Int. J. Electrochem. Sci., 9 **(2014)** 7587 - 7595

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Effects of Microorganism on Corrosion Performance of Zinc in Natural Seawater

Zhenhua Yu[1], Jie Zhang[2,], Xiaodong Zhao[3,*], Xia Zhao[2], Jizhou Duan[2] Xiuxia Song[2]*

[1] Qingdao Municipal Center for Disease Control and Prevention, Qingdao 266033, China
[2] Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[3] School of Naval Architecture and Ocean Engineering, Zhejiang Ocean University, Zhoushan 316022, China
[*]E-mail: zhangjie@qdio.ac.cn; danielxdzhao@aliyun.com;

Received: 2 September 2014 / *Accepted:* 14 October 2014 / *Published:* 28 October 2014

In this paper, the effects of the sterile seawater and natural seawater on corrosion of pure zinc sacrificial anode were investigated by using electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM) and fluorescence microscopy. The results showed in the natural seawater the biofilm could inhibit corrosion rate of the pure zinc sacrificial anode to some extent, compared with the sterile seawater. The reason is based on the consumption of oxygen and isolation from the seawater by forming biofilm. The variation of corrosion in the natural seawater showed a similar trend with the abiotic medium.

Keywords: Microbiologically influenced corrosion， zinc anode; electrochemical impedance spectroscopy; fluorescence microscopy

1. INTRODUCTION

In the marine environment, seawater corrosion and biofouling attachment are two important natural processes affecting the safe operation of metallic facilities. In seawater, bacteria and other microbe attach on the surface of the metal and form biofilm [1]. Biofilm is the interaction between different microorganisms in the environment with a very complex way, which creates a special environment on the interface of biological membranes and metal’s surface [2]. The special environment is different from the bulk solution, and a large number of complex chemical reactions are happened at the action of various microbes in this special environment, so the studies about the action of microbe on metal are very important for marine metallic facilities [3].

As we all know, microbiologically influenced corrosion (MIC) is the corrosion process which participated by the activities of microbial life [4]. In recent years, MIC has been concerned by a number of scientists. A lot of researches have been done about the microbial corrosion of metals in seawater [5], of which most studies are about carbon steel and stainless steel [6-10].

Sacrificial anodes are widely used for protection of steel structures in marine environment due to their high theoretical current efficiency, low active potential and low cost [11]. The outstanding advantage of zinc anode is its self-regulating features [12]. Zinc anode can provide better protection for metal facilities, so they are widely used on metal corrosion engineering [13]. But when the zinc anode was used in the marine environment, it was inevitably suffered from marine biofouling. The biofouling attachment and formation of microbial film could have certain influence on the performance of zinc anode. However, the influence of microbial film about the corrosion effects of pure zinc anodes in the marine environment has not been reported.

In this paper, the influences of sterile seawater and natural seawater on corrosion behavior of pure zinc sacrificial anode have been studied using electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM) and fluorescence microscopy. This work might be helpful on getting a better insight of evaluation of sacrificial anodes, and studying about the influence of microbial film on the anode.

2. MATERIAL AND METHODS

2.1. Material

Commercial pure zinc sacrificial anode plates (Pb: 0.00098, Cd: 0.005, Cu: 0.00012, Fe: 0.00045, Zn Bal) were used for this study. Samples with 10 mm×10 mm surface were used for surface analysis, fluorescence microscopy and as working electrodes for electrochemical measure. For electrochemical studies, one side of the sample was welded with the copper wire. Then the samples were embedded in a mold of PVC pipe, only one face kept as the working face. Samples for surface analysis and fluorescence microscopy were fixed a thread using silicon rubber. Before the experiment, the surfaces of all samples were treated according to GB 5776-86(China), polished with emery papers up to grit size of 1200#, rinsed with distilled water, degreased with ethanol using an ultrasonic bath, and finally dried by ethanol. Then they were kept in a silicagel desiccator and sterilized by ultraviolet lamp for 30 min before test.

2.2. Experimental medium

The seawater used in the experiment was taken from Huiquan Bay of Qingdao.

The seawater was filtered and let stand at room temperature for 5 days. Then it was autoclaved at 121°C for 30 minutes. 200ml was chosen as the sterile system, and 200ml natural seawater was chosen as the bacterial system. After cooling, the various indicators for the sterile seawater and natural seawater could be considered as the same.

2.3. Electrochemical experiments

The electrochemical tests were operated by SI 1287 potentiostat and SI 1260 frequency response analyzer. Electrochemical tests were carried out in the cell with three-electrode system consisting of the Zn anode sample as working electrode, a saturated calomel electrode (SCE) as the reference electrode and a Pt-plate with an area of 4 cm^2 as the counter electrode. The open circuit potential and electrochemical impedance spectroscopy were carried out at room temperature for 30 days. The EIS measurements were carried out over frequencies ranging from 100 kHz to 10 mHz with a 5 mV amplitude signal at OCP. The experimental data were analyzed by the software ZsimpWin [14].

2.4. Surface analysis experiment

The pure zinc sacrificial anode specimens immersed in sterile seawater and natural seawater respectively for 9 days and 23days were removed respectively from the system. All samples were immersed for 2 h in a 5% glutaraldehyde solution and then sequentially dehydrated using 50%, 75% and 100% ethanol–PBS solutions (15 min each). Afterwards, the samples were analysed by SEM to determine their corrosion morphologies.

2.5. Fluorescence microscopy of bacteria

Pure zinc sacrificial anode specimens immersed in natural seawater respectively for 5 days, 9days, 14days and 23days were removed aseptically from the system and gently rinsed in sterile seawater to remove any unattached cells. Then the samples were fixated with 5% glutaraldehyde (GA) solution for 30 minutes, stained in the dark for 15 minutes with 0.1% DAPI, then observed the bacteria with Zeiss Axioplan fluorescence microscope.

3. RESULTS AND DISCUSSION

3.1. Electrochemical measurements

The variations of the open circuit potential (E_{ocp}) with time were showed in Fig. 1 for samples immersed in sterile seawater (a) and natural seawater (b) respectively. The E_{ocp} of zinc samples in sterile seawater and natural seawater were significantly different. In sterile seawater, the E_{ocp} increased slowly from - 1.0531 to - 1.035V throughout the period of experiment, which indicating the electrochemical processes of the specimen surface was relative stable. While the E_{ocp} shifted increased from -1.07V to -1.025V during the primary stage in natural seawater, the increase could be due to bacterial adhesion and biofilm formation on the sample surface [15].

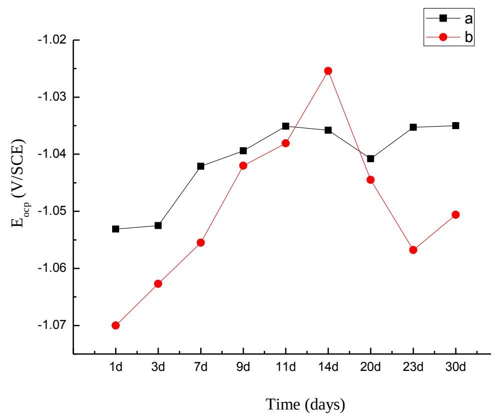

Figure 1. Time dependence of E_{ocp} for the sterile seawater (a) and natural seawater (b)

At the late of experiment, the E_{ocp} shifted in the negative direction from -1.025V to -1.0568V, which was because in the closed system, with the consumption of oxygen and nutrients, most of bacteria died and fell off from the surface of the sample. The surface of sample had changed.

Fig.2 shows the Nyquist plot obtained for the sacrificial anode immersed in the sterile seawater. For samples in the sterile seawater, the results of EIS were analyzed using ZSimpWin Software, the quality of the fit to the equivalent circuit was based on the χ^2-value between the experiment date and the fit results [16]. The EIS date for the sample immersed in the sterile seawater could be fitted by the equivalent circuit (Fig.4). As shown in the model, R_s is the solution resistance; Q_f is the capacitance of the surface layer; R_f is the resistance of the surface layer; Q_{dl} is the capacitance of the double layer; and R_{ct} is charge transfer resistance. The CPE impedance is described as: $Z_{CPE}=Y_0^{-1}(j\omega)^{-n}$ $(0<n<1)$, Y_0 is a constant phase element, and n is an empirical exponent, the value of n can reflect the degree of heterogeneities about the sample surface [17].

Table.1 shows the results of the fit with the equivalent circuit for the sterile seawater. From the Nyquist plot, the impedance represented by the diameter of the capacitive semicircle decreased from the 1st to the 11th day, and then increased from the 14th to the 23th day; in the 30th day, the diameter of the capacitive semicircle decreased again. The R_s increased from the 1st day to the 30th day because of addition of corrosion product. The R_{ct} for the sacrificial anode immersed in the sterile seawater decreased from the 1st to the 11th day, giving evidence to an increase of the dissolution of the sample; the corrosion rate of the sample increased gradually. From the 14th to the 23th day, the value of the R_{ct} increased gradually which implying the corrosion rate of the sample became relatively slow because addition of corrosion products on the sample surface, which could product the sample from aggressive to some extent. In the 30th day, the corrosion rate of the sample increased again because of loss of corrosion products. During the entire experiment time, the corrosion rate of the sample which immersed in the sterile seawater experienced a change process of increase, decrease, increase again.

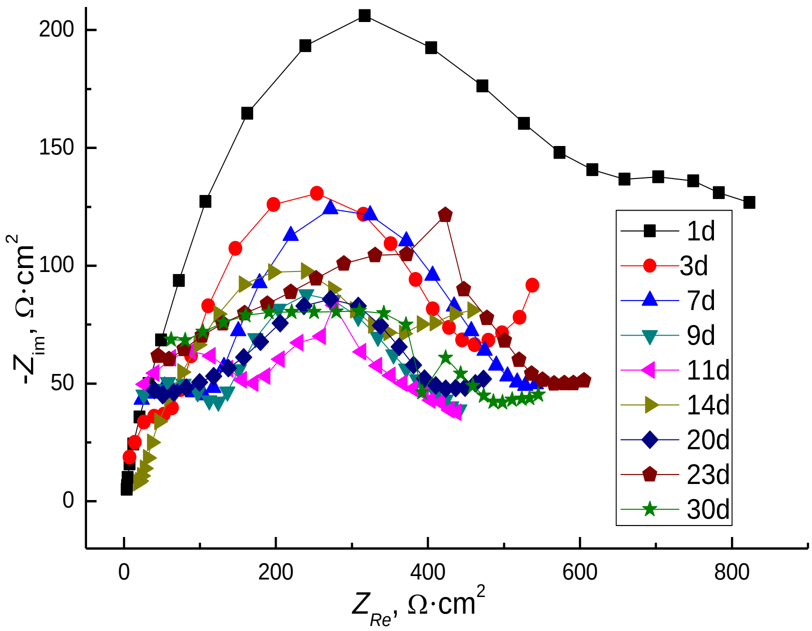

Figure 2. Nyquist plots for samples in sterile seawater

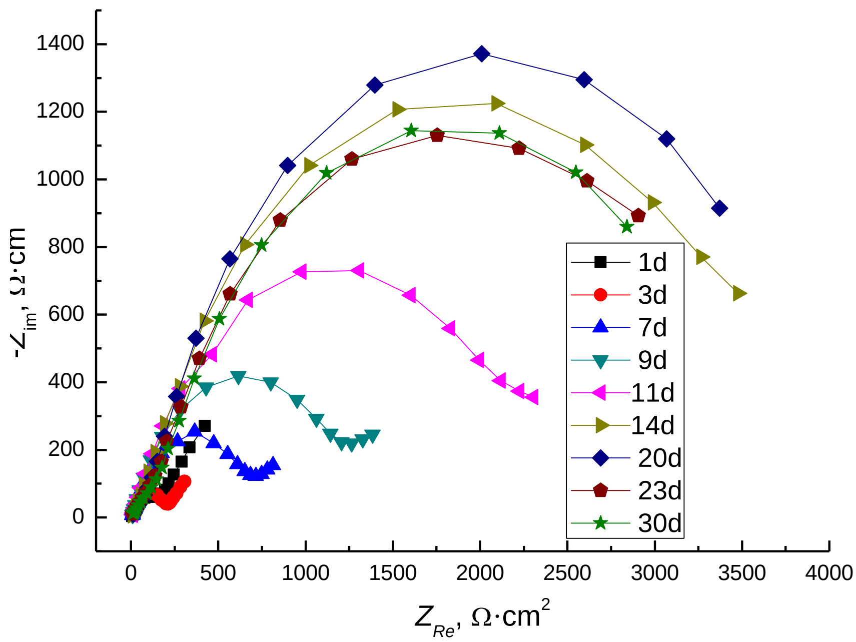

Figure 3. Nyquist plots for the samples in natural seawater

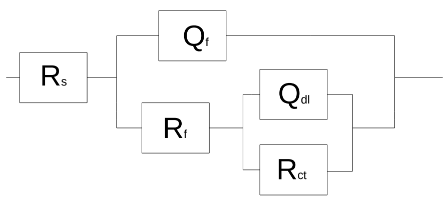

Figure 4. Equivalent circuits of the impedance diagrams of the samples

Table 1. Results of the fit with the equivalent circuit for samples in sterile seawater

Time	R_s (Ω·cm²)	Q_f (F/cm²)	R_f (Ω·cm²)	Q_{dl} (F/cm²)	R_{ct} (Ω·cm²)
1d	4.31	5.154×10^{-7}	130.5	2.674×10^{-6}	696.9
3d	4.739	9.945×10^{-8}	87.98	2.054×10^{-6}	520.5
7d	9.515	4.306×10^{-8}	121	2.626×10^{-6}	408.1
9d	11.34	4.101×10^{-8}	124.5	3.735×10^{-6}	293.5
11d	14.85	4.038×10^{-8}	159.8	5.196×10^{-6}	248.5
14d	25.3	9.607×10^{-7}	124.7	1.31×10^{-5}	338.3
20d	28.65	4.716×10^{-8}	125.8	2.786×10^{-6}	307.4
23d	31.16	3.588×10^{-8}	179	1.758×10^{-6}	379.2
30d	45.34	3.147×10^{-8}	188.6	1.775×10^{-6}	290.7

Fig.3 shows the Nyquist plot obtained for the sacrificial anode immersed in natural seawater. The EIS date for the sample immersed in the natural seawater could be fitted by the equivalent circuit (Fig. 4). Table.2 shows the results of the fit with the equivalent circuit for the samples in natural seawater. The value of R_{ct} reduced from 7048 Ω·cm² to 1789 Ω·cm² during the first 7 days, the reason was as the active anode sample, the sample could corrode rapidly when being contacted with the seawater. The value of R_{ct} increased from 1826 Ω·cm² to 5008 Ω·cm² from the 9th to the 20th day, which indicating a decreased corrosion rate. The reason was that the biofilm could inhibit corrosion to some extent. At the end of the experiment, the R_{ct} decreased to 4566 Ω·cm² because the drop of the biofilm and the loose corrosion products made the increase of corrosion.

According to the EIS date, the variation of corrosion in the natural seawater showed a similar trend with the abiotic medium, but the R_{ct} under aseptic conditions was significantly smaller than the natural seawater condition, which giving evidence to the bacteria could inhibit corrosion by forming biofilm and consuming oxygen.

Table 2. Results of the fit with the equivalent circuit for the samples in natural seawater

Time	R_s (Ω·cm²)	Q_f (F/cm²)	R_f (Ω·cm²)	Q_{dl} (F/cm²)	R_{ct} (Ω·cm²)
1d	8.08	1.704×10^{-6}	54.03	1.208×10^{-3}	7048
3d	12.32	1.193×10^{-6}	142.8	3.149×10^{-3}	2153
7d	11.07	6.587×10^{-7}	9.993×10^{-8}	3.782×10^{-4}	1789
9d	7.767	2.665×10^{-7}	6.12	3.423×10^{-5}	1826
11d	6.345	1.377×10^{-7}	7.649	1.921×10^{-5}	2810
14d	8.117	2.692×10^{-7}	21.72	2.575×10^{-5}	4409
20d	8.907	1.906×10^{-7}	25.13	4.83×10^{-5}	5008
23d	3.275	1.051×10^{-7}	17.09	7.282×10^{-5}	4920
30d	8.696	1.396×10^{-7}	24.9	7.753×10^{-5}	4566

3.2. Surface analysis

The results of the SEM about the samples in the sterile seawater and in the natural seawater were shown in Fig. 5. Fig5 (a) showed the SEM micrograph of the pure zinc sample immersed in the sterile seawater for 9 days. Some holes could be seen on the sample surface. The reason was that trace elements of the sample were corroded preferentially [18]. Moreover, some white corrosion products were observed. After 23 days, a lager corrosive hole was observed with some white floccus around the hole. Fig5 (c) showed the SEM micrograph of the pure zinc sample immersed in the natural seawater for 9 days. A large amount of bacteria could be seen on the sample surface, the shape of bacteria included sphere and bacilliform. EPS was formed by bacterial metabolic activity on the surface of the sample. EPS could be helpful to create an imhomogeneous biofilm on the metal surface. Because of the attachment of bacteria, the corrosion rate of metal was more uniform. Fig5 (d) showed the SEM micrograph of the sample immersed in the natural seawater for 23 days, in which almost no bacteria existed on the surface of the metal. The reason was because in the closed system, with the consumption of oxygen and nutrients, most of bacteria died and fell off from the surface of the sample. Some holes were also existed on the surface of the sample. The reason was that bacteria modified the physcicochemical environment and favor the electrochemical reactions, which lead to critical localized corrosion [19].

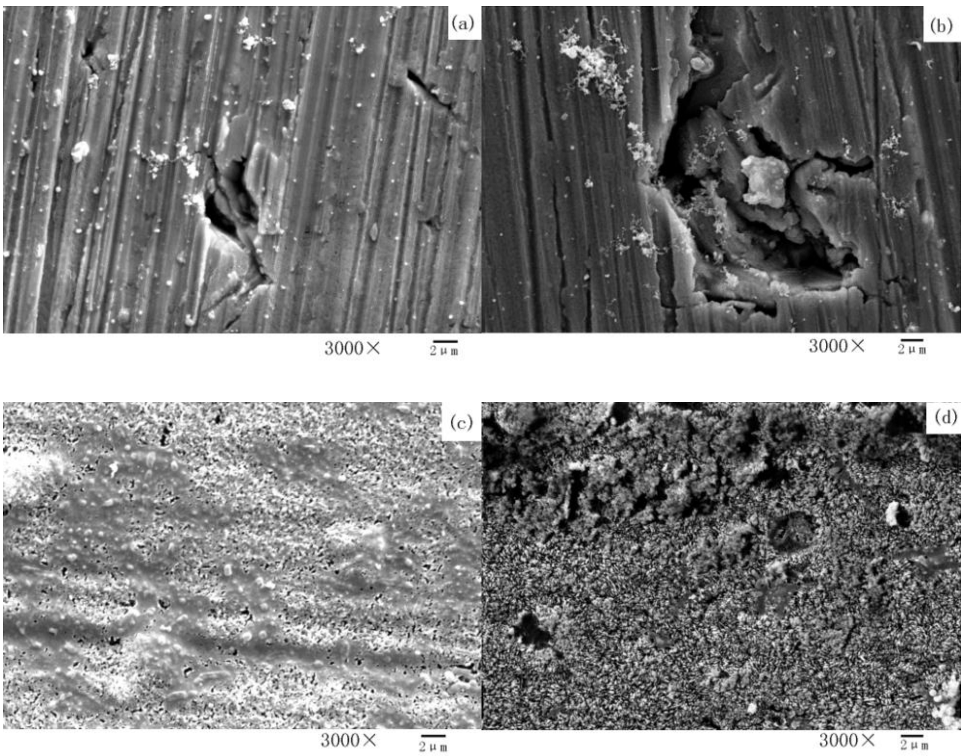

Figure 5. SEM images of the zinc samples immersed in the sterile seawater for 9 days (a) and 23 days (b), and in the natural seawater for 9 days (c) and 23 days (d)

3.3. Fluorescence microscopy results

Fig.6 showed the fluorescence microscopy of pure zinc samples immersed in natural seawater for 5 days, 9days, 14days and 20 days respectively. The examination of pure zinc samples exposed in the natural seawater showed bacterial attachment, growth, reproduction, biofilm formation and biofilm dropping. On the 5th day, most of the bacteria were monomer. But the bacteria had been inhomogeneous agglomerated after 9 days, which was consistent with the result of scanning electron microscopy. Because of the bacteria multiply metabolism, the biofilm generated in the 14th days. In the 23th days, the biofilm fell off from the surface of the sample, only little bacteria survival. That was because in the closed system, with the consumption of oxygen and nutrients, most of bacteria died.

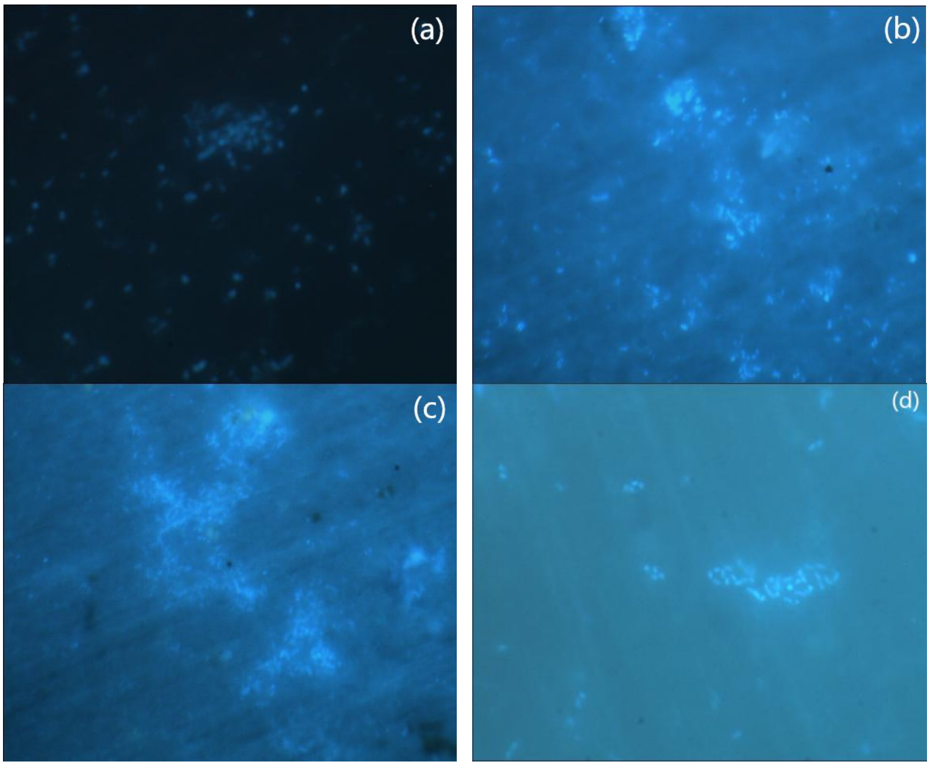

Figure 6. Fluorescence microscopy of zinc samples immersed in natural seawater for 5 days(a), 9days(b), 14days (c) and 20days(d)

4. CONCLUSIONS

1. The results of EIS measurements show in the natural seawater the biofilm could inhibit corrosion rate of the pure zinc sacrificial anode to some extent comparing with the sterile seawater system. During the entire experiment time, whether the samples immersed in the sterile seawater or in the natural seawater, the corrosion rate experienced a change process of increase, decrease, increase again. The R_{ct} under sterile conditions was significantly smaller than the natural seawater condition, which giving evidence to the bacteria could inhibit corrosion by forming biofilm and consuming oxygen.

2. The results of SEM micrograph show that a large amount of bacteria could be seen on the sample surface for the samples immersed in the natural seawater for 9 days, the shape of bacteria included sphere and bacilliform. EPS could be helpful to create an imhomogeneous biofilm on the metal surface.

3. The fluorescence microscopy results showed the process of bacterial attachment, growth, reproduction, biofilm formation and biofilm dropping during the entire exposure time in the natural seawater.

ACKNOWLEDGEMENT
This study was supported by National Natural Science Foundation of China (No. 41376003 and 41006054). The authors would like to acknowledge Zibo Hongtai Antisepic Co., Ltd for pure zinc sacrificial anode plates.

References

1. D. Xu, W. Huang, G. Ruschau, J. Hornemann, J. Wena and T. Gua, *Eng. Fail. Anal.*, 28 (2013) 149.
2. F. M. AlAbbas, C. Williamson, S. M. Bhola, J. R. Spear, D. L. Olson, B. Mishra and A. E. Kakpovbia, *Int. Biodeterior. Biodegrad.*, 78 (2013) 34.
3. M. Stipaničev, F. Turcu, L. Esnault, E. W. Schweitzer, R. Kilian and R. Basseguy, *Electrochim. Acta.*, 113 (2013) 390.
4. T. L. Dall'Agnol, C. M. Cordas and J. J.G. Moura, *Bioelectrochem.*, 97 (2014) 43.
5. S.Q. Chen, P. Wang and D. Zhang, *Corros. Sci.*, 87 (2014) 407.
6. Y.J. Chang, Y.T. Chang, C.H. Hung, J.W. Lee, H.M. Liao and H.L. Chou, *Int. Biodeterior. Biodegrad.*, xxx (2014) 1.
7. F. AlAbbas, C. Williamson, S. Bhola, J. Spear, D. Olson, B. Mishra and A. Kakpovbia, *J. Mater. Eng. Perform.*, 22 (2013) 3517.
8. T.Q. Wu, J. Xu, C. Sun,, M.C. Yan,, C.K. Yu and W. K, *Corros. Sci.*, 88 (2014) 291.
9. P.Y. Zhang , D.K. Xu, Y.C. Li, K. Yang and T.Y. Gu, *Bioelectrochem.*, 101 (2015) 14.
10. A.M. Awad, E.A. Ghazy, S.A. Abo El-Enin and M.G. Mahmoud, *Surf. Coat. Technol.*, 206 (2012) 3165.
11. F.L. Liu, J. Zhang, C.X. Sun, Z.H Yu and B.R. Hou, *Corros. Sci.*, 83 (2014) 375.
12. B.H. Wan, J.Y. Fei, S.P. Wang, L. Wang, Y. Chen, *Mater. Rev.*, 2010, *24*, 87.
13. S.M. Lee, Y.J. Kim, S.W. Eom, N.S. Choi, K.W. Kim and S.B. Cho, *J. Power Sources*, 227 (2013) 177.
14. F.J. Tang, G.D. Chen, J. S. Volz, R. K. Brow and M. L. Koenigstein, *Cem. Concr. Compos.*, 35 (2013) 171.
15. Y.B. Fu, *Dev. Appl. Mater.*, 21(2006) 38.
16. J. Santana Rodríguez, F. Santana Hernández, J. González González., *Corros. Sci.*, 2006, *48*, 1265.
17. S. Gaur, R.K. Singh Raman and A.S. Khanna, *Mater. Sci. Eng. C*, 42 (2014) 91.
18. D. Veys-Renaux, K. Guessoum, E. Rocca, N. David and K. Belhamel, *Corros. Sci.*, 77 (2013) 342.
19. P. Beese, H. Venzlaff, J. Srinivasan, J. Garrelfs, M. Stratmanna and K. J.J. Mayrhofer, *Electrochimica Acta* 105 (2013) 239.