Int. J. Electrochem. Sci., 14 **(2019)** 1610 – 1619, doi: 10.20964/2019.02.30

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Short Communication

Effect of Mixed Bacteria on Corrosion Properties of Zn-Al-Cd Sacrificial Anode

Jiwen Song[1], Jie Zhang[2,4,5], Jia Wang[2,3], Xiuxia Song[2], Shengli Chen[1]*

[1] CNOOC Information Technology Co., Ltd, Shenzhen, 518067, China
[2] Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China.
[3] University of Chinese Academy of Sciences, Beijing, 100049, China.
[4]Open Studio for Marine Corrosion and Protection, Pilot National Laboratory for Marine Science and Technology, Qingdao 266237, China
[5]Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, 266071, China
[*]E-mail: zhangjie@qdio.ac.cn

Received: 17 August 2018 / *Accepted:* 30 October 2018 / *Published:* 5 January 2019

In this paper, the mixed microorganism (sulfate reducing bacteria and Shewanella algae) on corrosion behavior of Zn-Al-Cd sacrificial anode has been studied using electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM) and fluorescence microscopy. The EIS results showed that the corrosion rate of mixed bacteria samples has experienced a process of increasing first and then decreasing as the test time is extended. SEM and fluorescence microscopy results showed that bacteria have undergone a process from mass propagation to the formation of a layer of biofilm on the surface of the sample. This layer of biofilm formed by bacteria, corrosion products and bacterial metabolites protects the sample, avoiding contact of corrosive media with the sample, thereby reducing the corrosion rate of the sample.

Keywords: Electrochemical impedance spectroscopy; Microbiologically influenced corrosion; Zn-Al-Cd sacrificial anode; Fluorescence microscopy

1. INTRODUCTION

Cathodic protection of sacrificial anodes has been widely used for corrosion protection of various marine equipments due to its advantages of good current dispersion, easy management and maintenance, and relatively low cost[1]. In the marine environment, biofouling and corrosion caused by the attachment of various microorganisms and marine organisms can significantly reduce the performance and life of

equipment and materials [2-4]. Sulfate reducing bacteria plays a critical role in the corrosion behaviors of the materials used in marine environment, thus effect of the sulfate reducing bacteria on the corrosion behavior of metallic materials has been studied in detail [5-7]. However, most researches focus on the corrosion behaviors of the carbon steels, ignoring that of the sacrificial anode systems. The influence of microorganisms on the sacrificial anode systems includes several aspects in marine environment. Usually a variety of microorganisms stick to the surface of the sample simultaneously, forming the biofilms to play a synergistic role. Recent studies have shown that Shewanella algae can inhibit the corrosion of metals[8, 9], lacking of the synergistic effects of the Shewanella algae and the sulfate reducing bacteria on sacrificial anode systems. This paper aims to study the effects of the mixed bacteria containing Shewanella algae and sulfate reducing bacteria on corrosion properties of the Zn-Al-Cd sacrificial anode systems using electrochemical impedance method, together with the microstructural characterization containing scanning electron microscopy and fluorescence microscopy [10, 11].

2. EXPERIMENTAL PROCEDURE

The sample used in this experiment was a Zn-Al-Cd sacrificial anode, and its chemical composition is shown in Table 1

Table 1. Chemical composition of Zn-Al-Cd sacrificial anodes (m %)

Element	Al	Cd	Pb	Cu	Fe	Zn
Content	0.36	0.045	0.00092	0.0012	0.0021	margin

The experimental seawater medium was taken from Qingdao Huiquan Bay and purified by coarse sand filtration. After standing for 5 days, a part of natural seawater was sterilized by high temperature and high pressure steam at 121 °C for 30 min. After cooling to room temperature, 200 ml was taken as a sterile system. 200 ml natural seawater is taken as the bacteria-based seawater system. The indicators of the sterilized seawater after cooling can be considered the same as the indicators of natural seawater[12].

2.1. Separation and purification of experimental strains

2.1.1. Separation and purification of Shewanella algae

The experimental strain was derived from the yellow rust layer of the Steel frame soaked in the sea. Shewanella algae[13]was cultured using revised Postgate's C medium. Medium composition: 1g yeast extract, 0.3 g sodium citrate, 1 g NH_4Cl, 0.06 g $CaCl_2 \cdot 6H_2O$, 0.5 g KH_2PO_4, 0.06 g $MgSO_4 \cdot 7H_2O$, 6 ml sodium lactate, 1000 ml of seawater, solid medium also needs to add 3% Agar. The medium used was sterilized at 121 ℃ for 30 min in a high temperature sterilizer and allowed to cool. About 5 g of

yellow rust was added to 100 ml of liquid medium, and cultured in a 30 ℃ biochemical incubator. After one week of culture, a small amount of the above-mentioned bacterial solution was taken and streaked on the revised Postgate's C solid medium, and then wrapped in plastic wrap and placed in a constant temperature culture at 30 ℃ for 4 days in an inverted culture, and the yellow colonies in the plate were picked again. After the first purification, a single colony was cultured, and then a single colony was picked and purified twice, and co-purified 5 times, and cultured in an inverted culture at 30 ℃ in an incubator[13, 14].

2.1.2. Separation and purification of sulfate reducing bacteria.

Revised Postgate's C medium was used for the enrichment culture of the sulfate reducing bacteria. The medium was sterilized in a high temperature sterilizer at 121℃ for 30 min. And 0.004 g/L $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ were added in the medium as the instruction of the sulfate reducing bacteria. The 100 ml medium was cultured in a biochemical incubator at 30 ℃ after putting 5 g yellow rust. After a week, the medium turned into black, with emitting pungent odor of the H_2S, which illustrates the growth of the sulfate reducing bacteria. The 50 μl bacteria solutions were sucked, separated and purified by coating separation methods. Proper amount of bacteria solution was sucked and evenly coated on the solid medium using glass rods following diluting the bacteria to the level of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and then inverted medium in a biochemical incubator at 30 ℃ without oxygen. After seven days, black colonies grew on the plates, and individual colonies in the plate were picked and streaked again. Then, the cells were inverted and placed in an anaerobic chamber and placed in an incubator at 30 ℃ for 5 times until a single colony was isolated, and then cultured in a liquid medium to prepare for the next experimental procedure[7].

2.2. Experimental system.

200 ml sterilized Postgate's C medium were added to 10 ml sulfate-reducing bacteria which have been cultured for 4 days, followed by adding an equal amount of overnight cultured Shewanella algae to use as a bacterial system. 200 ml sterilized Postgate's C medium was taken as a sterile system.

The specifications of the samples used in the experiment were 10 mm × 10 mm × 6 mm. According to the experimental requirements, one end of the experimental sample was welded with a copper wire, and one side was reserved as a working surface, and the other surface was sealed with an epoxy resin in a PVC pipe. All samples were prepared according to GB5776-86. Before the experiment, the working surface of the sample was ground to 1200# with water sanding paper, first rinsed with distilled water, then ultrasonically washed with ethanol, then rinsed with absolute ethanol, and the sample was placed in a clean bench and sterilized by UV for 30 min.

2.3. Electrochemical experiment

Electrochemical experiments [15] were carried out using Solartron's SI 1287 potentiostat and SI

1260 frequency response analyzer. The test system was a three-electrode system. The working electrode was a Zn-Al-Cd sample, the counter electrode was a platinum electrode (20 × 20 mm), and the reference electrode was a saturated calomel electrode (SCE). After the experimental system was assembled, the measurement was started after standing at room temperature for one day. The open circuit potential and the AC impedance spectroscopy were all performed at room temperature for a period of 18 days. The excitation signal of the AC impedance spectrum is a 10 mV sine wave voltage, and the scanning frequency ranges from 100 kHz to 10 mHz. When performing electrochemical tests, the measurement is started after the open circuit potential of the system changes less than 2 mV in 300 seconds. The data was collected by Zplot software, and the experimental results were fitted and analyzed by ZSimpWin electrochemical analysis software [16].

2.4. Surface analysis experiment

Take two 250 mL jars that have been autoclaved at 121 °C, add 200 mL of each of the above-mentioned sterilized and mixed bacteria seawater in a clean bench, and place two sterilized samples in the jars. Then, the jar is covered with a rubber stopper, and the rubber stopper and the mouth of the bottle are sealed with paraffin. After 4 days and 9 days, each of the sterilized and mixed bacterial seawater samples was taken separately for pretreatment in a clean bench. The sterile samples were immersed in 50% ethanol (solvent PBS) for 15 min, 75% ethanol soaked for 15 min, and 100% ethanol soaked for 15 min for dehydration. The mixed bacteria samples were treated with 5% glutaraldehyde solution (solvent as PBS). After soaking for 2 hours, it was then dehydrated stepwise using the same ethanol solution as above. After vacuum critical drying and gold spraying, the corrosion morphology was observed by SEM, and the acceleration voltage was 20 kV.

2.5. Fluorescence microscopy of bacteria

Take four pre-treated samples and hang them in a jar containing natural seawater. The rubber stopper is sealed with paraffin and placed at room temperature. Periodically, samples are taken from natural seawater for microbial fluorescence microscope observation. Before the experiment, the sample was washed three times with sterile seawater, fixed with 5% glutaraldehyde (diluted with PBS) for 30 min, and then stained with 0.1% 4,6-Diamidino-2-phenylindole (DAPI) for 15 min. After air drying in the dark, the stained sample was placed on a glass slide and placed under a BX51 fluorescence microscope in the dark.

3. RESULTS AND DISCUSSION

3.1. Open Circuit Potential

Figure 1 shows the open circuit potential (OCP) curves of Zn-Al-Cd sacrificial anodes as a function of time, which is significantly different in the sterile and mixed systems. The trend of the

sample in the bacterial system is about the same as that of the sterile system, but the OCP is significantly higher than the OCP of the sterile system, which is consistent with the report by A. Nagiub[8, 9]. Mainly because bacteria form aerobic and facultative biofilm on the surface of the electrode, it can reduce the dissolved oxygen content on the electrode surface by aerobic respiration. In addition, the biofilm itself and its secreted extracellular polymer (EPS) can act as a physical barrier to prevent dissolved oxygen in the bulk phase from diffusing toward the electrode surface.

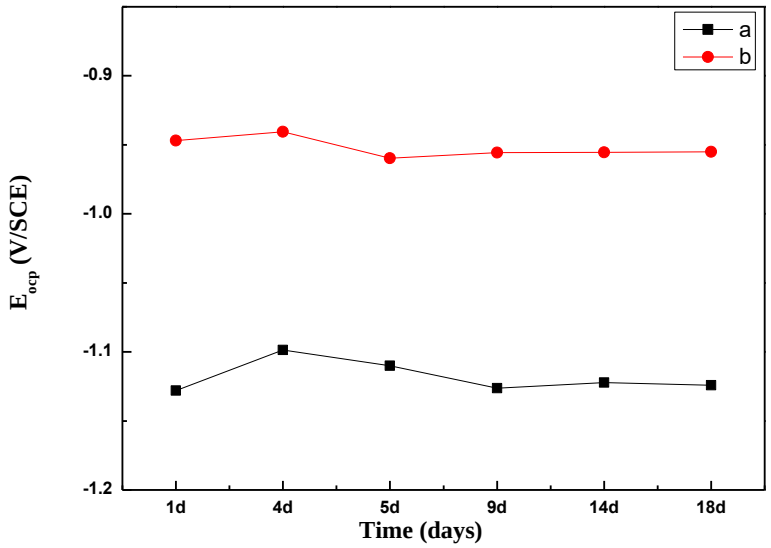


Figure 1. The open circuit potential-time curve of the samples in culture medium without (a) or with (b) bacteria

3.2. Electrochemical Impedance Spectroscopy

3.2.1. Electrochemical Impedance Spectroscopy Analysis of Samples in Sterile Bacteria Medium

Figure 2 (a) shows the AC impedance spectrum of the sample in a sterile system as a function of time and Figure 3 (a) shows its fitted circuit whose data is shown in Table 2. The R_s changed smoothly, indicating the stability of the experimental system during the entire experimental period. The Rct value has been on a decreasing trend within 9 days, which demonstrated the increase of the corrosion rate. The sample acted as an active anode and corroded rapidly when it contacted with corrosive media. During the period of 11 to 18 days, the value of R_{ct} began to increase again because corrosion products were continuously deposited on the surface of the sample with the corrosion reactions taking place. These corrosion conducts blocked the directly contact of the corrosive medium with the sample and reduced the corrosion rate. In short, the corrosion rate increased first and then decreased in the sterile system[17].

3.2.2. Electrochemical Impedance Spectroscopy Analysis of Samples in Mixed Bacteria Medium

Figure 4 (b) shows the impedance spectroscopy of the sample in the mixed bacteria including sulfate reducing bacteria and shewanella algae as a function of time. The curve radius was decreased firstly and then increased, showing that the corrosion rate increased firstly and then decreased.

According to the impedance spectroscopy, the AC impedance spectrum was fitted using the equivalent circuit in Figure 3 (b), and the obtained data was shown in Table 3. The Rs value fluctuated obviously, indicating the unstability of the electrochemical system during the whole experimental period, and this was related to the activity of bacteria. The Rct value decreased rapidly from 598.2Ω·cm^2 to 76.78 Ω·cm^2, which indicated that the sample corroded rapidly. The probable reason is that Shewanella algae consumed oxygen in the system, offering favorable condition for the rapid growth of the sulfate-reducing bacteria. During this period, the sulfate-reducing bacteria had a logarithmic growth phase, leading to a large number of sulfate-reducing bacteria attached to the surface of the sample, and then causing the rapid corrosion of the sample, which was consistent with the changed trend of the open circuit potential. Within the period of 5 to 18 days, the Rct value of the sample increased gradually, indicating that the corrosion rate of the sample decreased gradually. The possible reason was that as the bacteria multiplied, the oxygen in the system was continuously consumed, and a biofilm formed on the surface of the sample which weakened the corrosion process, combined with the deposition of corrosion products, to slow down the corrosion rate to a certain degree [18].

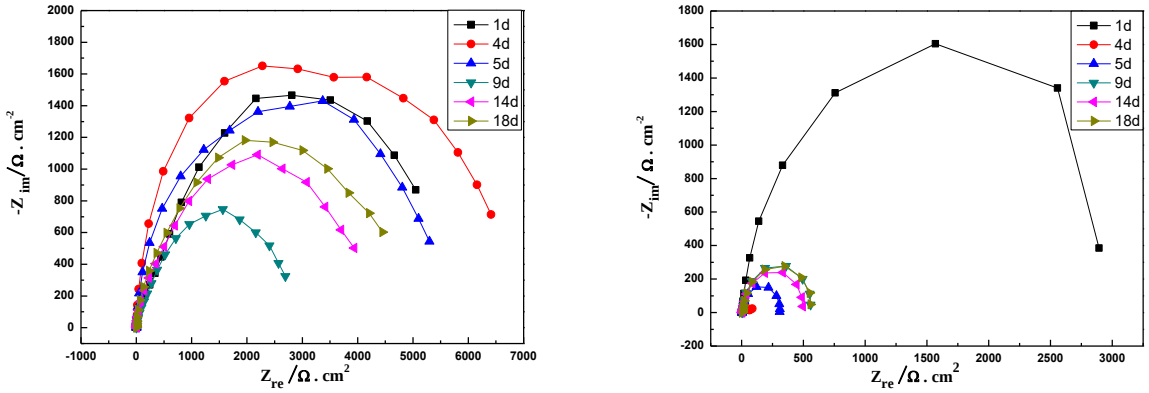


Figure 2. Nyquist plots for the samples in culture medium without bacteria (a) and culture medium with bacteria (b)

Table 2. Results of the fit with the equivalent circuit for culture medium without bacteria

Time/d	R_s/(Ω·cm^2)	C_f/(F·cm^{-2})	R_f/(Ω·cm^2)	C_{dl}/(F·cm^{-2})	R_{ct}/(Ω·cm^2)
1	1.676	1.581×10^{-5}	497.8	1.599×10^{-4}	3805
4	1.537	1.914×10^{-5}	278.3	2.108×10^{-4}	3203
5	1.597	2.01×10^{-5}	170.7	1.574×10^{-4}	3123
9	1.478	1.517×10^{-5}	289.4	1.326×10^{-4}	1981
14	1.675	2.009×10^{-5}	632.9	1.469×10^{-4}	2750
18	1.601	2.107×10^{-5}	803.5	1.383×10^{-4}	3036

Table 3. Results of the fit with the equivalent circuit for culture medium with bacteria

Time/d	$R_s/(\Omega\cdot cm^2)$	$Q_f/(F\cdot cm^{-2})$	n	$R_f/(\Omega\cdot cm^2)$	$C_{dl}/(F\cdot cm^{-2})$	$R_{ct}/(\Omega\cdot cm^2)$
1	0.9084	1.499×10^{-3}	0.9304	2.787	8.608×10^{-19}	598.2
4	2.479	5.282×10^{-3}	0.6603	3.807	1.9×10^{-3}	76.78
5	2.457	1.138×10^{-3}	0.8126	3.672	2.702×10^{-3}	319.3
9	5.883	1.266×10^{-3}	0.8376	5.018	2.78×10^{-3}	586.8
14	3.168	1.919×10^{-3}	0.8495	4.276	2.231×10^{-3}	530.6
18	3.351	1.938×10^{-3}	0.8535	4.175	2.052×10^{-3}	688.5

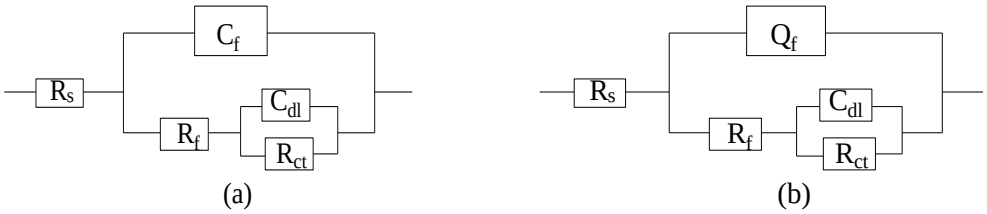
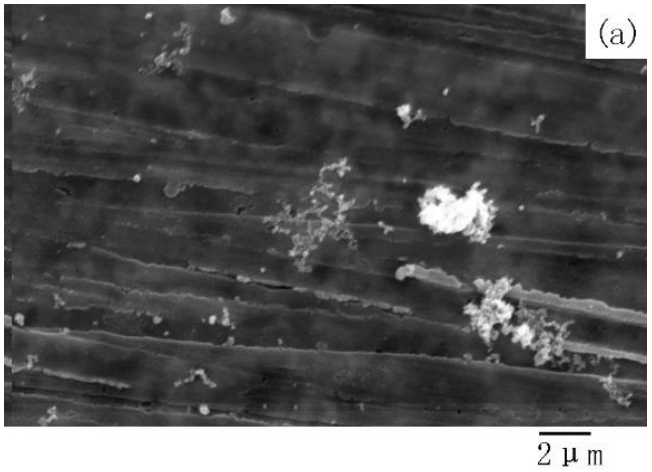


Figure 3. Equivalent circuits of the impedance diagrams of the sample in culture medium without bacteria (a) and culture medium with bacteria (b)

3.3. SEM and fluorescence microscopy analysis

Figure 4 shows the morphologies of Zn-Al-Cd samples soaked for 4 days (a) and 9 days(b) without bacteria medium. The corrosion of the sample surface occurred slightly with negligible change on the 4th day. On the 9th day, the corrosion happened severely and corrosion pits formed on the surface of the specimens, indicating the increase of corrosion rate of the specimens.



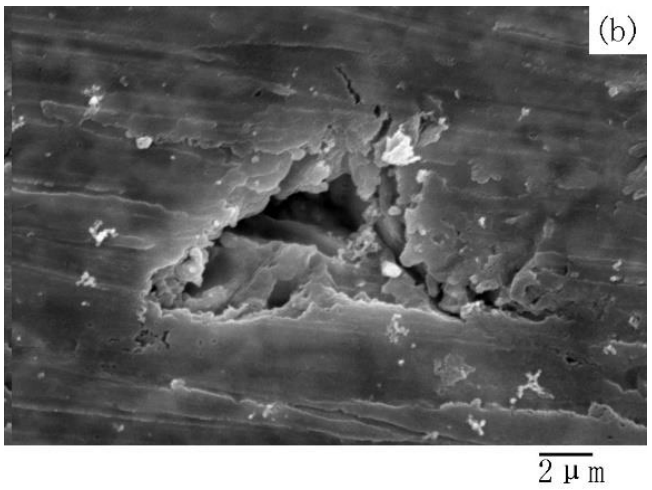


Figure 4. SEM images of the Zn-Al-Cd samples immersed in culture medium without bacteria for 4d (a) and 9d (b)

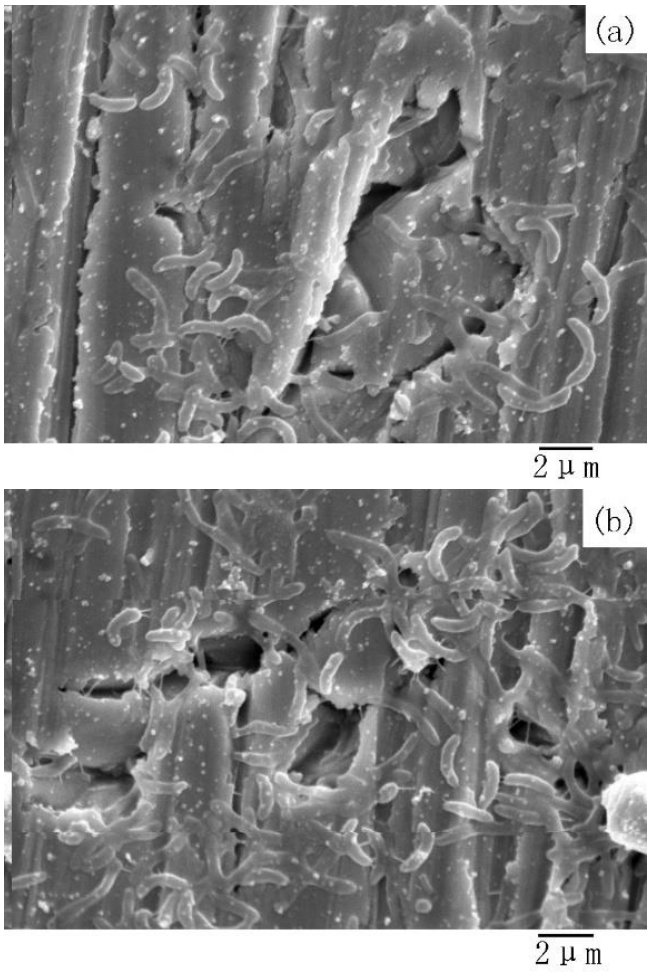


Figure 5. SEM images of the Zn-Al-Cd samples immersed in culture medium with bacteria for 4d (a) and 9d (b)

Figure 5 shows the SEM image of the sample soaked for 4 days. As can be seen from the figure, a large amount of bacteria was adhered to the surface of the sample, and the corrosion on the sample surface was severe and the white corrosion products were adhered to the surface of the sample. On the

9th day, the corrosion products and the metabolites of the bacteria depositing on the surface of the sample inhibited the corrosion of the sample[19].

Figure 6 is a fluorescence micrograph of the sample soaked in the bacterial solution for different times. As can be seen from the figure, the bacteria began to multiply and attach to the surface of the sample on the 1st day, appearing in the form of monomer. On the 4th day, the bacteria agglomerated gradually into a biofilm. After soaked for 9 days, a thicker biofilm formed on the surface of the sample. This layer of biofilm protected the sample to avoid the contact of the corrosive medium with the sample, so that the corrosion rate of the sample decreased, which is consistent with the electrochemical results and SEM results. On the 14th day, the bacteria died gradually and the residual bacteria on the surface of the sample decreased gradually with the consumption of nutrients and oxygen in the closed experimental system [20].

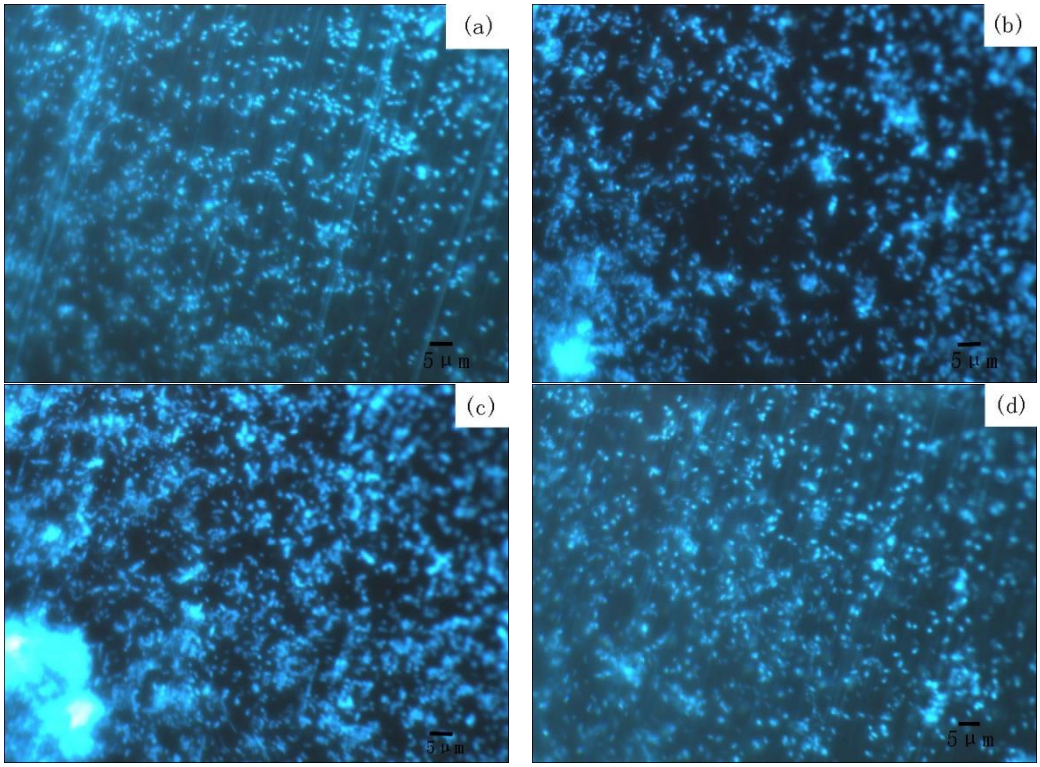


Figure 6. Fluorescence microscopy of Zn-Al-Cd exposed in culture medium with bacteria for 1d (a), 4d (b), 9d (c) and 14d(d)

4. CONCLUSIONS

Electrochemical results suggested that compared to the results of the sample in the medium without bacteria, the open circuit potential in the medium with mixed bacteria shifted positively. And the R_{ct} value was much smaller than that in the aseptic medium. This indicates that the mixed bacteria can accelerate the corrosion of the sample. The reason is that Shewanella algae consumed oxygen in the system, creating favorable conditions for the growth of sulfate-reducing bacteria and accelerating the corrosion of the sample.

SEM results suggested that after the sample soaked in the mixed bacterial system for 9 days, its surface was covered with a relatively dense biofilm. While in the system without bacteria, there were obvious corrosion pits and white corrosion products on the surface of the sample. Fluorescence microscopic results suggested that the bacteria enriched gradually and agglomerated on the surface of the sample in the mixed bacteria. After soaked for 9 days, a thicker biofilm formed on the surface of the sample. On the 14th day, the bacteria died gradually and the residual bacteria on the surface of the sample gradually decreased with the consumption of nutrients and oxygen in the closed experimental system.

ACKNOWLEDGEMENTS
This work was supported by the National Natural Science Foundation of China (Grant Nos. 41376003 and 41006054) and the Strategic Priority Research Program of the Chinese Academy of Sciences， Grant No. XDA13040405.

References

1. W.X. Guo, X.Y. Li, M.X. Chen, L. Xu, L. Dong, X. Cao, W. Tang and J. Zhu, *Adv. Funct. Mater*., 42 (2014) 6691.
2. S. Lorenzi, T. Pastore, T. Bellezze and R. Fratesi, *Corros. Sci*., 108(2016) 36.
3. L.Y. Xu, X. Su and Y.F. Cheng, *Corros. Sci.,* 66(2013) 263.
4. Ph. Refait, M. Jeannin, R. Sabot, H. Antony and S. Pineau, *Corros. Sci*., 90(2015) 375.
5. J. Carmona, P. Garcés and M.A. Climent, *Corros. Sci*., 96(2015) 102.
6. N. Parhizkar, T. Shahrabi, and B. Ramezanzadeh, *Corros. Sci.,* 123 (2017) 55.
7. B. Ramezanzadeh, and M. Rostami, *Appl. Surf. Sci*., 392 (2017) 1004.
8. A. Nagiub and F. Mansfeld, *Corros. Sci*., 43(2001) 2001.
9. A. Nagiub and F. Mansfeld, Ele*ctrochim. Acta*, 47(2002) 2319.
10. Y. Huang, S.J. Liu and C.Y. Jiang, *Wei shengwuxue Tongbao*, 44 (2017) 1699.
11. H.X. Wan, W.Z. Huangfu, Z.Y. Liu, C.W. Du, X.G. Li, D.D. Song and B. Cao, *Ocean Eng.* , 136 (2017) 11.
12. X.X. Song, J. Zhang, D.F. Yang and J.Z. Duan, *J. Mater. Eng.*, 3(2013) 58-63.
13. J. Zhang, X.X. Song, X. Luan, C.X. Sun, J.Z. Duan and B.R. Hou, *Acta Metall. Sin*., 48 (2012) 1495.
14. X.X. Song, Z.H. Yu, J. Zhang, C. Zhang and J.Z. Duan, *J. Guangxi Acad. Sci.* 33(2017) 291-297.
15. O.S. Fatoba, E.T. Akinlabi and S.A. Akinlabi, *Materialwiss. Werkstofftech.*, 49 (2018) 453.
16. R. Cao, L. Q. Zhu, H.C. Liu, W. Yang and W.P. Li, *Rsc Adv*., 5 (2015) 104375.
17. S. Khireche, D. Boughrara, A. Kadri, L. Hamadou and N. Benbrahim, *Corros. Sci*. 87 (2014) 504.
18. F.L. Liu, J. Zhang, C.X. Sun, Z.H. Yu and B.R. Hou,*Corros. Sci.*, 83 (2014) 375.
19. J. Ma, N. Yang, Y. Chen, F. Jiang and H. huang, *Agric. Biotechnol.*, 7 (2018) 74.
20. J.J. Wu, D. Zhang, P. Wang, Y. Cheng, S.M. Sun,Y. Sun and S.Q. Chen, *Corros. Sci*, 112 (2016) 552.