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Electrochemical Behavior of 316L Stainless Steel in f/2 Culture Solutions Containing *Chlorella Vulgaris*

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Microbiologically influenced corrosion is a serious problem for stainless steels in marine engineering. In this study, the attachment of a typical biofouling microalgae *Chlorella vulgaris* on the surface of 316L stainless steel was determined by a light microscopy. Its influence on the electrochemical behavior of this material was also evaluated by electrochemical techniques, including open circuit potential, electrochemical impedance spectroscopy and polarization curves. The results showed that this algal species could attach to the surface of 316L stainless steel without any extracellular polymeric substance. This action was not sensitive for the surface roughness but was influenced by the environmental pH value. The adhesion density reached a maximum at pH 9 value. Electrochemical results showed that this algal species had a corrosion inhibit efficiency for 316L stainless steel during a short period of immersion (14 d). This was mostly because of the oxygen produced by the photosynthetic activity of planktonic algae. The sessile algae species couldn't induce any influence on the electrochemical behavior of 316L stainless steel. All these results provide a basic knowledge on the influence of microalgae on the corrosion of stainless steel. This is meaningful for interpreting microbiologically influenced corrosion in situ for further.

Keywords: 316L stainless steel; *Chlorella vulgaris*; Open circuit potential; Electrochemical impedance spectroscopy; Polarization curve.

1. INTRODUCTION

Austenitic stainless steels are widely used because of their high strength, mechanical workability, and excellent electrical and thermal conductivities. The formation of a stable passive layer makes their strong corrosion resistance. Among them, 316L stainless steel (SS) is of great practical interest because it has good resistance to chloride corrosion resistance with high concentration of Mo

[1]. As a result, this type of material is often used in marine engineering, including port facilities, cooling water circuit and ships and related equipments.

However, they are improved to be susceptible to various corrosion processes once immersed in marine environment due to the aggressive bulk they are exposed to. Microbiologically influenced corrosion (MIC) is a serious problem during these corrosion processes. This kind of corrosion is not an actual form of corrosion but a process which involves micro-organisms that may initiate or otherwise contribute to the propagation of corrosion [2, 3]. The microorganisms involved in this process are referred to bacteria, algae and fungi. Most of them can attach to the surfaces of substratum. Researchers thought that the growth of these microorganisms on the surface after adhesion could change the local environment and then induced the corrosion of the metal alloys indirectly.

Up to now, many studies have been conducted on MIC on 316L SS [4-10]. The most of these researches have demonstrated the role of bacteria in biocorrosion [11]. By contrast, microalgae have attracted little interest. In fact, the role of them in such processes cannot be excluded [12]. Previous studies have clearly demonstrated that there is an exoelectrogenic activity present in microalgae conducted by a photosynthetic metabolism [13, 14]. Photosynthetic metabolic activity can also produce oxygen, which is important to the electrochemical behavior of SS. Moreover, some species of algae can produce H_2O_2 or other reactive oxygen species under the oxidative stress [15, 16]. All these compounds are thought to influence the electrochemical behavior of SS. However, the influence of them needs to be improved with relative experiments, especially those experiments with unialgal cultures.

Chlorella vulgaris (*C. vulgaris*) is a typical species of algae contributed to a biofilm in situ [17, 18]. In recent years, this species of algae has been expected to construct a high efficient microbe fuel cell [14]. In this study, this species of algae was isolated from the coast of China and cultured in lab. The influence of roughness and environmental pH value on the adhesion of this species to the surface of 316L SS was evaluated by a light microscopy. Electrochemical techniques, including open circuit potential, electrochemical impedance spectroscopy (EIS) and polarization curve, were used to determine the electrochemical behavior of 316L SS in the culture solutions with *C. vulgaris*. The results in this study would not only provide knowledge on the influence of microalgae on MIC of SS, but also gave useful information in the design of new microbial fuel cells.

2. EXPERIMENTAL

2.1 Algal culture and the pre-treatment of specimen

The species of *C. vulgaris* was isolated from the coast of China. Unialgal, non-axenic cultures were cultured at a temperature of 20 ± 2 °C and a salinity of 30 under a 12:12 light:dark cycle with an irradiance of 6000 lux during the light period. Sea water from the Huiquan Bay was filtered and then sterilized at 121 ºC for 30 min in a pressure vapor sterilizer. The sterilized sea water was enriched with f/2 medium ($NaNO_3$: 8.83×10^{-4} M; $NaH_2PO_4 \cdot H_2O$: 3.63×10^{-5} M; $Na_2SiO_3 \cdot 9H_2O$: 1.07×10^{-4} M; $FeCl_3 \cdot 6H_2O$: 1×10^{-5} M; $Na_2EDTA \cdot 2H_2O$: 1×10^{-5} M; $CuSO_4 \cdot 5H_2O$: 4×10^{-8} M; $Na_2MoO_4 \cdot 2H_2O$: 3

$\times 10^{-8}$ M; $ZnSO_4 \cdot 7H_2O$: 8×10^{-8} M; $CoCl_2 \cdot 6H_2O$: 5×10^{-8} M; $MnCl_2 \cdot 4H_2O$: 9×10^{-7} M; Vitamin B_{12}: 1×10^{-10} M; Thiamine·HCl: 3×10^{-7} M) for the species.

The 316L SS (Table 1, 20 mm×20 mm×4 mm) was polished with silicon carbide. It was ultrasonically cleaned in analytical reagent grade ethanol and Milli-Q water for 10 minutes, respectively. After that, the specimens were immersed into the culture solutions.

Table 1. Chemical compositions of specimens (wt.%)

Ni	Si	P	Mn	S	Cr	C	Mo	Ti	Fe
13.308	0.926	0.042	1.837	0.026	17.030	0.026	2.260	2.130	Rest

2.2 Adhesion test and electrochemical experiments

Different roughness was obtained by polishing the specimens using different grit of silicon carbide. 1 M HCl or 1 M NaOH solutions were used to adjust different pH values of mediums. The surface roughness polished by 800 grit of silicon carbide was used in cultures of different pH values. After 48 hours, triplicate coupons were put in a beaker containing 10 mL of salted water (20 g L^{-1} of NaCl solution) and placed at the ultrasound bath for 10 min. Cell density (number of cell mL^{-1}) was measured by a light microscopy counting in a 0.1 mL plankton counting chamber. At the same time, one specimen was used to evaluate the attachment state of *C. vulgaris* on 316L SS by a scanning electron microscopy (SEM). This specimen was taken from culture solutions and fixed with 1% glutaraldehyde in a phosphate buffer solution (PBS) for 1 hour, and then dehydrated with an ethanol gradient (at 50%, 75% and 90% for 15 minutes respectively, and 100% for 0.5 h). The specimens were observed using a Jeol JSM5900 LV scan electron microscope (Tokyo, Japan) at an acceleration of 25 kV.

Electrochemical experiments were conducted by using a CHI760C control system (CH Instruments, Inc.). They were implemented in traditional three electrodes system, in which, the working electrode was the 316L SS, the counter electrode was a platinum wire, and the reference electrode was a silver/silver chloride electrode (Ag/AgCl, 3 M KCl). The results of EIS were analyzed by fitting the data using Zsimpwin software.

3. RESULTS AND DISCUSSION

3.1 Adhesion Assay

The attachment of microalgae on surfaces had received considerable attention in the past years. Previous studies showed that attachment was influenced by a serious of parameters, such as surface property (wettability and roughness), pH, illumination, culture density, bacterial film and so on [17,

19]. Fig. 1 showed the influences of surface roughness of 316L SS on the adhesion density of *C. vulgaris*. The adhesion density was the highest on the smoothest surface of 316L SS with a roughness of 6 μm. The lowest adhesion density was obtained on the surface of specimen with a roughness of 38 μm. The adhesion density of this species of algae on specimen with a roughness of 10 μm was also relatively lower. All these showed that there was no inconsistent relationship between adhesion density and roughness of specimen. It seemed that the species of *C. vulgaris* was not sensitive for the surface roughness of 316L SS. Erable et al. [20] thought that the influence of roughness on micro-organic adhesion was based on the size of micro-organisms. The size of *C. vulgaris* in this study was about 2 μm (see Fig. 3), which was much lower than the roughness of the surface obtained by silicon carbide.

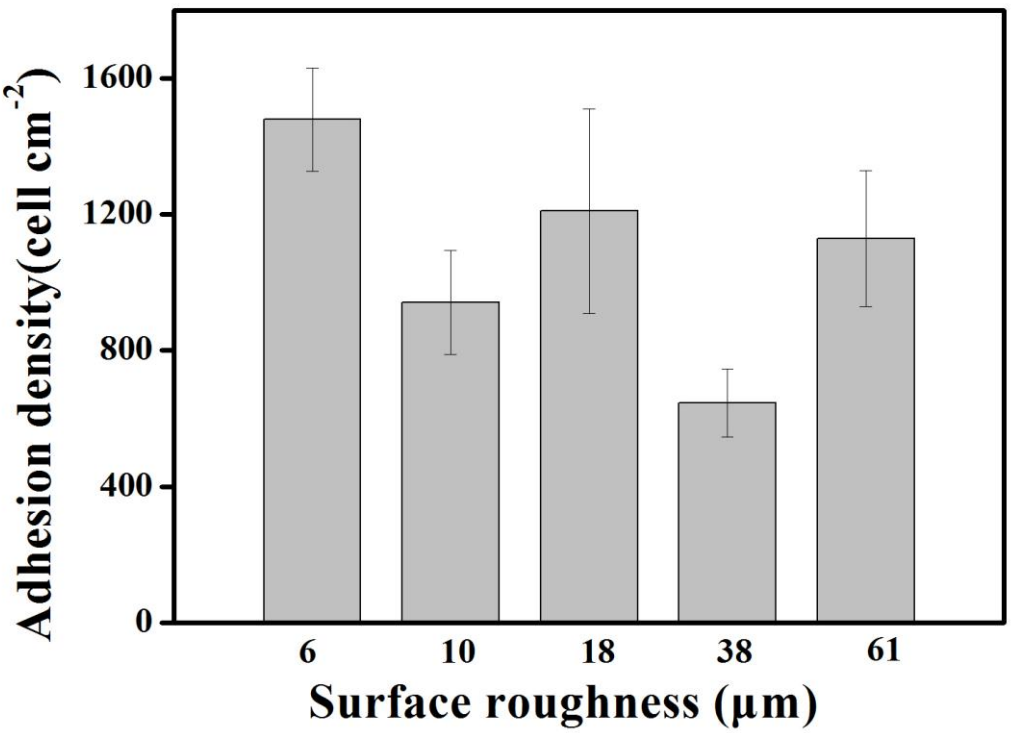

Figure 1. Influence of surface roughness of 316L SS on the mean (± S.D.) adhesion density of *C. vulgaris*.

Fig. 2 showed the influences of environmental pH value on the adhesion density of *C. vulgaris* on the surface of 316L SS. The adhesion density at pH 6, 7 and 8 was qualitatively similar with each other. It reached a maximum at pH 9, which was about two times than those obtained at the other pH values. This was presumed that pH 9 was favorable for the growth of *C. vulgaris* that the culture density kept a higher value at this pH value during 48 hours. The higher culture density had been improved to increase the attachment of algal species on the surface of substratum [17].

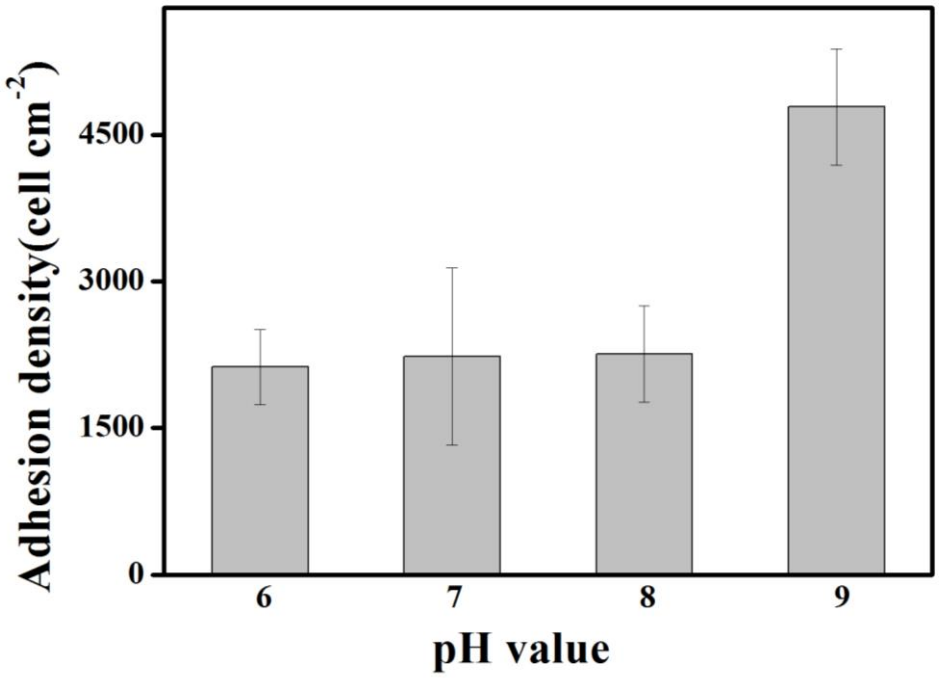

Figure 2. Influence of culture pH value on the mean (± S.D.) adhesion density of *C. vulgaris*.

Fig. 3 was a SEM picture of 316L SS specimen immersed in f/2 culture solutions with *C. vulgaris* after 48 h. This picture showed that this species of algae did not produce any extracellular polymeric substances, which was commonly for the biofouling micro-organisms. However, the adhesion mechanism of this species could not be exactly defined by the present study.

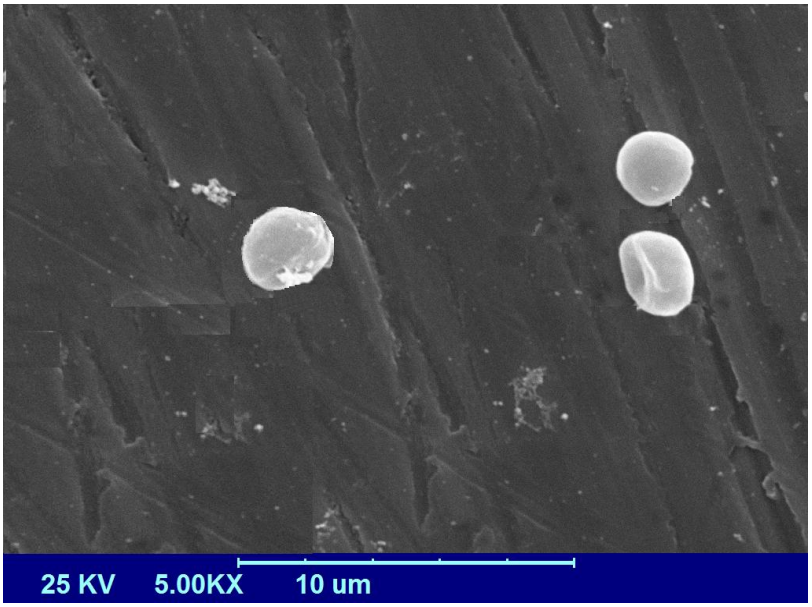

Figure 3. SEM of 316L specimen immersed in f/2 culture solutions with *C. vulgaris*.

3.2 Electrochemical behavior of 316L SS in culture solutions

Fig. 4 was the open circuit potential (E_{OC}) of 316L specimen immersed in f/2 culture solutions with and without *C. vulgaris* within 14 days. The E_{OC} of specimen in medium without algal species showed a slight increasing intendancy during the first two days. The value increased to -0.102 V from -0.148 V. After that, it stayed at -0.100 V for about one week and began to show a decreasing tendency with immersion time. Compared with this, the relationship between the E_{OC} of specimen immersed in medium with *C. vulgaris* and immersed time was more complex. The E_{OC} of specimen increased with immersed time during the light time while it decreased with immersed time during the dark time. However, the E_{OC} of specimen immersed in culture solution with *C. vulgaris* showed a slight variation after 14 days.

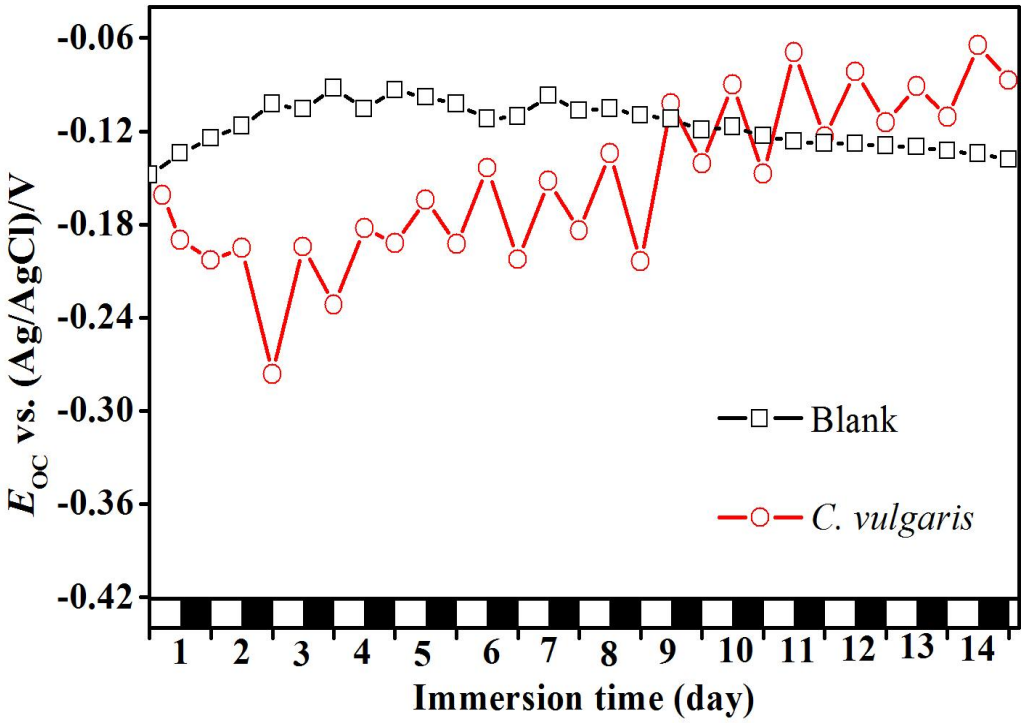

Figure 4. The variation of open circuit potential (E_{OC}) of 316L SS specimen immersed in f/2 culture solutions with and without *C. vulgaris* with immersion time. (X axis: Blank means light period; Dark means dark period.)

Fig. 5 showed the electrochemical impedance spectroscopy obtained on 316L SS immersed in f/2 culture medium without (A) and with (B) *C. vulgaris* during 14 days. The impedance magnitude of SS increased with immersion time in the two kinds of culture solutions. With the same immersion time, the impedance magnitude of SS in the culture solution with algal species was higher than that in solution without algal species.

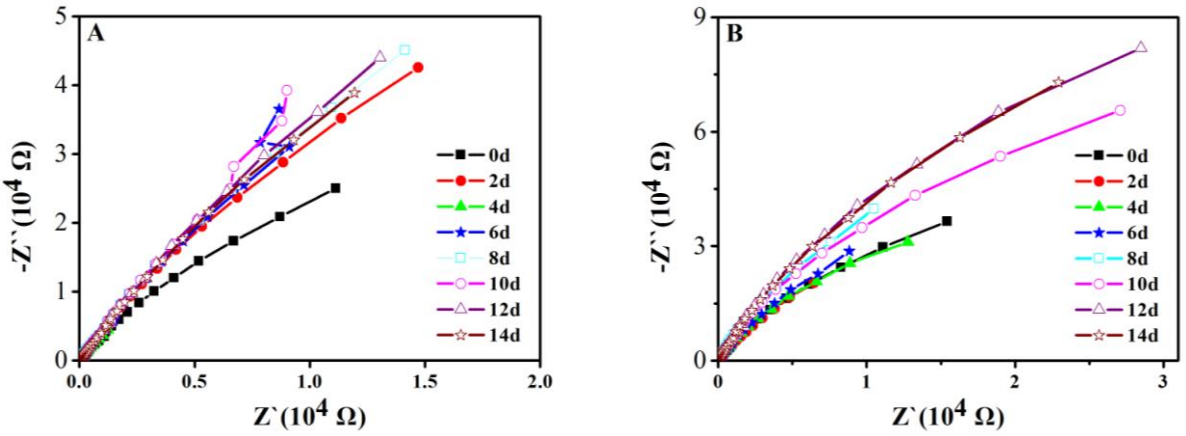

Figure 5. The electrochemical impedance spectroscopy (EIS) obtained on 316L SS immersed in f/2 culture medium without (A) and with (B) *C. vulgaris* during 14 days.

Considering the duplex nature of the formed passive films on 316L SS substrate [21], the EIS results were modeled according to an equivalent circuit consisting of two parallel combination R_1C_1 and R_2C_2 pairs arranged in series and in series connection with the ohmic impedance (Rs) (Fig. 6) [22]. The first inner barrier layer was a thin and compact, which was followed by a relatively thick porous outer layer facing the solution. Two constant phase elements (CPE) here were used instead of the two ideal capacitors to account for the deviations observed as capacitive slopes and phase angles lower than -1 and 90°, respectively. In this model, the total film resistance ($R_t = R_1 + R_2$) were used to evaluate the corrosion resistance of SS.

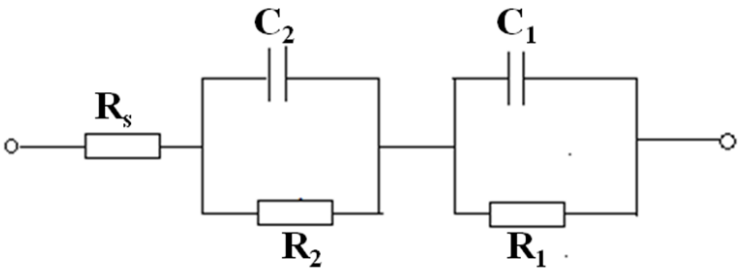

Figure 6. The equivalent circuit model used for impedance data fitting.

Fig. 7 showed the variations of R_t with immersion time. R_t of specimen in culture solutions without *C. vulgaris* was relatively stable during the whole immersion period with a value of about 1×10^5 Ω cm^{-2}. R_t of specimen in culture solution with *C. vulgaris* was similar with that without algal species at the beginning of immersion time. However, after 14 days immersion time, this value was nearly two times of that in culture solutions without algal species. This indicated the metabolic activity of *C. vulgaris* could inhibit electrochemical corrosion process of SS. This was consistent with the results of the E_{OC} of specimen.

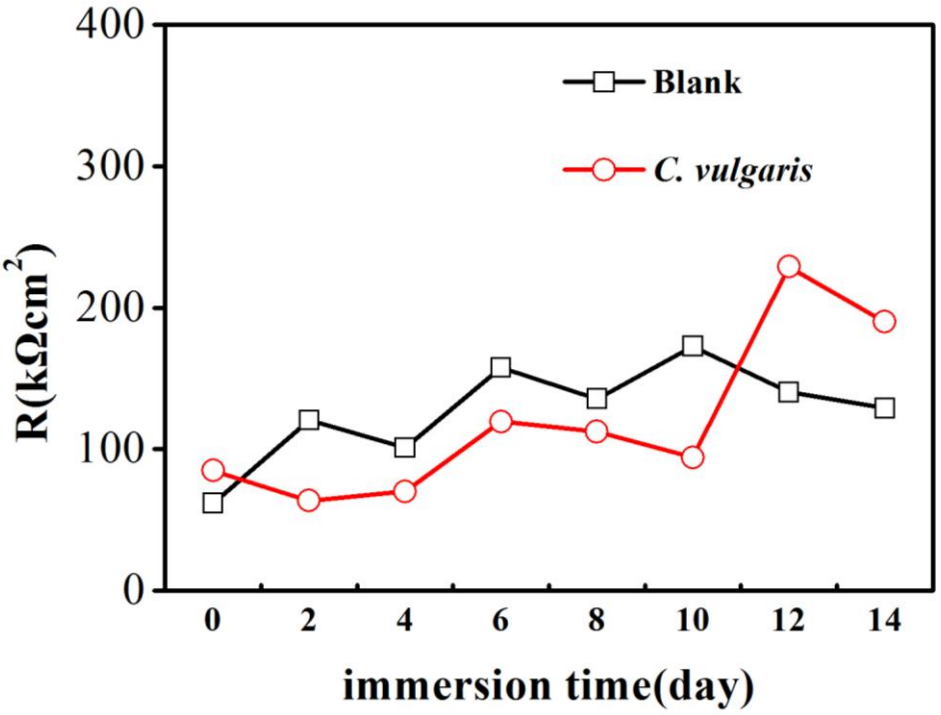

Figure 7. The variations of R_t with immersion time.

Fig. 8 showed the polarization curves of the 316L SS immersed in f/2 culture solutions with and without *C. vulgaris*.

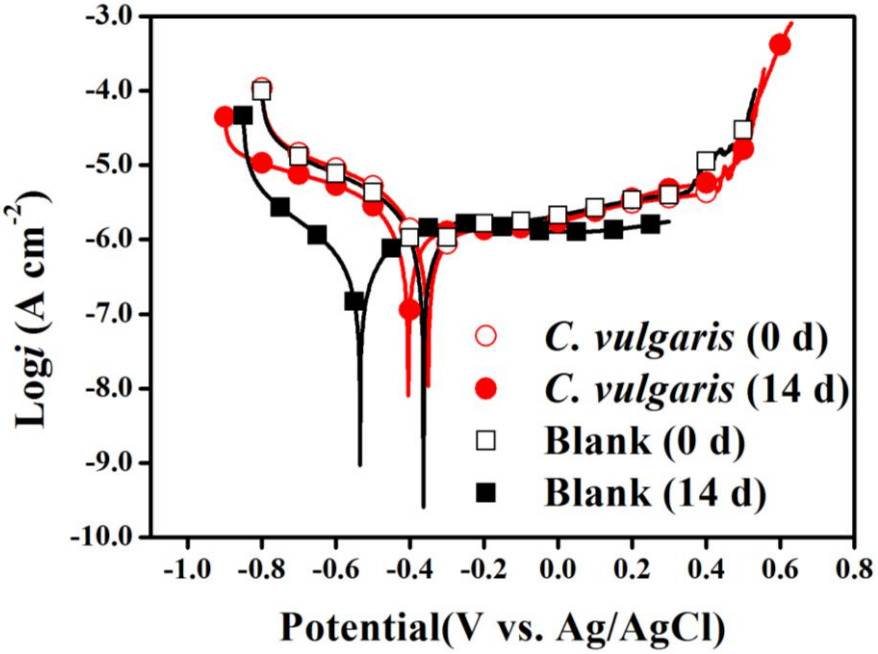

Figure 8. Polarization curves of the 316L SS immersed in f/2 culture solutions with and without *C. vulgaris*.

The polarization curves in culture solutions with and without algal species were qualitatively similar at the first immersion day. After 14 days, there were few changes for the anodic part of the polarization curve of 316L SS immersed in two kinds of culture solutions. The polarization curve in culture solutions without algal species displaced to more negative potentials by about 100 mV. Compared with this, the polarization curve displaced slightly, and showed a different cathodic current. All these features indicated that addition of the microalgae to the culture media could reduce the corrosion rate of 316L SS.

3.3 Influence of Oxygen

Oxygen was considered to be an important parameter for influence of photosynthetic organism on the electrochemical behavior of SS. Considering the polarization curves (Fig. 8), it was referred that the oxygen played an importance role in this process. In order to improve this, the environmental oxygen concentration was determined with real-time during the whole immersion time. The variation of dissolved oxygen in the culture solutions with *C. vulgaris* with culture time was shown in Fig. 9. The concentration of dissolved oxygen in the first culture days was very low, about 4 mg L^{-1}, which was lower than a saturation concentration at this temperature. This was because the seawater used for the culture solutions was sterilized at 121 ºC for 30 min. It began to increase with culture time after four days. This was resulted from the growth of *C. vulgaris* with culture time. Moreover, the concentration of dissolved oxygen oscillated with light similar with the variation of the E_{oc} of the 316L SS with immersion time. With one way ANOVA analysis, the E_{oc} of the 316L SS had a good linear relationship with the concentration of dissolved oxygen (F test, $P<0.01$).

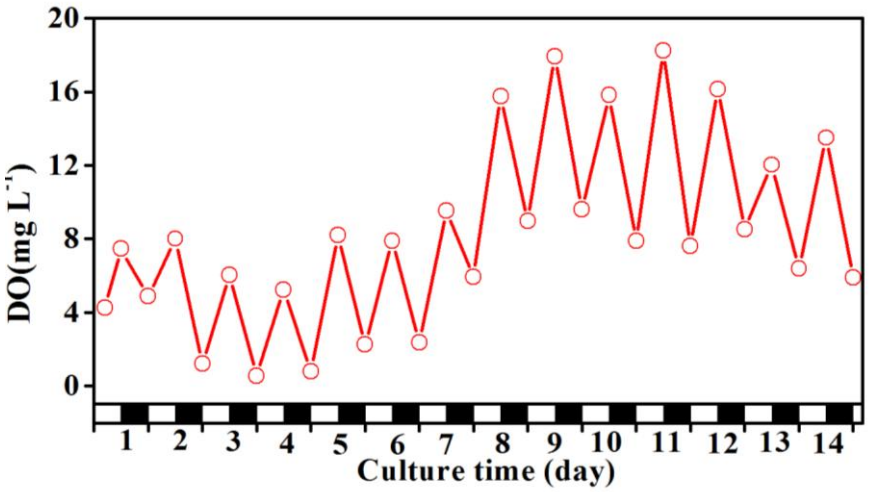

Figure 9. The variation of dissolved oxygen in the culture solutions with *C. vulgaris* with culture time. (X axis: Blank means light period; Dark means dark period.)

For a further study, the variation of concentration of dissolved oxygen in culture solutions with *C. vulgaris* was mimicked by blowing oxygen and nitrogen smoothly into solution with tubes close to

the inner side of the vessel. The results were shown in Fig. 10. During the process of blowing air, the solution stirring caused by the air bubble moving upward to fluid level was ignored because of blowing slowly enough and the far distance between the tube and working electrode. It was obvious that the OCP values decreased sharply from -0.17 V to -0.26 V with the increase of concentration of nitrogen. It could increase to the initial stage with the increase of concentration of oxygen.

The pH in the culture solutions in this study was about 8.17 and it kept nearly stable during the whole process. At the medium of this pH, the predominant cathodic reaction of surfaces in oxygenated media was oxygen reduction:

$$O_2+2H_2O+4e^- \longrightarrow 4OH^-. \quad (2)$$

According to the Nernst equation the reversible potential (E^0) for the oxygen reduction reaction was given by

$$E=E^0(O_2/OH^-)+RT/(nF)\ln pO_2/(OH^-)^4, \quad n=4 \quad (3)$$

Where, R, F were both constant, and T was 293K in this study. Therefore, the theoretical ΔE could be calculated. However, the result calculated seemed not enough to cause such a big variation of E_{oc} of the 316L SS. We presumed that the introduction of oxygen was a complex process for the SS because it could also change the surface state of passive film. The higher oxygen in solution most likely resulted in more oxygen vacancies in the surface of passive film. Such vacancies served as a kind of trap accumulating negative charges in the surface of passive film, which caused the more negative potential drop in Helmholtz double layers. Thus, the OCP values of 316L SS decreased with the dissolved oxygen. This had been improved by other literature [23].

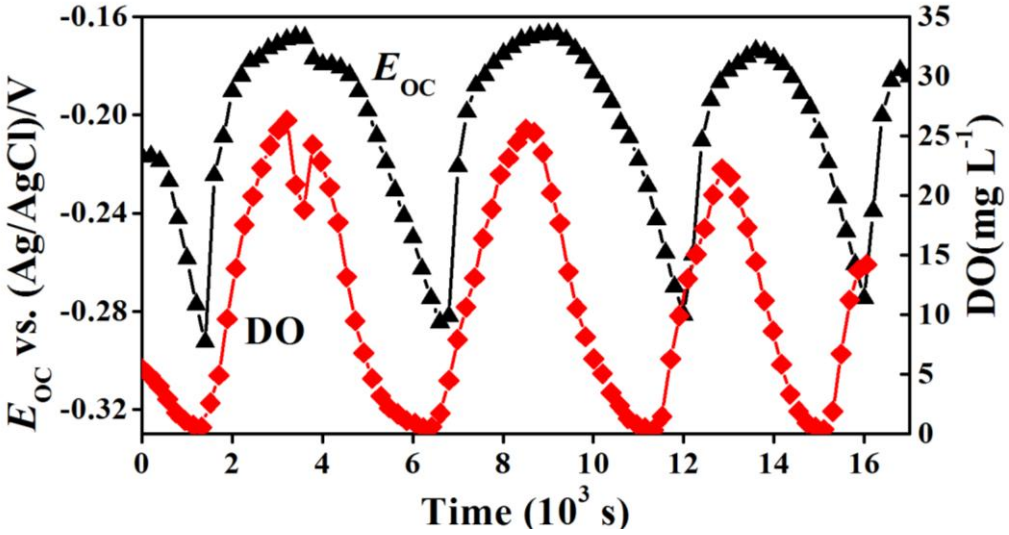

Figure 10. The variation of E_{oc} of the 316L SS and dissolved oxygen in culture solutions with immersion time.

The mimicked experiment improved the positive correlation between the concentration of environmental oxygen and the E_{oc} of the 316L SS immersed in the aquatic environment. This could explain most variation of E_{oc} of the 316L SS immersed in the culture solution with *C. vulgaris*. During the last two days in this study, the E_{oc} of the 316L SS immersed in the culture solution with *C. vulgaris* kept an increasing tendency while the concentration of oxygen began to show a slight decreasing tendency. This could not be explained by the above experiments. At the last two days, parts of algal species began to descent, and some compounds within the algal species began to flow out of the algal cells. High content of some compounds, e.g. β-carotene, which had been improved to have a corrosion inhibit efficiency [22], played important roles during this period of time.

3.4 Influence of sessile C. vulgaris

As for the influence of microalgae on the electrochemical behavior of specimen, many researchers referred that the sessile algae played the most important roles [4, 14]. These literatures showed that the photosynthetic activity of sessile microalgae could influence the electrochemical behavior of substratum by producing oxygen or an exoelectrogenic activity.

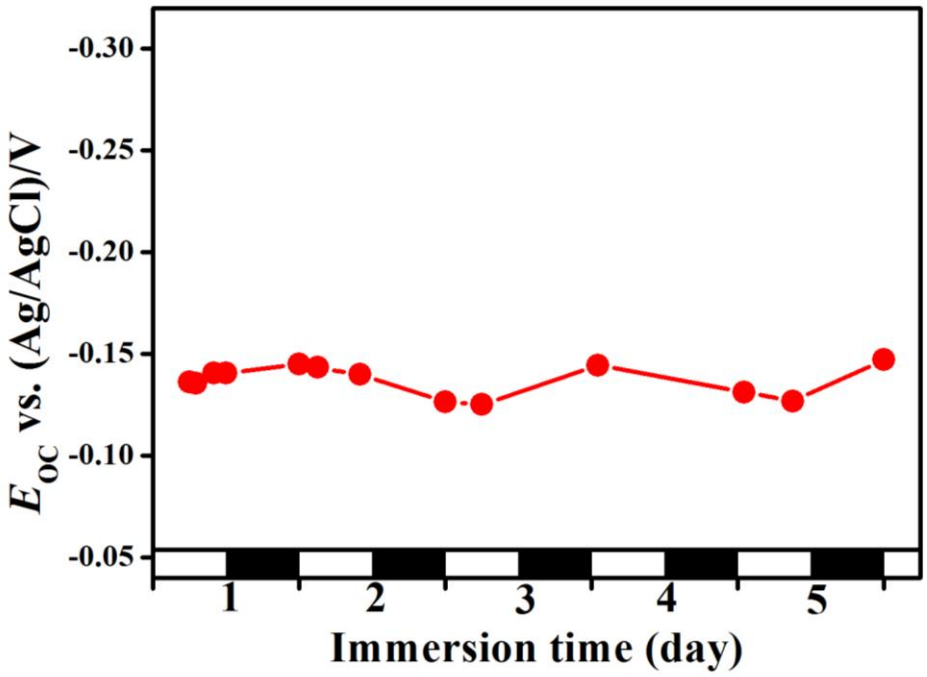

Figure 11. The variation of open circuit potential (E_{OC}) of 316L specimen immersed in f/2 culture solutions without *C. vulgaris* with immersion time. (X axis: Blank means light period; Dark means dark period.)

These processes were expected because microbial biofilms were naturally occurring cell aggregations that had been shown to produce current in microbe fuel cells in the absence of mediators

[24]. In order to evaluate the influence of the sessile microalgae on the electrochemical behavior of 316L SS in this study, an electrode of 316L SS with an adhesion density of 5×10^4 cell cm^{-2} of *C. vulgaris* was introduced to a fresh f/2 medium without algal species. The E_{oc} of this electrode was determined in the following five days under a 12:12 light:dark cycle with an irradiance of 6000 lux during the light period (Fig. 11). The oscillation of E_{oc} of 316L SS in culture solutions with *C. vulgaris* with immersion time depended on light was not seen in this process. Therefore, it was presumed the influence of *C. vulgaris* on 316L SS in this study was conducted mostly by the planktonic algae. This was possibly because the less adhesion density on the surface of SS. We thought it was necessary to improve the adhesion density of microalgae in order to construct a high efficient microbe fuel cell.

4. SUMMARY

The present study showed that the *C. vulgaris*, which was a common member of biofilm on the surface of substratum immersed in seawater, had an inhibit efficiency for SS corrosion as inferred from OCP, EIS measurements and polarization scans. This was conducted by the influence of oxygen produced by the photosynthetic activity of planktonic algae on the passive film of SS. The influence of sessile algae on the electrochemical behavior of 316L SS was not obvious during a short immersion time. This study provided knowledge on the influence of microalgae on the corrosion of SS, which was important to interpret MIC process in situ for further.

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References

1. A. Pardo, M. C. Merino, A. E. Coy, F. Viejo, R. Arrabal and E. Matykina, *Corros. Sci.*, 50 (2008) 1796
2. B. Little, P. Wagner and F. Mansfeld, *Electrochim. Acta*, 37 (1992) 2185
3. R. A. Lane, *Journal of Failure Analysis an.*, 5 (2005) 33
4. N. J. E. Dowling, J. Guezennec, J. Bullen, B. Little and D. C. White, *Biofouling*, 5 (1992) 315
5. G. G. Geesey, R. J. Gillis, R. Avci, D. Daly, M. Hamilton, P. Shope and G. Harkin, *Corros. Sci.*, 38 (1996) 73
6. M. Geiser, R. Avci and Z. Lewandowski, *Int. Biodeterior. Biodegrad.*, 49 (2002) 235
7. L. R. Hilbert, D. Bagge-Ravn, J. Kold and L. Gram, *Int. Biodeterior. Biodegrad.*, 52 (2003) 175
8. C. M. Xu, Y. H. Zhang, G. X. Cheng and W. S. Zhu, *Mater. Sci. Eng. A*, 443 (2007) 235
9. M. Moradi, J. Duan, H. Ashassi-Sorkhabi and X. Luan, *Corros. Sci.*, 53 (2011) 4282
10. Y. Djemai-Zoghlache, A. Isambert and N. Belhaneche-Bensemra, *J. Ind. Microbiol. Biotechnol.*, 38 (2011) 1969
11. B. Little, J. S. Lee and R. I. Ray, *Electrochim. Acta*, 54 (2008) 2
12. J. Landoulsi, K. E. Cooksey and V. Dupres, *Biofouling*, 27 (2011) 1105
13. J. M. Pisciotta, Y. Zou and I. V. Baskakov, *PLoS ONE*, 5 (2010) e10821
14. A. J. McCormick, P. Bombelli, A.M. Scott, A. J. Philips, A. G. Smith, A. C. Fisher and C. J. Howe, *Energ. Environ. Sci.*, 4 (2011) 4699

15. C. Marconnet, C. Dagbert, M. Roy and D. Fe′ron, *Corros. Sci.*, 50 (2008) 2342
16. J. Liao, H. Fukui, T. Urakami and H. Morisaki, *Corros. Sci.*, 52 (2010) 1393
17. R. Sekar, V. P. Venugopalan, K. K. Satpathy, K. V. K. Nair and V. N. R. Rao, *Hydrobiologia*, 512 (2004) 109
18. T. E. Irving and D. G. Allen, *Appl. Microbio. Biotechnol.*, 92 (2011) 283
19. S. Cao, J. D. Wang and D. R. Chen, *Microb. Ecol.*, 62 (2011) 931
20. B. Erable, I. Vandecandelaere, M. Faimali, M. L. Delia, L. Etcheverry, P. Vandamme and A. Bergel, *Bioelecchem.*, 78 (2010) 51
21. M. Da Cunha Belo, M. Walls, N. E. Hakiki, J. Corset, E. Picquenard, G. Sagon and D. Noël, *Corros. Sci.*, 40 (1998) 447
22. F. E. Heakal, M. M. Hefny and A. M. A. El-Tawab, *J. Alloys Compd.*, 491 (2010) 636
23. Z. C. Feng, X. Q. Cheng, C. F. Dong, L. Xu and X. G. Li, *J. Nucl. Mater.*, 407 (2010) 171
24. A. E. Franks, N. Malvankar and K. P. Nevin, *Biofuels* 1 (2010) 589