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A microbial biosensor for hydrogen sulfide monitoring based on potentiometry

Ehsan Ebrahimi [a], Fatemeh Yazdian [b,c], Ghasem Amoabediny [a,b,*], Mohammad Reza Shariati [a], Behdokht Janfada [b,c], Mohammad Saber [d]

[a] Faculty of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
[b] Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
[c] Department of Life Science Engineering, Faculty of New Science and Technologies, University of Tehran, Tehran, Iran
[d] Department of Mechanical Engineering, University of Kashan, Kashan, Iran

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ABSTRACT

In this study, a microbial biosensor for hydrogen sulfide (H_2S) detection based on *Thiobacillus thioparus* immobilized in a gelatin matrix was developed. The *T. thioparus* was immobilized via either surface adsorption on the gelatin matrix or entrapment in the matrix. The bacterial and gelatin concentration in the support were then varied in order to optimize the sensor response time and detection limit for both methods. The optimization was conducted by a statistical analysis of the measured biosensor response with various bacterial and polymer concentrations. According to our experiments with both immobilization methods, the optimized conditions for the entrapment method were found to be a gelatin concentration of 1% and an optical density of 82. For the surface adsorption method, 0.6% gelatin and an optical density of 88 were selected as the optimal conditions. A statistical model was developed based on the extent of the biosensor response in both methods of immobilization. The maximum change in the potential of the solution was 23.16 mV for the entrapment method and 34.34 mV for the surface absorption method. The response times for the entrapment and adsorption methods were 160 s and 105 s, respectively. The adsorption method is more advantageous for the development of a gas biosensor due to its shorter response time.

1. Introduction

Sulfide compounds, such as hydrogen sulfide (H_2S), are versatile reagents that are used in many industries, such as the petrochemical, food, textile, refinery, gas and oil industries. Hydrogen sulfide is a toxic, flammable and colorless gas, and therefore it is necessary to control even its low concentrations in the environment [1]. An H_2S concentration of 15–50 ppm causes headache, eye irritation, and dizziness. At a concentration of 200–300 ppm, H_2S may have an anesthetic effect, and a several-minute exposure to greater than 700 ppm may lead to death [2]. These lethal effects are due to the inhibition of enzymatic activities. Hydrogen sulfide acts as an inhibitor of several types of enzymes, including ascorbate oxidase and cytochrome C oxidase [3–5]. This harmful compound can be detected by various means, including microbial biosensors [2,6].

The species *Thiobacillus thioparus*, *Thiobacillus thiooxidans*, and *Thiobacillus ferrooxidans* can be used as H_2S receptors in microbial biosensors [2,6]. There are different methods of immobilizing biocatalysts on supports [7]. Bacterial biocatalysts are able to self-aggregate or attach to exterior agents, such as gelatin and collagen polymers. Depending on the agents, grafting occurs through covalent bonds or Van der Waals forces [8–11]. Entrapment and surface adsorption are two common methods of microorganism immobilization. Shoichet and coworkers [12] studied the stability of agarose and alginate for the immobilization of microorganisms. These polymer beads were stable for approximately 90 days with no significant effects on the microorganisms. Camelin and coworkers [13] immobilized *Bifidobacterium longum* cells to gellan gum beads with citrate. Babu and coworkers [10] used 10% gelatin and 5% glutaraldehyde to immobilize *Pseudomonas alcaligenes* on a membrane surface. Immobilized microorganisms in biocompatible polymers can be used as receptors in microbial biosensors. Other biological receptors, including enzymes, antibodies, and nucleic acids, have been developed as well [7,14–16]. Microbial biosensors have been successfully utilized for the detection of special substances in liquid

* Corresponding author at: University of Tehran, Chemical Engineering, 16 Azar, Tehran, Iran. Tel.: +98 61112215; fax: +98 2188631400.
E-mail address: amoabediny@ut.ac.ir (G. Amoabediny).

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or gas components, such as in CO_2, NO_2, CH_4 and H_2S [7,24]. The transducers in these biosensors function based on electrochemical, calorimetric, optical or light scattering convertors. Among these, electrochemical transducers can be further categorized as amperometric, voltammetric, or conductometric convertors [5,10,17–20]. Akyilmaz and coworkers [21] designed a microbial amperometric biosensor for the detection of Vitamin B1 by measuring the dissolved oxygen concentration in the environment. Wen and coworkers [5] used oxidative bacteria immobilized on polymeric beads to measure methane concentrations based on the dissolved oxygen gradient. Shahidi Pour Savizi and coworkers [32] fabricated an amperometric biosensor based on the inhibition of *Coprinus cinereus* peroxidase by sulfide for sulfide detection. The sensor had a linear response in the range of 1.09–16.3 μm, a detection limit of 0.3 μm, and a response time of 43 s. Kariminia et al. [33] constructed an optical biosensor based on fungal peroxidase inhibition for sulfide detection as well.

To the best of our knowledge, no studies have investigated the use of biosensors based on alternating potentials in solution for the rapid and sensitive detection of H_2S. In this article, the variables influencing the bacterial biosensor fabrication were optimized using statistical analysis. The biosensor response time and calibration data were measured for both entrapment and surface adsorption methods.

2. Materials and methods

T. thioparus (PTCC 1668) was purchased from the Persian type culture collection and was cultured in a medium (pH 7) containing 2 g of KH_2PO_4, 2 g of K_2HPO_4, 0.4 g of NH_4Cl, 0.4 g of Na_2CO_3, 0.2 g of $MgCl_2{\cdot}6H_2O$, 5 g of $Na_2S_2O_3{\cdot}5H_2O$, 1 ml of a trace metal solution and 3 ml of a vitamin solution per 1000 ml of distilled water. The trace metal solution comprised 50 g of Na_2-EDTA, 11 g of $ZnSO_4{\cdot}7H_2O$, 7.34 g of $CaCl_2{\cdot}2H_2O$, 2.5 g of $MnCl_2{\cdot}2H_2O$, 0.5 g of $CoCl_2{\cdot}6H_2O$, 5 g of $FeSO_4{\cdot}7H_2O$, 0.5 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.2 g of $CuSO_4{\cdot}5H_2O$, and 11 g of NaOH per 1000 ml distilled water and was adjusted to pH 6. All the minerals were purchased from Merck and were used without further purification. The components of the vitamin solutions, namely, 10 mg of thiamine-hydrochloride·$2H_2O$, 20 mg of nicotinic acid, 20 mg of pyridoxine-hydrochloride (Vitamin B6), 10 mg of p-aminobenzoic acid, 20 mg of riboflavin, 20 mg of Ca-pantothenate (vitamin B5), 1 mg of biotin and 1 mg of vitamin B12 per 1000 ml distilled water, adjusted to pH 7, were bought from Sigma.

For the immobilization media, porcine gelatin was obtained from Fluka, and sodium alginate was obtained from Sigma–Aldrich. Glutaraldehyde, sodium chloride and calcium chloride were purchased from Merck, and the silicon membrane was obtained from the Iranian Polymer Institute (IPI). The feed gas was a mixture of H_2S (1%) and N_2 (99%).

2.1. Cultivation and harvesting conditions

T. thioparus was cultured at 30 °C and 180 rpm for 4 days. Bacteria were separated from the medium by centrifugation (Hettich, UNIVERSAL320) at 4 °C and 8000 × *g* for 15 min and washed two times with saline solution (0.9%) for immobilization.

2.2. Entrapment immobilization

A highly adhesive gelatin polymer was used to prepare the immobilized beads for the entrapment method. Gelatin, 1–10% and sodium–alginate, 0.15% were dissolved completely in 4 ml distilled water for 10 min at 60 °C. The homogenized polymeric mixture was then cooled to 40 °C. A 1 ml volume of the harvested cell solution at a predefined concentration determined based on the optical density at 600 nm was then added to the mixture. A 5 ml volume of the mixture was injected into a $CaCl_2$ solution with a syringe pump to generate beads less than 1 mm in diameter. Finally, the cell-entrapped beads were stirred for 2 h to obtain the necessary strength for the beads to maintain their shape in the biosensor apparatus and then stored at 4 °C.

2.3. Surface adsorption immobilization

Silicon surfaces of 0.1 mm thickness were cut into 2 cm × 2 cm pieces. The silicon surfaces were washed with deionized water in an ultrasonic device with 15 W of power for 10 min to obtain varnished surfaces. To activate and sterilize the silicon pieces, they were exposed to ultraviolet radiation for 2 h. Gelatin (1 ml) was prepared at concentrations ranging from 0.1 to 1% and placed on the silicon surfaces. A layer of 50 μm of gelatin was measured by a thickness gauge (CHY115, CHY Company, Taiwan). The gelatin surfaces were stored either at 4 °C or at room temperature for 6–8 h, depending on the gelatin concentration. The 1-ml cell solution was placed on the gelatin surface, then dried for 2 h at room temperature. Before the drying of the surface, 400 μl of a 5% glutaraldehyde solution in distilled water (pH 5.7) was added to the surface. Then, the microorganisms were immobilized on the gelatin surface at room temperature and in the dark for 4–5 h to complete the surface adsorption reactions.

Table 1
Experimental levels with CCD method in entrapment method.

No. run	Gelatin concentration (X1)	*T. thioparus* concentration (X2)
1	5.5	10
2	2.3	30
3	8.7	30
4	1	80
5	5.5	80
6	5.5	80
7	5.5	80
8	10	80
9	2.3	130
10	8.7	130
11	5.5	130

2.4. H_2S biosensor apparatus

As shown in Figure S1, the immobilized microorganisms were transferred to the biosensor apparatus, which had a 50 ml volume and was isolated with a Teflon cap to prevent the exit of H_2S and air. A pH electrode (AZ Instrument, Taiwan) was used to measure alterations in the H^+ ion concentration. A saline solution was used as the carrier electrolyte in the biosensor. First, in order to reach the maximum activity of the bacteria, the biosensor was saturated with air for several minutes, and the temperature of the vessel was set to 29–31 °C. After venting the biosensor, the concentration of H_2S in the biosensor was adjusted according to the feed time, the volume of the apparatus, and the flow rate of the gas stream. Next, the immobilized *T. thioparus* were inserted into the biosensor apparatus (in one step we aerated the biosensor and in another H_2S was introduced). Measuring the alterations in the H^+ concentration simultaneously with low-speed aeration required up to 15 min. The changes in the H^+ concentration were then recorded online by a computer for each test.

2.5. Viability experiments

To determine the viability of the microorganisms in each immobilization method, the alterations in potential were measured using the biosensor apparatus. After the experiments, the bacteria were extracted from the apparatus and maintained at 4 °C. This experiment was performed for both immobilization methods until the biosensor activity level reached 70%. The "activity level" was defined as the ratio of the change in potential of the immobilized biosensor to the change in potential in the presence of free microorganisms at a particular bacterial concentration.

2.6. Control tests

It was noted that H_2S can affect the biosensor potential even in the absence of microorganisms. Furthermore, the immobilized bacteria could change the potential in an H_2S-free environment. Two types of control tests were therefore conducted for the H_2S biosensor. The first control measured the change in H^+ concentration when 1 ml of bacterial solution was added. The second control probed changes in the H^+ concentration in the presence of H_2S and in the absence of microorganisms.

2.7. Experimental design

The gelatin and bacterial concentrations were optimized to obtain the maximum change in potential. For both immobilization methods, a 1 ml cell solution with an optical density of 10–150 was used. The concentration of gelatin was chosen as a variable parameter due to discrepancies and limitations among the common methods. Concentrations of 1–10% (w/v) gelatin solution, 4 ml, and 0.1–1% (w/v) gelatin solution, 1 ml, were used for the entrapment and the surface adsorption methods, respectively. The parameters are shown in Tables 1 and 2, respectively. The levels for the different factors were selected based on previous studies [24,32–33].

3. Results and discussion

3.1. Strain selection for the biosensor receptor

T. thioparus is a chemoautotroph microorganism that consumes H_2S as an electron source. Due to its optimum temperature (29–31 °C) and optimum pH (6.5–7), it is an appropriate candidate for H_2S biosensors [22]. Hydrogen sulfide can be metabolized