Microorganism based biosensor for monitoring of microbiologically influenced corrosion caused by fungal species

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The metallic corrosion by organic acids, produced by microbial activities of microfungi, have been reported by many investigators. Microbial corrosion can be influenced either by aerobic or anaerobic activity which is dependent upon the presence or absence of oxygen in the environment. It has been well-recognized that microfungi produce organic acids such as, acetic acid, citric acid, glutamic acid, carboxylic acid, etc. which create problems in many industries.

Corrosion is influenced by the presence of microorganisms on the metallic surface, through the electrochemically active species secreted by microorganisms in the corrosion process. It is well-known that corrosion reactions are subject to electrochemical polarization phenomenon, which are monitored systematically by electrochemical techniques. However, in the case of microbiologically influenced corrosion (MIC), electrochemical and surface analytical techniques are not adequate in explaining the extent and type of microorganisms involved in the MIC problems. Due to lack of knowledge about exact microbial species involved in microbial corrosion process, the monitoring of MIC is very difficult. The products secreted during MIC are not thoroughly understood. Therefore, the mechanisms involved are still controversial and conflicting about microbes and their metabolic activities.

In view of the above constraints, the online measurement of corrosive species secreted by the fungal species during microbe-metal interaction is rather difficult. In this regard, microbial biosensors are very useful for monitoring different microbial metabolites, which directly participate in MIC. Over the last few years, several biosensors have been developed for the detection of organic substrates. Many techniques have been reported for the immobilization of microorganisms. Enzyme based biosensors are generally specific and more expensive. Many microbial biosensors consist of immobilized microorganisms placed under suitable environment, with electrochemical device and thus, are suitable for online monitoring of biochemical processes.

In the present investigation, a microorganism-based biosensor consisting of immobilized Acetobacter sp. on porous cellulose acetate membrane attached with oxygen electrode has been developed and its application in monitoring of microbiologically influenced corrosion caused by the group of microfungi has been investigated.

Experimental Procedure

Culture of bacteria

The Acetobacter sp. was isolated from the corroded metal surface. Details of isolation and identification
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are given elsewhere\(^8\). These bacteria were used for the construction of microbial biosensor. The bacteria were cultured in growth medium (dextrose, 2; casein, 0.2; \(\text{K}_2\text{HPO}_4\), 0.05; \(\text{FeCl}_3\), 0.06 and \(\text{MgSO}_4\cdot7\text{H}_2\text{O}\), 0.20 g per litre in triple distilled water) under aerobic conditions at pH 6.5-6.8.

**Immobilization of the microorganism**

Fifteen mL of the culture broth of *Acetobacter* sp. grown for 48 h was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded. The pellet was washed three times with sterile triple distilled water, and finally suspended in 5 mL distilled water, and 4 mL of suspension was dripped onto a porous acetylcelullose membrane (Genei Co., 0.45 \(\mu\)m pore size, 4.7 mm diameter, 150 \(\mu\)m, thickness). The microbes were allowed to be retained over the membrane.

**Assembly of the microbial electrode**

The scheme of the microbial biosensor is shown in Fig. 1. The oxygen electrode (Elico Instrumentation Ltd., Hyderabad, India) consisted of gold cathode and a silver anode. The porous acetylcelullose membrane retaining *Acetobacter* sp. was cut into a circular disc and soaked in the alkaline buffer (0.1 M \(\text{KH}_2\text{PO}_4\)-\(\text{NaOH}\) and pH), adjusted to pH 8.2 for better performance. The circular membrane was attached to the cathodic surface of the oxygen probe. The microbe layer was then covered with gas permeable synthetic net and held in place by the help of an O-ring.

**Experimental set-up and method**

The experimental set-up is depicted in Fig. 2. All measurements were performed in 250 mL Borosil glass-cell containing microbial electrode and magnetic stirrer (1000 rpm). The cell was directly attached to interface (Wenking POS-73) and recorder (Servogor XY 733).

The working volume of the solution containing acetic acid was 100 mL and temperature of the cell was maintained by the electronically controlled thermostat at 25±0.5°C. The pH of the solution was adjusted with 0.05 M \(\text{H}_2\text{SO}_4\) and saturated with air. Then, it was added periodically in the cell for measuring the current value.

**Results and Discussion**

**Response of microbial biosensor**

The experiments were performed to investigate the effect of various parameters on the response time. Fig. 3 shows typical response curves of microbial bio-

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*Fig. 1—Scheme of the microorganism-based biosensor*

*Fig. 2—Schematic diagram of the amperometric microbial biosensor*
The steady state of the current revealed, that, the consumption of oxygen by the microorganism and diffusion of the oxygen to the immobilized microbe from the cell containing test solution were in equilibrium state, which depends on the concentration of acetic acid, injected into the cell and the amount of the microorganism immobilized on the membrane surface. The pH of the sample solution containing acetic acid had to be kept below pH value for acetic acid (4.74 at 28°C), because acetic ions cannot pass through the membrane. When microbial biosensor was inserted in distilled water, the sensor returned to its normal state. These results are in good agreement with the observations reported by other workers.

When sample solution was added to test solution during the experiment, a calibration curve was obtained as shown in Fig. 4. A linear relationship was obtained between the concentration of acetic acid and the decrease in current. The total decrease in oxygen current is 0.4 μA and response time is 10 min. The minimum concentration of acetic acid for measurement was 8 mg/L. The sensitivity of microbial biosensor for acetic acid is 0.04 μA/mM. The reproducibility of the current difference was determined under influenced response of the microbial biosensor.

**Monitoring of MIC by microbial biosensor**

The microbiologically influenced corrosion (MIC) is a common problem in many industries. Electrochemical process of corrosion is accelerated by metabolic activities of microorganisms. If biosensors and electrochemical techniques are combined together it can play a significant role for monitoring of MIC. In the case of *Acetobacter* sp. and other microfungi involved in the MIC, microorganisms produce organic acids, which influence corrosion process. According to Coponhegen, most of the organic acids produced by microfungi are more detrimental to metals.

It is well-known that organic acids are more corrosive for all types of metallic structures. When microbial biosensor was inserted into the culture medium contaminated by fungal strains for the determination of organic acids, the response was found to be very good in the presence of fungal species. In other environments (culture medium) affected by bacteria *Pseudomonas* sp., *Thiobacillus* sp., *Desulphovibrio* sp., and nitrifying bacteria, etc., however, the response of biosensor was very poor. The response of microbial biosensor depends on the assimilation of organic acid by immobilized microbes. The results obtained by microbial biosensor and those obtained by conventional methods were found to be in good agreement and were not affected by other metabolic products and organic materials. The selectivity of microbial biosensor was determined. The sensor did not respond to other compounds produced by microorganisms or nutrients present in the environment. Since, the work still is under progress, the detailed data has not been given here. For investigations of actual cause (type of microorganisms), which influences MIC, microbial biosensor may be very helpful. The microbial electrodes are stored below 4°C in order to prolong the life of the electrode for more than two weeks.

**Conclusions**

The amperometric microbial biosensor was developed using immobilized *Acetobacter* sp. The microbial biosensor has good stability, response time, reproducibility and is cheaper than enzyme based bio-
sensors. This may prove to be an ideal analytical tool for online monitoring of MIC. It may be applied for the identification of microorganisms and microbial problems caused by fungal species in many industries.

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