Development of a Sensor for Acetic Acid Based on *Fusarium solani*

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Abstract
Electrochemical biosensors have become very important tools in analytical chemistry because of their advantages like accuracy, high sensitivity and easy handling. This article describes electrochemical detection of acetic acid based on the fungus *Fusarium solani.* The biosensor employed for the study of assimilation of substrates was fabricated by coupling the immobilized membrane with the DO probe using dialysis tubing. A microbial dispersion containing 0.2 g of wet weight of the organisms per mL was used for immobilization upon cellulose nitrate membrane. The membrane retaining the fungus was placed on the Teflon membrane of the oxygen electrode so that the fungus was trapped between the two membranes. The linear range was found to be between 2 and 70 ppm (v/v) of acetic acid. A polypropylene net increases the lifetime of the sensor due to its retention of humidity in the net. By dipping the electrochemical probe onto buffer (pH 7.2), the sensor was found to have an extended lifetime of 120 days with about 800 determinations.

Keywords: Acetic acid, Biosensors, *Fusarium solani*, Isolation, Assimilation

Dedicated to Professor T.V. Subramanian on the Occasion of His Retirement from the Department of Chemical Engineering at Anna University, Chennai, India

1. Introduction

Biosensors are becoming more and more important, taking into consideration the interdisciplinary areas, they are put to use now, like environment, biotechnology, chemical analysis and online measurement of production processes, to name a few. Microbial sensors for monitoring various pollutants have been developed, where the microorganisms are in direct contact with a transducer, converting the biochemical signal into an electrical response. Microbial characterizations have been studied based on the assimilation of substrates, recording the respiratory activity. Microbial sensors comprising immobilized whole cells and an oxygen probe have been used for determination of sugars, acetic acid, alcohol, vitamins, glutamic acid, antibiotics, peptides, enzyme activities, biochemical oxygen demand, cofactors and inhibitors. Assimilation characteristics of various microorganisms such as molds, yeasts, bacteria, actinomycetes and activated sludges have been tested with various substrates and the key metabolites or rate of their overall metabolic reactions ATP, NADH, cytochrome content and DNA, are suitable for describing the physiological state. There have been reports on simultaneous measurement of two parameters, the content of proteins and nucleic acids and the determination of complex variables like biodegradable compounds and mutagenic substances in wastewater. It also has been found that a very low microbial loading of the biosensor is a prerequisite for a kinetically controlled respiration electrode and such sensors coupled with suitable immobilization of the microorganisms as well as thin membranes have to be used. The sensitivity of this type of sensor is mainly determined by the cell activity but not by diffusion limitation and the advantage is that one could attain response times of about 15 s by very low 'microbe loading' of the sensor. A combination of the electrode system with very low amounts of whole cells, resulting in a decrease of diffusion resistance leading to very fast detection of changes in the respiration rates (1–5 s), in contrast to microbial electrodes for assimilation tests, with response times from 5 to 30 min has also been reported. Several sensors for acetate and acetic acid have been developed before. A detailed investigation on the biochemical characteristics of the microorganisms in question is necessary in order to develop a biosensor for enhanced stability and selectivity [1–5].

Acetate sensor based on *Acetobacter* has been reported based on NADH-fluorescence for online monitoring of biotechnological process [6]. Acetate sensor based on *Trichosporon brassicae* had a response time of between 6 and 10 minutes with a stability of 21 days and a range linear up to 22.5 mg/dm^3 [7, 8]. A microbial sensor for acetic acid based on *Trichosporon brassicae* immobilized in acetyl cellulose had a measuring range of 0.08–1.2 $mmol/dm^3$ and a very quick response time of 8 s. It has been reported that this sensor had a precision of 6% and a stability of 21 days [9–11]. A similar sensor was reported to be used for monitoring of glutamic acid fermentation [12, 13]. Recently online process monitoring of acetic acid in *Escherichia coli* cultivation using electrochemical detection in flow injection system was reported, and there have been reports of acetate kinase used for development of such sensors. A comprehensive biochemical characterization of this fungus was done along with voltammetric studies of the fungus [14–21], and it was found that the response for acetic acid for this fungus was very high and hence was studied further for development of sensor for acetic acid. The measurement of acetic acid is important for fermentation process because, during the process of cultivation of microorganisms with acetic acid as the carbon source, it inhibits the growth of the organisms above a certain concentration, and hence it is very important to control the concentrations of acetic acid formed in the production broth. By monitoring the acetic acid concentration, we could prevent the inhibition of growth of organisms in the fermentation broth, aiding in higher production

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of acetic acid in production processes. This article discusses the possible applications of the fungus *Fusarium solani*, which showed very high assimilation for the acetic acid, and hence was further studied for specific assimilation of acetic acid and subsequent development of a sensor for the same.

2. Experimental

2.1. Materials

Potassium dihydrogen orthophosphate was purchased from Ranbaxy Laboratories, Bombay, India, and acetic acid and other chemicals were purchased from Loba Chemie, Bombay, India. All the analyses were done using millipore water.

2.2. Isolation and Identification of the Fungus

The fungus *Fusarium solani,* was isolated from soil sample from a factory in Vellore, Tamil Nadu, India, which manufactured polyurethane. The fungus was identified at Indian Agricultural Research Institute, (IARI), Pusa, New Delhi, India.

2.3. Culture of the Fungus

The fungus was cultured on 12 mL of the slant culture medium in the test tube of size 15 mm × 160 mm at 27 °C for 2 days prior to conducting assimilation tests. The fungus was grown in Czapek-Dox media (KH_2PO_4 (1 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5 g), KCl (0.5 g), $FeSO_4$ (0.01 g), Sucrose (30 g) and Agar (15 g) in 1 liter of distilled water at pH 7.3).

2.4. Maintenance of Cell Temperature

The sensor unit was kept at constant temperature during all the experiments, by passing air through a rotameter dry column before the silicone tube.

2.5. Fabrication of Oxygen Electrode

A dissolved oxygen (DO) probe was fabricated using a gold cathode (area 0.03 cm^2), and a platinum counter electrode. The gas permeable membrane and the dialysis tubing used in the probe were purchased from Century Instruments Co., Chandigarh, India. The biosensor, employed for the assimilation of alcohol was fabricated by coupling the immobilized membrane with the dissolved oxygen (DO) probe using dialysis tubing. Wenking potentiostat Model POS 88 Japan, was used with a Rikadenki *X–Y–t* recorder, Japan.

2.6. Assembly of Microbial Electrode

Cellulose nitrate membrane (pore size <0.25 micron) from Millipore was used as the matrix for immobilization. The fungus was immobilized by physisorption, after which the membrane was washed thoroughly with buffer to remove any unbound fungus on the membrane. A fungal dispersion (harvested during stationary growth phase) containing 0.2 grams of wet weight of the fungus/cm^3 was used for immobilization. The membrane retaining the fungus was placed on the Teflon membrane of the oxygen electrode trapping the fungus between the two membranes. The membranes were fastened with rubber rings. The immobilized membranes were stored at 4 °C in phosphate buffer when not used.

2.7. Assay Procedures

A typical response curve for acetic acid has been given in Figure 1. The initial current at zero time (i_0) was obtained with buffer solution saturated with dissolved oxygen, which indirectly shows the endogenous respiration of the fungus. The assimilable substrate was injected into the system the substrate permeated through the porous nitrocellulose membrane and was assimilated by the immobilized fungus. The enhanced assimilation results in the increase in the respiratory activity of the fungus resulting in the decrease of dissolved oxygen (DO) at the microbial membrane/dissolved oxygen sensor interface. Hence, the current of the electrode decreased markedly with time until a steady state was reached since the respiration of the fungus was activated. The steady state indicated the diffusion of O_2 from a sample solution and O_2 consumption by fungus is in equilibrium. The steady state current depends on numerous factors among others such as concentration of substrate, the assimilation activity of the immobilized fungus and the rate of metabolism (oxidation) of substrate.

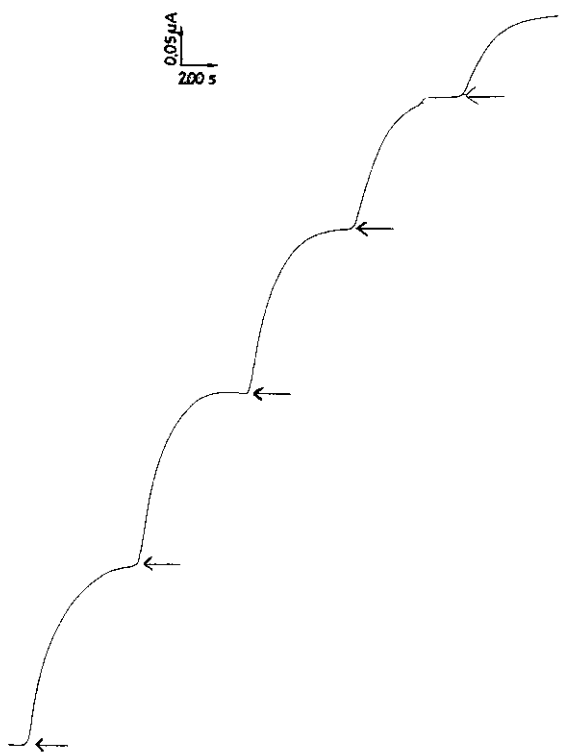

Fig. 1. Response curve for acetic acid. Every stage of the response curve had the following experimental conditions: current 5 µA, concentration of acetic acid used was 20 ppm.

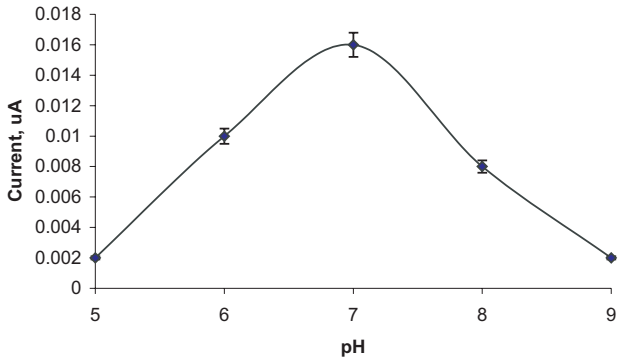

Fig. 2. Effect of pH. Concentration of acetic acid used was 43 ppm. Results portray the average values of three measurements. Error bars show error values of less than 3 %.

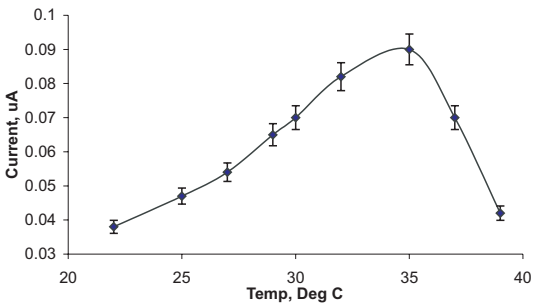

Fig. 3. Effect of temperature. Concentration of acetic acid used was 40 ppm. Results portray the average values of three measurements. Error bars show error values of less than 5 %.

Table 1. Selectivity of the substrates by *Fusarium solani* using the microbial probe. The concentration of all the substrates used for measurement was 50 ppm.

Substrates	*Current (μA)*
Methyl alcohol	20
Ethyl alcohol	120
Propyl alcohol	50
Isobutyl alcohol	80
Isoamyl alcohol	40
Sorbitol	60
Mannitol	40
Glycerol	40
Fumaric acid	24
Citric acid	16
Acetic acid	380
Lactic acid	70
Ascorbic acid	10
Formic acid	30

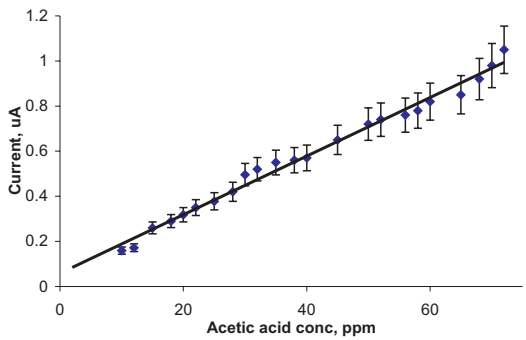

Fig. 4. Calibration plot for acetic acid assimilation by *Fusarium solani.* Error bars show error values of less than 7 %.

3. Results and Discussion

3.1. Effect of pH and Temperature on the Sensor Response

Figures 2 and 3 depict the effect of pH and temperature. The optimal temperature for the growth of the organism was found to be 34 °C and the sensor studies were performed at this temperature. As temperature influences the performance of the sensor, affecting the response, it was kept constant during all the experiments. Death of the fungus was found to be at 47 °C. The optimal pH for the growth of the fungus was found to be pH 7 but the measurements were done below pH 4.75 at 30 °C, which is the pK_a value for acetic acid, because acetate ions cannot pass through the oxygen permeating membrane. Buffer adjusted to 3.5 with 0.05 M sulfuric acid was used to adjust the pH of the experimental sample.

3.2. Performance of the Microbial Sensor

Table 1 reports on the selectivity of various substrates. In terms of selectivity, the sensor response was weak for volatile compounds like methanol and formic acid. The sensor response was relatively high for ethanol. Though the responses for ethanol, sorbitol, propyl alcohol and formic acid are high, these analytes are fortunately not present in fermentation. The sample mixtures were not studied for their response, as we propose this sensor for monitoring acetic acid levels in fermentation, because high levels of acetic acid inhibits organisms growth. The current responses obtained had a very good linearity with acetic acid concentrations in the samples as can be seen in the Figure 4.

3.3. Lifetime of the Microbial Sensor

The polypropylene net increases the lifetime (the time period during which the fungus was active without any decrease in either selectivity or sensitivity to a considerable extent) of the sensor due to its retention of humidity in the net that is essential to the life of the organism. By dipping the assembly with the fungus onto a buffer (pH 7.2), when the system is not in use, the sensor was found to be having an extended lifetime of 120 days with about 800 determinations. The sensor was reproducible during this lifetime and the number of determinations. It is worth mentioning here that moisture in the polypropylene net on which the organism is immobilized needs to be monitored and it should never be allowed to dry.

4. Conclusions

By coupling *Fusarium solani* onto an oxygen electrode, we were able to obtain a highly selective acetic acid sensor. (For the same concentrations of various substrates, 50 ppm, the response signal obtained for acetic acid was much higher than the other substrates studied (Table 1)). The detectable concentration of acetic acid was 72 ppm, higher than those obtained by previous researchers. The volatile substrates like methanol seem to have less effect on the performance of the sensor. Since we were able to obtain reproducible results, we strongly believe that acetic acid can be monitored and controlled in production broth using this technique and this fungal strain. Besides, the selectivity and sensitivity of the sensor was reasonably high, which we believe is another advantage of this sensor.

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