Int. J. Electrochem. Sci., 7 **(2012)** 5331 - 5338

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Technical Report

Experimental Study on Hypochlorous Acid Blocking the Marine Diatom Adhesion

Shuangkou Chen [1], *Janfang Zhu*[1,*], *Bochu Wang*[2], *Huoan Liu*[1], *Bai He*[1]

[1] College of Chemistry and Chem-engineering, Chongqing University of Science and Technology, Chongqing 401331, China;
[2] Bioengineering College, Chongqing University, Chongqing 400044, China.
[*]E-mail: cqzhujianfang@sina.com

Received: 11 April 2012 / *Accepted:* 16 May 2012 / *Published:* 1 June 2012

We investigated the effect of hypochlorous acid (HOCl) on biofouling diatom adhesion by using sea-water antifouling electrolysis method. Results revealed that the diatom killing effect of HOCl generated by electrolysis device was positively correlated with the applied current density and electrolysis time, negative correlated with diatom concentration. When the current density was 20mA/cm2 and treatment time was 4min, the diatom-killing percentage reached 94.57%, indicating the successful killing of diatoms by HOCl, which subsequently blocked the diatom adhesion from its root. FDA fluorescent staining of the HOCl treated diatom showed faint red fluorescence of the treated cells, suggesting diatom cell membrane damage after treatment, which could be part of the HClO adhesion blocking mechanism.

Keywords: HOCl; antifouling; marine diatom

1. INTRODUCTION

Many marine devices often have biofilm forming on the submerge surface area which is mostly made up of marine bacteria and diatoms [1]. Horbund et al. recommended to separate the bacteria biofilm from the diatom layer, and they reported that large organisms preferentially adhere to surfaces with diatom layers [2]. The diatom layers not only provides the anchor point for the planktonic larva of adhesive organisms, but can serve as baits for barnacles, mussels, larva and adults of zooplanktons as well. Meanwhile layers can decompose organic matters and increase concentrations of CO_2 and NH_3 required for diatom growth. In return, these diatom become the food source of adhesive animals [3]. So the diatom layers play a key role in the adhesion of large organisms.

Moreover, the marine organisms often secret special proteins with super adhesion properties, known as mussel adhesive protein-Maps [4,5,6], which could tightly adhere to any kinds of materials,

such as the surface of a moving ship and petroleum pipelines in the ocean. These attachments would cause a loss of efficiency or cause destructions and then the marine bio-fouling happened [7].

In order to hinder marine fouling, researchers have developed a new technology by using sea water electrolysis equipment [8,9], which could generate hypochlorous acid (HOCl) and if HClO's concentration in the sea water is between 0.1 and 0.3 mg/L [8], it could block or inhibit the growth of marine organisms and prevents their adhesion. Relevant theoretical investigations also demonstrated that after HOCl water solution modification [10,11,12], the adhesion strength between marine organism and surface would be reduced.

However, the detailed experimental effect of HOCl on blocking adhesion had never been fully explored. Since diatoms had an important function to the adhesion, we reported a study on hypochlorous acid blocking the marine diatom adhesion. Some common diatoms, such as *Navicula sp.*, *Achnanthes sp.*, *Nitzschia sp.* and *Melosira sp.*, have the ability of excreting mucus and adhesion to available surfaces. But *Navicula sp.* is the major biofilm forming species. Therefore we selected *Navicula sp.* as target organism and carried on laboratory culturing and HOCl blocking adhesion experiments.

2. EXPERIMENTAL

2.1. Diatom culture

The benthic diatom, *Navicula sp.* (*Navicula incerta*, No. 371), was provided by Diatom Culture Bank of Aquatic Biology Research Institute, Chinese Academy of Sciences. The diatom nutrient medium was based on the f/2 [13] recipe. The prepared medium was autoclaved at 121℃ for 20min. Vitamins were filter sterilized and added to the autoclaved medium after it has cooled down. The diatom culture was incubated in a climatic cabinate with a light intensity of 3000 lx, cycle of 14:10 and temperature of 20±1℃.

2.2. HClO production system

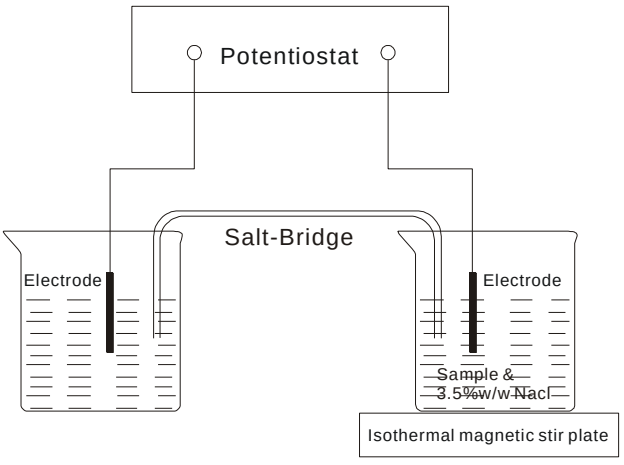


Figure 1. Schematic diagram of the micro-electrolysis device

The HOCl producing system is mainly made up of a potentiostat and a self-made working electrode. This device (as shown in Figure 1) was placed on an isothermal magnetic stir plate during the experiment.

2.3. Sample analysis

Diatoms on the plates were thorough washed for several times with 30 mL washing buffer to remove all diatoms. Absorbances of the obtained diatom suspensions were then measured with a spectrophotometer following the acetone method [14]. The steps are as followings: adding 1 to 2 drops of magnesium carbonate suspension into each 10mL sample, then take some samples to filter by using microporous membrane (0.45μm), put microporous membrane as well as these filtrate attached on the membrane into a centrifuge tube, and then add the 10mL acetone (90%), vibration sometime, put into the refrigerator (4℃) for about 24h in order to extract chlorophyll *a*; after the settlement, put the sample in the centrifuge (4000r/min speed, 15min). take the supernatant, measure the absorbance at the wavelength of 750nm,663nm,645nm and 630nm (90% acetone as reference). The content of Chlorophyll *a* is calculated as follows:

$$\text{Chl-a}(\mu\text{g/L}) = (11.64*\text{OD663} - 2.16*\text{OD645} + 0.1*\text{OD630})*\frac{V_{acetone}}{V_{water}*\delta}$$

Where, $OD_L = E_L - E_{750}$, E_L is the absorbance when the wavelength is L (nm), $V_{acetone}$ is the volume of extracted liquid by using acetone (mL) , V_{water} is the water filtration volume (L), and δ is the optical path of cuvette (cm).

The adhesion blocking percentage was calculated with the previously mentioned equation to reflect the effect of treatment on diatom adhesion. Additionally, diatom suspension (30 mL) of the same concentration from each sample was treated with HOCl and incubated at 20℃ for 24 h at a condition of light: darkness of 14h:10h, light intensity of approximate 3000 lx for measurement of chlorophyll *a* content.

3. RESULTS AND DISCUSSION

3.1. Concentration of HClO in test solution

We measured the concentration of HOCl in 3.5 wt % NaCl solution (100 ml volume) by HClO Rapid Tester (Type S-CL501,QingShiJie Technology Co., Ltd, Shenzhen). As the instrument range limits was (0-2mg / L), the HOCl concentrations were measured under a lower current density and electrolysis time and the date were showed in Table 1. When the current density was low (1mA/cm^2), the concentrations of HOCl were not observed to be significant with increasing electrolysis time. While the current density increased, the concentrations of HOCl were observed to be notable with increasing electrolysis time. At the same electrolysis time, the concentrations of HOCl were observed to be notable with the current density raised. From the table we could find that in order to obtain a higher HOCl concentration, the Electrolysis time and current density should be greater than 4 minutes and 5mA/cm^2.

Table1. Concentration of HOCl under different electrolysis time and current density

Time	Current Density			
	1A/cm2	5A/cm2	10A/cm2	15A/cm2
2mins	0.01mg/L	0.02mg/L	0.54mg/L	0.64mg/L
4mins	0.02mg/L	0.77mg/L	1.38mg/L	1.78 g/L
6mins	0.03mg/L	1.41mg/L	-	-
8mins	0.05mg/L	-	-	-

3.2. Electrolysis time on the killing effect of diatoms

As mentioned before, when current density was greater than 5mA/cm^2, a higher HOCl concentration could be obtained. So we kept the current density (i＝10 mA/cm^2) was the same. When diatom concentration was also the same, diatom-killing percentage increased when more time was allowed for micro-electrolysis to produce HOCl. The diatom-killing percentage increased rapidly from 40.51% at 2 min to 91.42% at 4 min. After the time was greater than 10 min, the diatom-killing percentage remained stable, good antifouling effect could be gained.

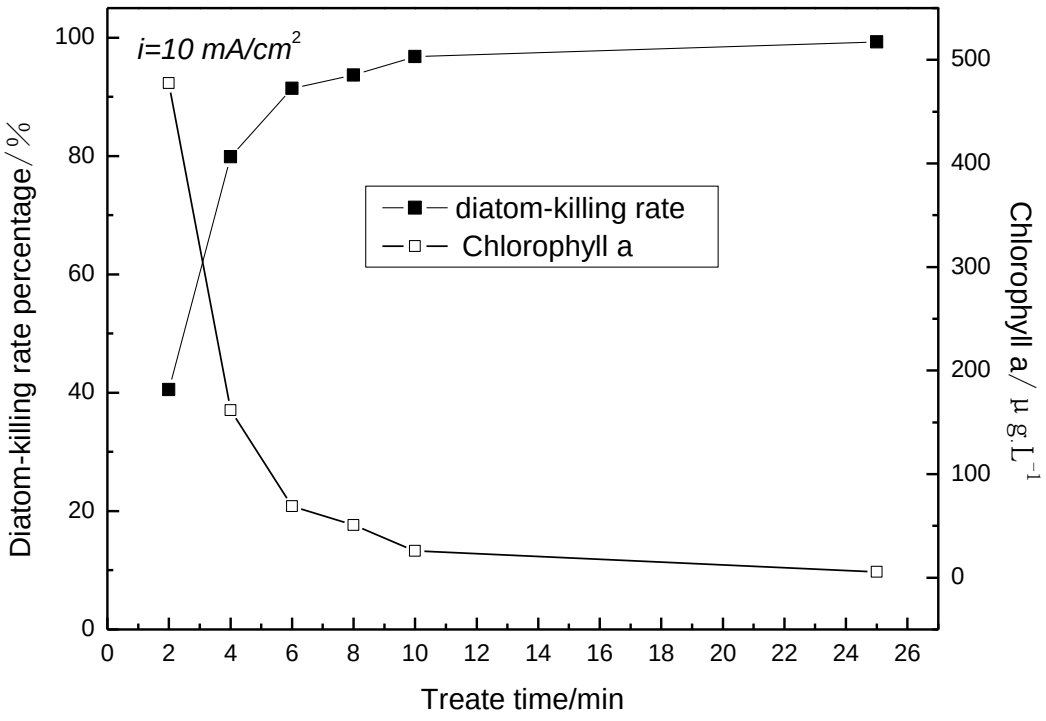


Figure 2. Effect of electrolysis time on diatom-killing rate

3.3. Current density on the killing effect of diatoms

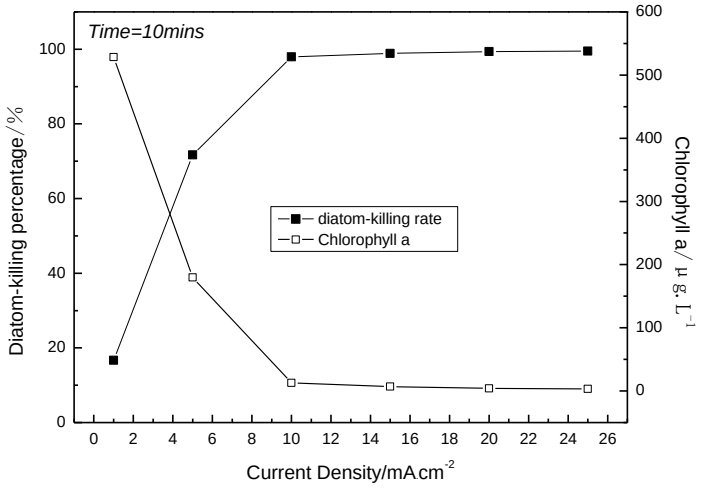


Figure 3. Effect of current density on diatom-killing rate

As seen in Figure 3, when diatom concentration and electrolysis time (10 min) was held constant, diatom-killing by HOCl produced from micro-electrolysis increased as the current density increased. Chlorophyll *a* values of the remaining cells decreased as the current density increased. When the current density was 1-10 mA/cm^2, the diatom-killing percentage increased from 16.67% to 97.98%. When the current density exceeded 10 mA/cm^2, the killing percentage was more than 98%, with almost all diatoms being killed.

When electrolysis time was the same, with the increasing of current density, more diatoms were killed (Figure 4). In addition, under different current density, the diatom killing percentage trend did not varied, i.e., the killing percentage presented more rapid rising in the first 6 min. When the current density was 10 mA/cm^2, and the electrolysis time was 4 min, diatom-killing could reach 79.86%. However, when the current density was 20 mA/cm^2, and the treatment time was 4 min, diatom-killing percentage reached 94.57%.

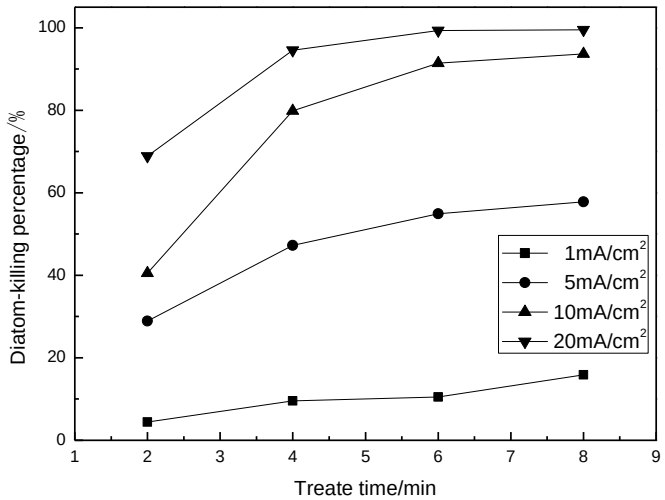


Figure 4. Effect of current density and electrolysis time on diatom-killing rate

3.4. Diatom concentration on diatom-killing rate

In Figure 5, the initial chlorophyll *a* concentration was 678.05 μg/L for diatom culture A and 244.24 μg/L for diatom culture B.

It demonstrated that when current density (i= 5 mA/cm^2) and electrolysis time kept constant, diatom concentration could significantly affect the diatom-killing rate. After 6 min of electrolysis, culture B reached 94.49% of killing rate, while the diatom-killing percentage of culture A was only 30.04%.

These results suggested that HOCl could effectively kill diatoms, therefore blocking their adhesion from the root. The diatom-killing effect of HClO is positive correlation with the electrolysis time and current density, but negatively correlated with initial diatom concentration.

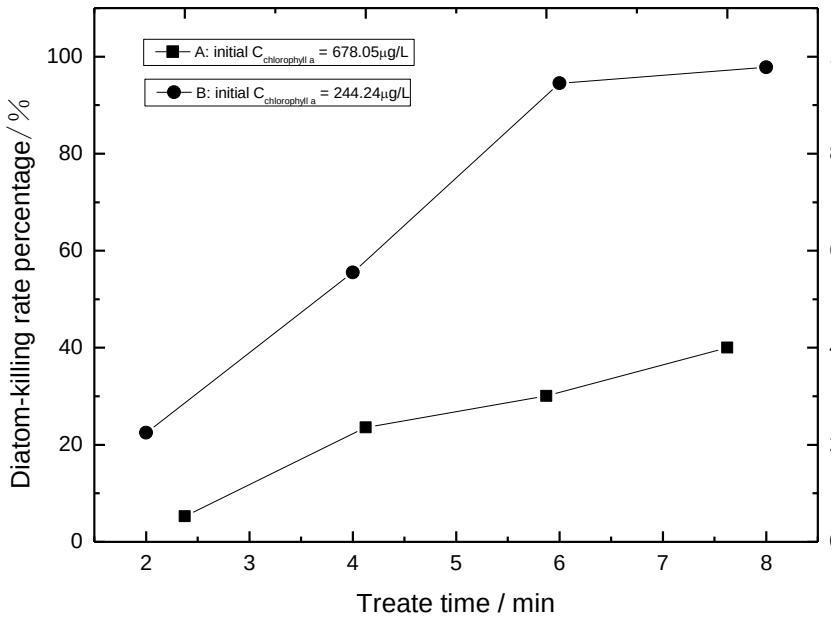
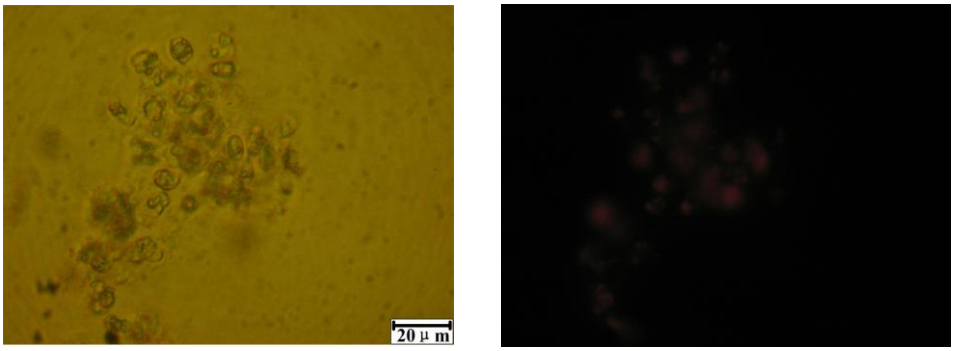


Figure 5. Effect of diatom concentration and electrolysis time on diatom-killing rate

3.5 FDA fluorescent staining

Fluorescein Diacetate (FDA) is a fat-soluble molecule that does not fluorese itself. FDA can diffuse into cells and get hydrolyzed by esterase to produce fluorescein. When the sample istreated with FDA, viable cells with complete cell membrane could accumulate fluorescein and emit yellowish green fluorescence. If cell membranes were damaged, fluorescein could no longer accumulate in cells, therefore cells could not emit fluorescence. However, chlorophylls in the cell could give out red fluorescence if they are not completely decomposed. As shown in Figure 6.(b), the HOCl treated cells emitted faint red fluorescence, suggesting damaged cell membrane of the diatoms after HOCl treatment from electrolysis.



(a) Light microscopy **(b)**. Fluorescent Microscopy.

Figure 6. Florescent staining of FDA.

4. CONCLUSIONS

The potential of the adhesion blocking effect of HOCl on *Navicula sp.* diatom has been investigated. Data demonstrated that the diatom-killing effect of HOCl was positivley correlated with the exerting current density and treatment time, negatively correlated with initial diatom concentration. When current density was 20 mA/cm^2 and electrolysis time was 4 min, the killing percentage reached 94.57%, indicating a successful diatom-killing by HOCl, thereby blocking the adhesion from its root. FDA fluorescent staining of the HOCl treated diatoms showed that these diatoms could emit faint red fluorescence, suggesting cell membrane damage after HOCl treatment. Therefore, it is likely that HOCl blocks microbial adhesion by penetrating through the cell wall, impairing cell membrane and oxidizing its enzymatic system to cause loss of enzymatic activity. When this happens, protein, RNA, DNA and other macromolecules would be released to the environment, leading to microbial death.

ACKNOWLEDGEMENTS
This work was supported by Natural Science Foundation Project of CQ CSTC(No.2010BB5297) and Research Foundation of CQUST (No.CK2010B16).

References

1. Q. Wang, *Shanghai Coating* 1(2000)20-23.
2. H.M. Horbund, A. Freiberger, *Ocean Eng*.1(1970)631-634.
3. D.M. Yebra, S. Kiil, K.D. Johansen, *Prog. Org. Coat.* 50(2004)75-104.
4. H. Lee, N.F. Scherer, P.B. Messersmith, *PNAS* 103(2006)12999-13003.
5. J.L. Dalsin, B.H. Hu, B.P. Lee, *Am. Chem. Soc.* 125(2003)4253–4258.
6. A.M. Baty; P.A. Suci; B.J.Tyler, G.G.Geesey,*Colloid. Interface. Sci.* 177(1996)307-315.
7. W. Maja, W. Burkard, *J. Adhes. Sci. Technol.* 17(2003)1957-1977.
8. W.L. Hu, L.L. Wu, J. Zhou, J.X. Li, *J. Electrochem*, 5(1999)299-303.
9. H.A. Liu; S.K. Chen, *Advanced Materials Research* 345(2011)189-194.
10. S.K. Chen, B.C.Wang, T.G.Zhou, *Colloids and Surfaces B: Biointerfaces* 70(2009)243-247.
11. S.K. Chen, B.C.Wang, T.G.Zhou, *Journal of Molecular Science*, 24(2008) 42-45.

12. S.K. Chen, B.C.Wang, T.G.Zhou, *Computer and Applied Chemistry*, 26(2009)917-920.
13. R.L. Guillard, J.H. Ryther, *J. Microbiol*, 8 (1962)229-239.
14. D.I. Arnon, P*lant Physiology*, 24(1949)1-15.