygen Ingress. **Environ. Sci. Technol.,** v. 46, p. 9183 – 9190, 2012.

(3) MELO, R. S., NEVES, M. H. C. B., BAPTISTA, O. R. Cultivo axênico das cianobactérias marinhas *Aphanothece halophytica* Frémy, 1933 e *Chroococcus minutus* (Kützing) Nägeli, 1849. **Acta Botanica Brasilica,** v. 25, p. 234 – 240, 2011.

(4) MORRIS, G. A., LI, P., PUAUD, M., LIU, Z., MITCHELL, JR., HARDING, S. E. Hydrodynamic characterisation of the exopolysaccharide from the halophilic cyanobacterium Aphanothece halophytica GR02: a comparison with xanthan. **Carbohydrate Polymers,** v. 44, p. 261 – 268, 2001.

(5) RICHERT, L. Caractérisation de souchesetd`exopolysacharidesde cyanobactéries et de micro-algues du ‘‘kopara’’ de Rangiroa-Tuamotu, Polynésie franc¸ aise. Doctoral dissertation, Papeete,PolynésieFrançaise: Université de PolynésieFrançaise, pp 229, 2004.

(6) STOWE, S. D., RICHARDS, J. J., TUCKER, A. T., THOMPSON, R., MELANDER, C., CAVANAGH, J. Anti-Biofilm Compounds Derived from Marine Sponges. **Mar. Drugs,** v. 09, p. 2010 – 2035, 2011.

(7) YEBRA, D. M., KILL, S., DAM-JOHANSEN, K. Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. **Progress in Organic Coatings,** v. 50, p. 75–104, 2004.