Electrochimica Acta 54 (2009) 5598–5604

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

The influence of local glucose oxidase activity on the potential/current distribution on stainless steel: A study by the wire beam electrode method

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ARTICLE INFO

Article history:
Received 19 December 2008
Received in revised form 18 April 2009
Accepted 25 April 2009
Available online 3 May 2009

Keywords:
Wire beam electrode
Glucose oxidase
Potential/current distribution
Hydrogen peroxide
Biofilm

ABSTRACT

The wire beam electrode (WBE) method was first used to study the activity of local glucose oxidase (GOD) on stainless steel surface in seawater. Glucose oxidase was immobilized in calcium alginate gel capsules, which were embedded in a layer of artificial biofilm (calcium alginate gel) on the WBE surface. The potential/current distributions on the WBE surface were mapped using a newly developed device for the WBE method in our lab. The results demonstrated that the catalysis of H_2O_2 formation by GOD can produce local noble potential peaks and cathodic current zones on the stainless steel surface. An interesting fluctuant current distribution around cathodic zones was observed the first time. The potential and current maps showed that the enzyme heterogeneity of the artificial biofilm caused a corresponding electrochemical heterogeneity at the biofilm/metal interface. The application of the WBE method to ennoblement study enables us to observe the heterogeneous electrochemistry at biofilm/stainless steel interface directly, providing us with a powerful tool to investigate other biofilm-related processes such as microbially influenced corrosion (MIC).

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1. Introduction

"Enzymatic mechanism" is one of the mechanisms that has been proposed to explain the electrochemical parameters changes, e.g., open circuit potential (OCP) noble shift (ennoblement), cathodic current increase, after biofilms colonization on the passive metals in seawater [1]. Glucose oxidase (GOD) is one of the enzymes produced by microorganisms, which has been detected in natural marine biofilm [2]. GOD has been particularly used in microbially influenced corrosion (MIC) and ennoblement studies [3–6]. GOD can catalyze the oxidation of glucose to form hydrogen peroxide (H_2O_2) and gluconic acid according to the following reaction [5]:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \quad (1)$$

H_2O_2 has also been detected in natural marine biofilm [7]. The high standard potential of H_2O_2 ($E^0(H_2O_2/H_2O)$) is believed to be a main reason for the ennoblement and cathodic current increase after marine biofilm formation on passive metal surface [8].

In the past decades, conventional electrochemical techniques, such as open circuit potential measurement, polarization curves, electrochemical impedance spectroscopy (EIS), cyclic voltammetry, Mott-Schottky measurement, have been widely applied to study the ennoblement mechanism [9–12,3,13–17]. Although having been proven very useful, these techniques have a common limitation where only average electrochemical information can be obtained and the spatial electrochemical information, which is believed to be the essential electrochemical parameters at biofilm/metal interface, is hard to investigate. One basic characteristic of bacterial and biofilm adhesion is their heterogeneous spatial distribution on metal surface, which can lead to electrochemistry heterogeneity and induce or accelerate localized corrosion. Another concern is the change of non-uniform electrochemical distribution with time progression during which the growth and reproduction of microorganisms vary greatly [18]. A better understanding of the spatial relationship between heterogeneous biofilms and local electrochemical information can allow further insights into the classical electrochemical information obtained and possibly into some new micro-scale electrochemical mechanisms from a view of distribution.

Local electrochemical techniques are critical to measure the heterogeneous electrochemistry at the biofilm/metal interface. To date, several local electrochemical techniques have been used for this purpose. The application of scanning electrochemical microscopy (SECM) for the study of enzymes in biological system drew considerable attention recently [19,20]. Lewandowski's group used the scanning vibrating electrode technique (SVET) to map the electrochemistry of metal surfaces covered by single-species biofilms or by abiotic biofilm matrix analogs (polysaccharides) [21]. The relation-

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0013-4686/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2009.04.064

ship between *Oceanospirillum* biofilms and anodic or cathodic zones on copper was investigated with the SVET [22]. More recently, the SVET was used to investigate the influence of an enzyme (hemin) on the local electrochemical behaviour of stainless steel in an aerobic medium [23]. The results showed that hemin deposited on the electrode surface led to a local increase in the cathodic current, demonstrating the contribution of the enzyme to the creation of galvanic cells in biofilms.

The WBE method has been proven to be a new powerful tool to investigate the electrochemical behaviour at metal/biofilm interface in our lab [18]. In that work, chemically added H_2O_2 (30 ppm and about 0.05 ml) in the simulated biofilm (Ca alginate gel, the same as that in this test) was used to mimic the ennoble micro-environment in biofilm. The potential and current distributions were mapped after injecting H_2O_2 solution into the artificial biofilm on the WBE immersed in seawater. The WBE method has been applied to investigate many types localized corrosion [24–30]. One advantage of this technique is that this method can directly measure the potential distribution and electronic currents flowing in the metallic phase. Another advantage is that the test can be easily performed when a thick coating or artificial biofilm is deposited on the WBE surface. This is in contrast to other local techniques such as scanning probe techniques because they require extra attention when performed on thick simulated biofilms. Therefore, the WBE method is potentially a powerful tool to investigate the biofilm-related studies.

Furthermore, the WBE method can be regarded as a technique at micro-scale. The resolution of the WBE measurement is lower than that of scanning probe techniques, e.g., the SECM and the SVET. But the key factor determining the resolution of biofilm-related studies using these high resolution techniques is the dimension of micro-environments in the biofilm system designed and investigated, not the resolution of these techniques. On the other hand, the physical, chemical and biological micro-environments in natural biofilms are hard to simulate and from the published literatures we can see that the dimension of the electrochemical heterogeneity at the artificial biofilm/metal interface is usually at a scale of about mm^2 or cm^2. From this point of view, the WBE method can also be deemed as a local technique at micro-scale.

In our lab, the WBE method has been used to investigate the relationship between the local H_2O_2 distribution and the electrochemical heterogeneity on stainless steel surface [18]. In this work, chemically generated H_2O_2 in the simulated biofilm was used to mimic the micro-environments in biofilm. The results showed that the spatial distribution of H_2O_2 can cause a corresponding electrochemical heterogeneity at the biofilm/stainless steel interface. This test model is simple to simulate the complex environment in the natural marine biofilm. Therefore, it is necessary to use an “active” biofilm model in a test to efficiently simulate the natural marine biofilm.

The purpose of this work is to investigate the local H_2O_2 formation by catalysis of GOD capsule embedded in an artificial biofilm and the corresponding heterogeneous electrochemistry at the biofilm/stainless steel interface using the WBE method, a new technique applied in this field. Because natural marine biofilms are very complicated, we have chosen calcium alginate as the matrix of biofilms (artificial biofilm) to define the factors operative in this electrochemical heterogeneity study. GOD was immobilized in calcium alginate gel capsules [31,32], which were covered by the artificial biofilm on the WBE surface. This technique allows the immobilized enzyme to have good storage stability.

2. Experimental

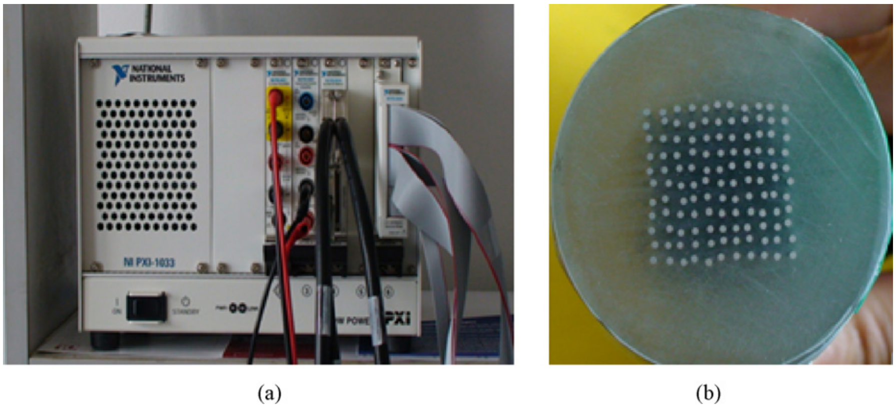
2.1. The device for the WBE method

Fig. 1(a) shows the newly developed device for the WBE method in our lab. This WBE test device consists of NI PXI 1033 chassis and the modular instruments: NI PXI 2535, 4071 and 4022.

(a) PXI-1033: 5-slot PXI chassis with integrated MXI-Express controller.
(b) PXI 4071: 7.5 digit digital multi-meter, voltage measurement range 10 nV–1000 V, input resistance more than 10^{10} Ω.
(c) PXI 4022: high-speed, high-precision guard and current amplifier, ability to detect picoampere current level with femtoampere noise with PXI 4071.
(d) PXI 2535: high-density FET switch matrix module features 544 cross-points, 4 × 136 one-wire matrix configuration (136 channels), switching speed is as high as 50,000 cross-points/s, unlimited simultaneous connection. More channels can be achieved by easy expansion of switch modules. This device is directly controlled by a PC with a transparent, high-speed serial link.

2.2. The WBE fabrication

The WBE was fabricated from 121 stainless steel wires regularly arranged as a 11 × 11 matrix (304 SS, 1 mm diameter), embedded in epoxy resin with an interval of 1 mm from each other, as shown in Fig. 1(b). The chemical composition of the stainless steel: C ≤ 0.08, Si ≤ 1.00, Mn ≤ 2.00, P ≤ 0.035, S ≤ 0.03, Ni 8.00–10.50, Cr 18.00–20.00, Fe balance. The WBE was polished with metallurgical paper up to no.1000 grade, and cleaned with acetone. The WBE was stored in a desiccator until use.



(a) (b)

Fig. 1. Experimental setup of the WBE method (a) and the wire beam electrode (b).

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