"Mircea cel Batran" Naval Academy Scientific Bulletin, Volume XVIII – 2015 – Issue 1
Published by "Mircea cel Batran" Naval Academy Press, Constanta, Romania // The journal is indexed in: PROQUEST SciTech Journals, PROQUEST Engineering Journals, PROQUEST Illustrata: Technology, PROQUEST Technology Journals, PROQUEST Military Collection PROQUEST Advanced Technologies & Aerospace

EFFECTS OF SULPHATE REDUCING BACTERIA ON THERMOSETTING POLYMERS/ZN COMPOSITE COATINGS

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Abstract: *Ships and barges are exposed to a lot of corrosion environments, an important role being microbiologically induced corrosion that attack ballast tanks and void spaces, cargo holds in commercial ships. The rate of corrosion increased quickly and it necessary repair and replacement of structural details, incurring very considerable cost penalties due to direct repair costs and to delay costs. Sulphate Reducing Bacteria is a group of phylogenetically diverse anaerobic microorganisms that were first discovered by Beijerinck in 1895. At present, 14 genera have been identified, the two most established genera of SRB being Desulfovibrio and Desulfotomaculum. Corrosion induced by Sulphate Reducing Bacteria has made high losses in shipping and gas industry every year. The paper evaluate the attachment effect of Sulphate Reducing Bacteria and the variation of roughness values before and after the attachments on the surfaces of zinc and zinc – thermosetting polymers composite coatings obtained by electro co-deposition. For testing it was used two types of thermosetting polymers as disperse phase in zinc matrix. It was used zinc matrix because this metal is the most widely used material for protection of steel against corrosion. The success of using zinc as a coating on steel can be attributed to its sacrificial nature, low cost and ease of application. Investigations of the surfaces were made using atomic force microscopy method and epifluorescence method. Sessile bacteria on samples were stained with 4', 6-diamidino-2- phenylindol (DAPI). After testing it was observed that attachment of bacteria and the roughness of the composite coatings surfaces are lesser than on zinc surface. By decrease the roughness the pitting attack will be better controlled. Those facts indicated that the thermosetting polymers/Zn composite coatings are more resistant to the attack of microorganisms like Sulphate Reducing Bacteria.*
Keywords*: Sulphate Reducing Bacteria, thermosetting polymers, composite coatings, atomic force microscopy, roughness*

INTRODUCTION

Attachments of bacteria on different materials and biofilm formations determine seriously problems such as various problems such as medical infections, fouling of water cooling system, product contamination and microbiologically influenced corrosion (MIC). Attachments of bacteria on different surfaces create a biofilm formed by microbial aggregates and extracellular polymeric substances (EPS). The EPS creates a microenvironment for sessile bacteria and allow the development of synergistic relationship.

Sulphate Reducing Bacteria (SRB) is an anaerobic bacteria using organism as nourishment. SRB can reduce sulphate to sulphide, and then form hydrogen sulphide or iron sulphide. SRB exists extensively in seawater, river water, underground piping and oil spirit wells, causes microbiologically influenced corrosion and a huge economic loss [1 – 3].

When MIC is present it can be observed some typical signs: clusters of pits several cm in diameter found under a cover of organic deposits (dirt and rust scale mixed with oil spills); high local corrosion rates in pit clusters; in oil cargo tanks, flat bottomed pits may show a stepwise development with "stairs" at the pit edge; black color of iron sulphides appearing during removal of cover (quickly disappearing when uncovered due to oxidation); sulphidic smell, quickly disappearing after ventilation.

If bacterial corrosion process is superposed upon the common electrolytic process the effects are more dramatically: severe corrosion appears in double hull tankers, especially in cargo oil tanks. In single hull tankers, pitting may represent a pollution hazard, if pits penetrate the bottom plating. In double hull tankers, pitting also represents a safety hazard, due to leakage of oil cargo and hydrocarbon vapors into ballast spaces [4].

In literature was been reported some corrosion rate for tanks of ships determined by MIC. So that, it was reported an excessive pitting corrosion up to 2mm/year on the uncoated carbon steel bottom plating [5] and 6mm/year for the bottom plating which is an extremely high rate if the construction material is considered which has in general a thickness of 10–15mm for a tank vessel [6]. Water ballast tanks are an integral part of every ship and although these areas are not revenue earning, they can be a critical expense item particularly if steelwork replacement as a result of corrosion is required. Due to the fact that large cargo vessels and oil tankers have hundreds of thousands square metre ballast tank surface, corrosion prevention and even more reconstruction if prevention fails is extremely costly [7].

The atomic force microscope is a mechanical imaging device that requires minimal sample preparation and creates three-dimensional images with high spatial resolution. Combining atomic force microscopy (AFM) and epifluorescence microscopy (EFM) are joined to yield a powerful tool for the investigation of biological samples [8]. Using these methods, it was reported the attachment of SRB on different type of steels: carbon steel [9], 304 stainless steel [10], 316 stainless steel [11], D36 carbon steel [12], alloy 625 and austenitic stainless steel [13], ASTM grade 2 titanium [14] etc.

The informations in literature about attachment of SRB on metal and especially on composite layers are penurious. In the present paper the work was focus on performing AFM coupled with EFM methods in order to evaluate the influence of materials pure zinc and thermosetting polymers/Zn composite coatings on SRB attachment.

MATERIALS AND METHODS

For evaluate the effect of sulphate reducing bacteria on zinc and thermosetting/Zn composite coatings were coated by electro-codeposition three types of surfaces: pure zinc coatings, phenol formaldehyde resin/Zn composite coatings and epoxy resin/Zn coatings. The electrolyte bath were the following composition: 310g/L $ZnSO_4 \times 7H_2O$; 75g/L $Na_2SO_4 \times 10H_2O$; 30g/L $Al_2(SO_4)_3 \times 18H_2O$. The pH of the solution was 3.8. Sulphate electrolyte was preferred for electrodeposition as zinc sulphate electrolyte has higher cathodic potential than zinc chloride electrolyte. Sodium sulphate increases the conductivity of electroplating solution. To stabilize the electrolyte acidity it was used aluminium sulphate as buffering agent, which has also an effect to obtain more shinning layers. Surfactants were not used in the bath as they reacted with resin particles. These layers were electrodeposited on DC04 steel as substrate.

Suspension for electro co - deposition of composite layers was prepared by adding phenol formaldehyde resin particles, respectively epoxy resin particles (mean diameter size 0.1 – 5μm) to the solution to give a concentration of 10g/L in the zinc electrolyte plating bath. Electro co - deposition took place in the bath at a temperature of 25^0C, current density of 5A/dm^2, time for electrodeposition 30min. The suspension bath was stirred by a mechanical stirrer at a constant rotational speed of 800rpm.

Phenol – formaldehyde resin and epoxy resin are thermosetting polymer witch become plastic with increasing temperature, irreversible solidifies and become insoluble. PF resins type novolac can be obtained from phenol and formaldehyde in acid medium. The properties of PF resin particles type novolac used as dispersed phase for electrodeposition are: molecular weight 3392 – 3816 g/mol; melting point 70 – 80ºC, viscosity at 120 ^{0}C 35 – 45 Pa s, free phenol < 1 %, water content < 0,5 %. The resin is not esterified. The epoxy resins (also widely known as epoxide resins) are characterized by the possession of more than one 1,2 - epoxy group per molecule. This group may lie within the body of the molecule but is usually terminal. The large family of epoxy resins represents some of the highest performance resins of those available at this time. Epoxies generally out-perform most other resin types in terms of mechanical properties and resistance to environmental degradation. The properties of epoxy resin particles used as dispersed phase for electrodeposition are: molecular weight 500 g/mol; density 1.18 – 1.25g/ cm^3, melting point 64 – 76ºC, epoxy parameter 0,185 – 0,220 equivalents in 100g resin 100%, volatile substances contained - maximum 1%.

To evaluate the effect of SRB on coatings, the microorganisms were prepared at University of Duisburg Essen Biofilm Centre, Aquatic Biotechnology. pH of solution with SRB cells suspension was 6.2. Attachment of SRB was made in a few steps: putting a drop from prepared solution cells on the tested surfaces; waiting to dry (15 - 20min). After that coupons were incubated in bacterial suspension of SRB (1 ppb organic matter 10^9cells/mL) for 24h to allow attachment and biofilm formation with 2.5% glutaraldehyde.

Biofilm and attached cells on coatings were investigated combined AFM and EFM methods. Subsequently, they were stained with 0.01% (wt/vol) DAPI for 10min and visualized at the epifluorescence microscope.

A NanoWizardII atomic force microscope (JPK Instruments, Germany) and an upright epifluorescence microscope (AxioImager A1m; Zeiss, Germany) were combined using the BioMaterialWorkstation (JPK Instruments). Throughout the present study the prototype of this new system was used. The key feature of the BioMaterialWorkstation was a shuttle stage that carried the actual sample precisely fixed on a glass slide. This shuttle stage could be transferred between the atomic force microscope and the epifluorescence microscope, giving a precise positioning of the stage on both microscopes.

For AFM images, silicon cantilever CSC37 A (Mikromasch, Estonia) with the following features was used: typical length, 250 μm; width, 35 μm; thickness, 2 μm; resonance frequency, 41 kHz; and nominal force/spring constant, 0.65 N/m. Each AFM image consists of 512 by 512 pixels. AFM images were performed by contact mode in air.

RESULTS AND DISCUSSION

The 2D topography of pure zinc layer and thermosetting polymers/Zn composite layers under atomic force microscope are presented in Figs. 1 – 3.

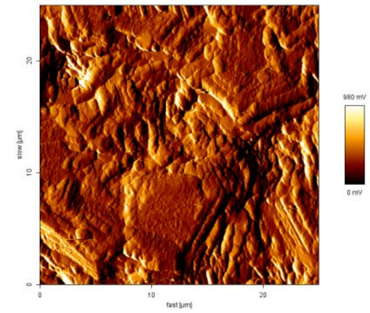


Figure 1. 2D - AFM image of untreated pure zinc surface (25μm x 25μm)

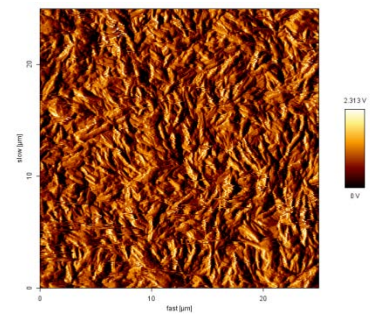


Figure 2. 2D - AFM image of untreated PF resin/Zn composite coating surface (25μm x 25μm)

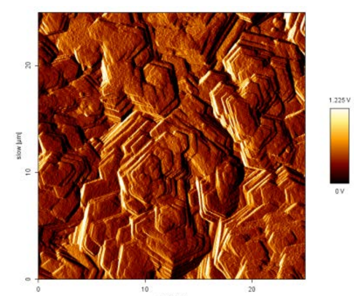


Figure 3. 2D - AFM image of untreated epoxy resin/Zn composite coating surface (25μm x 25μm)

It can be concluded that pure zinc is made up of hexagonal and regular crystals. By adding thermosetting polymers in electrolyte bath, the topography of composite coatings obtained is changed. Regular crystals of zinc will be disorder and crystals becomes more finely. Pure zinc coatings have a rather regular surface and composite coatings surfaces have finer grains structure, particles of polymers are distributed uniform on the surfaces. Phenol formaldehyde resin and epoxy resin polymers could be an inhibition effect on metal matrix crystals growth and a catalytic effect in increasing nucleation sites.

Epifluorescence microscopy images of a DAPI – stained biofilm sample of SRB on the surface of coatings are presented in Figs. 4 – 6.

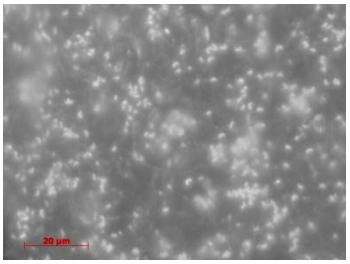


Figure 4. EFM image of the SRB attachment and EPS formed on pure zinc surface

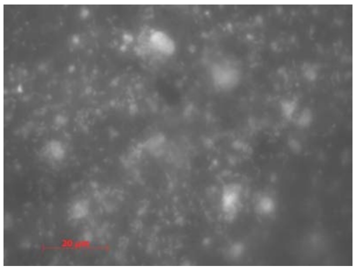


Figure 5. EFM image of the SRB attachment and EPS formed on PF resin/Zn composite coating surface

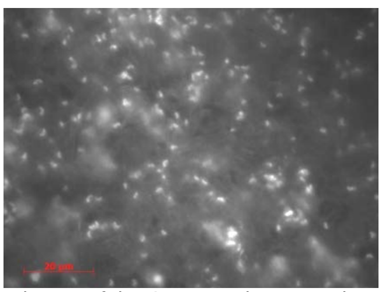


Figure 6. EFM image of the SRB attachment and EPS formed on epoxy resin/Zn composite coating surface

A high number of bacteria attached on pure zinc coating indicate that this type of coatings is not resist at microbiological attach. From Figure 5 – 6 can observed that the attachment of sulphate reducing bacteria on thermosetting/Zn composite coatings surfaces is lesser, so that could be concluded that this type of coatings are more resistant at microbiological corrosion induced by this type of bacteria.
The 3D images of the AFM scan acquired by contact mode in air on pure zinc layers and thermosetting polymers/Zn composite surfaces untreated and after SRB attachment with biofilm and EPS formation are presented on Figures 7 – 9.

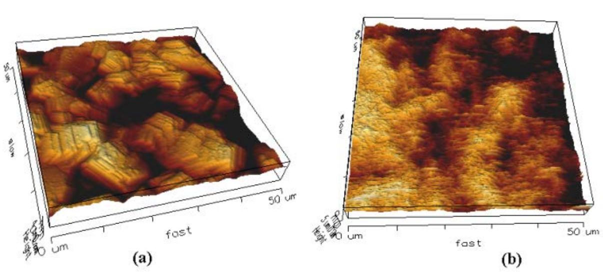


Figure 7. 3D - AFM images of pure zinc surface: (a) - untreated; (b) - with SRB bacteria attached and EPS formed

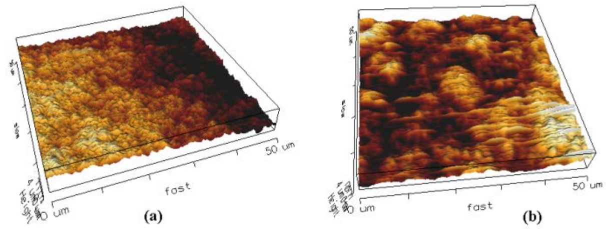


Figure 8. 3D - AFM images of PF resin/Zn composite coatings surface: (a) - untreated; (b) - with SRB bacteria attached and EPS formed

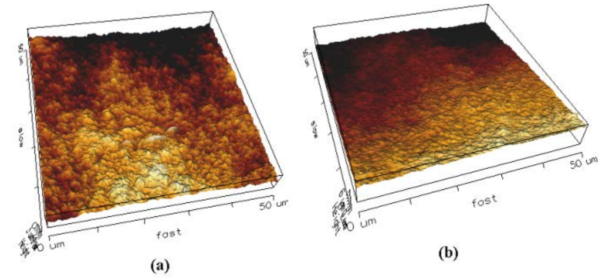
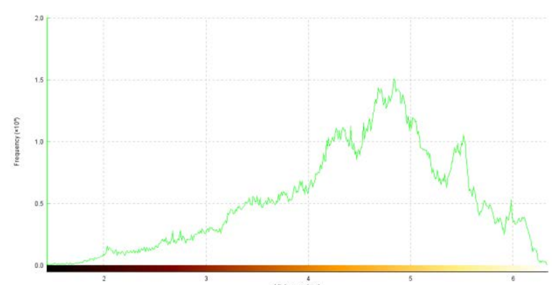
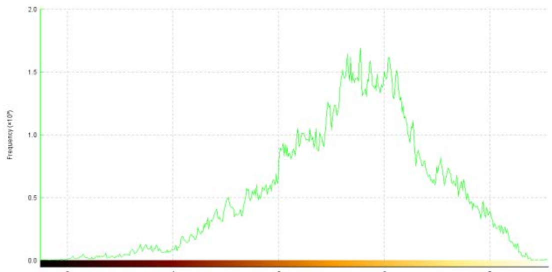


Figure 9. 3D - AFM images of epoxy resin/Zn composite coatings surface: (a) - untreated; (b) - with SRB bacteria attached and EPS formed

From Figures 7 – 9 could be concluded that exist visible differences between untreated and treated surfaces with SRB. These modifications of surfaces topography results from attachments of SRB cells on coating surfaces, biofilm and EPS formation. By using atomic force microscopy coupled with epifluorescence microscopy was demonstrate that surfaces where microorganism was attached have imperfections that protect them against removal by swab or rinse.
Variation of the surfaces roughness for pure zinc and thermosetting polymers/Zn composite layers before and after attachment of SRB, biofilm and EPS formation are shown in Figures 10 - 12. Roughness has an important role in determining how a real object will interact with its environment. AFM is considered as one of the most powerful methods for surface roughness characterization, because of its accuracy, non-damage to the surface and non-vacuum condition [15]. Roughness is quantified by the vertical deviations of a real surface from its ideal form. If these deviations are large, the surface is rough; if they are small the surface is smooth.

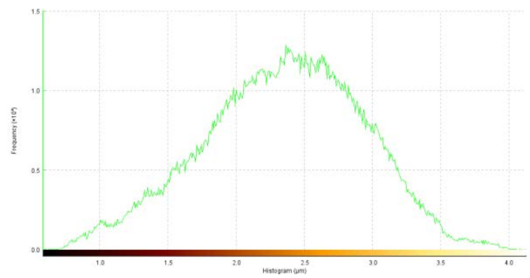


(a)

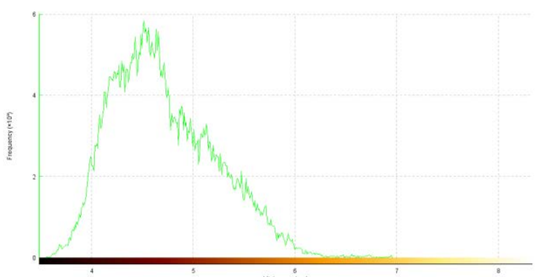


(b)

Figure 10. Histograms of the scanned surfaces for pure zinc surface: (a) - untreated; (b) - with SRB bacteria attached and EPS formed

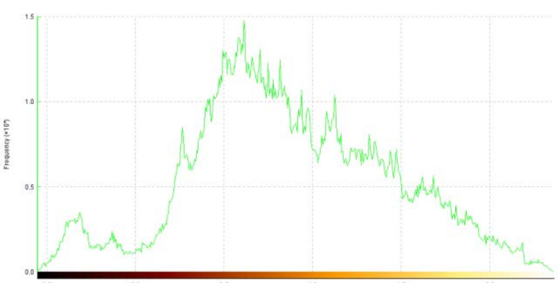


(a)

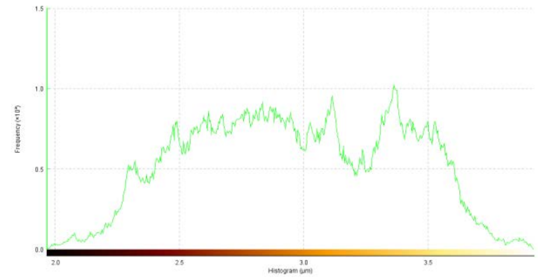


(b)

Figure 11. Histograms of the scanned surfaces for PF resin/Zn composite coatings surface: (a) - untreated; (b) - with SRB bacteria attached and EPS formed



(a)



(b)

Figure 12. Histograms of the scanned surfaces for epoxy resin/Zn composite coatings surface: (a) - untreated; (b) - with SRB bacteria attached and EPS formed

The values of surface roughness as R_a – average roughness and R_{RMS} (root – mean – squared roughness) that takes as reference the root mean square of the spacing between peaks and valleys weighted by their individual frequencies and amplitudes, evaluated by AFM method from histograms of the scanned surfaces are presented in Table 1.

Table 1. Values of R_a and R_{RMS} evaluated by AFM method

Type of coatings	R_a, nm	RMS, nm
Pure Zn coating untreated	723.2	907.5
Pure Zn coating with SRB	585.6	742.5
PF resin/Zn composite coatings untreated	473.1	584.9
PF resin/Zn composite coatings with SRB	412.8	507.7
Epoxy resin/Zn composite coatings untreated	453.7	563.2
Epoxy resin/Zn composite coatings with SRB	346.9	407.5

From data presented in Table 1 it could be observed that the values of R_a and RMS roughness for composite coatings untreated and treated with bacteria are lesser than roughness of pure zinc. For pure zinc coatings, the difference between the value of roughness for untreated and surface with SRB is bigger than other tested surfaces. That indicates a lot of bacteria attached on this surface, creating biofilm, EPS and corrosion product that conducting to a smooth surface

For all systems tested the surfaces roughness decrease after the attachments of bacteria. That could indicate an increase of the uniformity for all tested surfaces after the attachments of SRB, biofilm and EPS formation.

The roughness of the surfaces evaluated from AFM histograms confirmed the optical aspect of surfaces and indicates that the inclusion of thermosetting polymers particles in zinc matrix reduces the dimension size of zinc crystals. The zinc coatings untreated and treated with SRB exhibit a higher roughness among all other coatings. This is because of the random distribution of thin hexagonal plates developed in the absence of polymers particles parallel to the substrate surface.

From the EFM images presented in Figures 4 – 6 and from the values of the roughness were observed that attachment of SRB on thermosetting polymers/Zn composite coatings is lesser than on pure zinc. Those facts indicated that the thermosetting polymers/Zn composite layers are more resistant to the attack of microorganisms like SRB.

Conclusions

The attachment of Sulfate Reducing Bacteria on thermosetting polymers/Zn composite coatings is lesser than attachment on the pure zinc coatings. Bacterial attachment, biofilm and EPS formations on the surfaces are complex processes that are affected by material surface (pure metal or composite coatings).

Thermosetting polymers like phenol- formaldehyde resin and epoxy resin included in zinc matrix seems to be antibacterial properties and better resistance to microbiologically influenced corrosion than pure zinc coatings.

The surface roughness of the coatings decreases after the attachments of bacteria, biofilm and EPS formation.
From the epifluorescence images, surface topography and roughness values obtained from histograms could conclude that the thermosetting polymers/Zn composite layers are more resistant to the attack of the Sulphate Reducing Bacteria than pure zinc layers.
This type of new coatings, thermosetting polymers/Zn could be used, in the future, to protect ships against microbiologically influenced corrosion induced especially by sulphate reducing bacteria.

Acknowledgments

The investigations were financially supported by the National Research Programs UEFISCDI–Romanian Ministry of Education and Research–Research Projects: CPE–PN II 10 / 30-08-2013 (2013–2016)–HyBioElect.

Bibliography

[1] Liang C., Wang H., Huang N.B., *Effects of Sulphate Reducing Bacteria on corrosion behaviour of duplex stainless steel*, Journal of Iron and Steel Research International, vol.21 (4), pp. 444 – 450, 2014.
[2] Fushao L., Maozhong A., Guangzhou L., Dongxia D., *Effects of sulfidation of passive film in the presence of SRB on the pitting corrosion behaviors of stainless steels*, Materials Chemistry and Physics, Vol. 113 (2 – 3), pp. 971 – 976, 2009.
[3] Baker P., Ito K., Watanabe K., *Marine prosthecate bacteria involved in the ennoblement of stainless steel*, Environmental Microbiology, Vol. 5 (10), pp. 925 – 932, 2003.
[4] http://www.dnvusa.com/Binaries/Bacterial%20corrosion%20brochure_tcm153-416324.pdf
[5] Huan R.T., Mc Farland B.L., Hodgman R.Z., *Microbial influenced corrosion in cargo oil tanks of crude oil tankers*, NACE, ISBN 97535 1997 CP, Houston, USA, 1997.
[6] Cleland J.H., *Corrosion risks in ships ballast tanks and the IMO pathogen guidlines*, Engineering Failure Analysis, Vol. 2 (1), pp. 79 - 84, 1995.
[7] Heyer A., D'Souza F., Leon Morales C.F, Ferrari G., Mol J.M.C., de Wit J.H.W., S*hip ballast tanks a review from microbial corrosion and electrochemical point of view*, Ocean Engineering, Vol. 70, pp. 188 – 200, 2013.
[8] Zhang W., Stack A. G., Chen Y., *Interaction force measurement between E. coli cells and nanoparticles immobilized surfaces by using AFM*, Colloids and Surfaces B: Biointerfaces, vol. 82 (2), p.316 - 324, 2011.
[9] Javed M.A., Stoddart P.R., Wade S.A., *Corrosion of carbon steel by sulphate reducing bacteria: Initial attachment and the role of ferrous ions,* Corrosion Science, vol. 93, pp. 48 – 57, 2013.
[10] Yuan S., Liang B., Zhao Y., Pehkonen S.O., *Surface chemistry and corrosion behaviour of 304 stainless steel in simulated seawater containing inorganic sulphide and sulphate-reducing bacteria*, Corrosion Science, Vol. 74, pp. 353 – 366, 2013.
[11] Xua C., Zhanga Y., Chenga G., Zhu W., *Pitting corrosion behavior of 316L stainless steel in the media of sulphate-reducing and iron-oxidizing bacteria*, Materials Characterization, vol. 59 (3), p. 245 – 255, 2008.
[12] Jie W., Kaili Z., Tingyue G., Issam I. R., *A green biocide enhancer for the treatment of sulfate-reducing bacteria (SRB) biofilms on carbon steel surfaces using glutaraldehyde*, International Biodeterioration & Biodegradation, vol. 63 (8), p. 1102 – 1106, 2009.
[13] Wan Y., Zhang D., Liu H., Li Y. and Hou B., *Influence of sulphate-reducing bacteria on environmental parameters and marine corrosion behavior of Q235 steel in aerobic conditions*, Electrochimica Acta, vol. 55 (5), p. 1528 – 1534, 2010.
[14] Wanga H., Wang Z., Honga H., Yina Y., *Preparation of cerium-doped TiO_2 film on 304 stainless steel and its bactericidal effect in the presence of sulfate-reducing bacteria (SRB)*, Materials Chemistry and Physics, vol. 124 (1), p. 791 – 794, 2010.
[15] Feng F., Shi *K., Xiao S.-Z., Zhang Y.-Y., Zhao Z.-J., Wang Z., Wei J.-J., Han Z., Fractal analysis and atomic force microscopy measurements of surface roughness for Hastelloy C276 substrates and amorphous alumina buffer layers in coated conductors, Applied Surface Science, Vol. 258, pp. 3502 – 3508, 2012.*