Biosensors and Bioelectronics 70 (2015) 106–114

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Investigating the effect of design parameters on the response time of a highly sensitive microbial hydrogen sulfide biosensor based on oxygen consumption

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ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form
9 March 2015
Accepted 10 March 2015
Available online 11 March 2015

Keywords:
Hydrogen sulfide
Thiobacillus thioparus
Design parameters
Agarose
Sodium alginate
Response time

ABSTRACT

A novel hydrogen sulfide microbial biosensor was developed based on investigating the influence of four design parameters: cell concentration, immobilization bed type, hydrogen sulfide concentration, and geometrical shape of the biosensor. *Thiobacillus thioparus* was used as the recognition element and it was immobilized on sodium alginate as well as agarose bed. The results were optimized by the application of statistical optimization software based on response time of the system. Oxygen reduction was considered as the detection sign. Sodium alginate solution with a concentration of 2.3% (w/v) and optical density of 10 at 605 nm was found as the optimum conditions for immobilization with response time of 72 s. Optimum response time of immobilized *T. thioparus* on agarose was also found equal to 120 s at agarose concentration of 1.2% (w/v) and optical density of 10.83. Performance of the biosensor in different temperatures, pH and agitation speeds was also analyzed. The designed biosensor could detect concentrations of hydrogen sulfide as low as 0.5 ppm. *T. thioparus* could retain 99% of the original activity in both systems, after ten days passing the fabrication. A fractal analysis was also done theoretically to investigate the diffusion of oxygen in immobilized cells which showed a satisfactory value of oxygen take up by the immobilized cells.

1. Introduction

Since the last four decades, an immense research has been done in the field of detecting hydrogen sulfide. H_2S is a venomous, flammable and colorless gas with a malodor of rotten egg. It is swiftly absorbed by the respiration system causing neurological sequelae which finally leads to death. It can also cause a malodor nuisance problem even at relatively low concentration of 2 ppm. At moderate concentrations of 50–150 ppm it causes headaches, eye irritation and dizziness. It is largely produced in many industries such as petroleum and gas refining (Pandey et al., 2012; Kim et al., 2008). Hence, a preliminary step toward handling this hazardous is to monitor and detect its existence in the environment. Therefore, novel and accurate techniques such as biosensors have been suggested for detection of low concentrations of H_2S. Proper selection of the biosensor receptor is of great importance. Both phototrophic and chemotrophic bacteria are good choices for receptor, since they use hydrogen sulfide as the energy source and through this process they consume oxygen (Oprime et al., 2001).

Bacteria such as *Thiobacillus denitrificans*, *Thiobacillus ferroxidans* and *Thiobacillus thioparus* can use H_2S as energy source (Pancrazio et al., 1999). *T. thioparus* is chosen among them, due to its low nutrients requirements and high rate of H_2S consumption. Moreover, *T. thioparus* has the ability to grow under rough environmental conditions such as oxygen deficiency and acidic solutions, which is a great privilege in fabrication of biosensors. Its optimum pH range is 6.6–7.2 and it grows well in 28 °C. The overall equation for oxidation of H_2S is as following (Oyarzun et al. 2003; Caceres et al., 2010):

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (1)$$

Along this reaction H_2S acts as electron donor and *T. thioparus*

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http://dx.doi.org/10.1016/j.bios.2015.03.025
0956-5663/

consumes oxygen. Hence, the oxygen concentration of the solution declines. The existence of H_2S in the solution can be perceptible with the aim of this detection sign. A biosensor system based on this logic was designed and investigated (Kubo et al., 1995).

In the present study, the effect of four designing parameters, including cell, bed and H_2S concentration, along with system scale, on the response time of the biosensor was studied. Response time is defined as the time when the concentration of dissolved oxygen in the system starts to decline until it approaches to a constant value. Enduring harsh conditions is of the main traits of a good biosensor. Thus, *T. thioparus* species were immobilized on sodium alginate and agarose. In addition to being an excellent matrix, agarose and sodium alginate have high porosity which results in high capacity for cell entrapment (Yujian et al., 2006; Parkash and Jaisawal., 2011). Moreover, biosensors should possess low detection limits; therefore, concentrations of 0.5–20 ppm of H_2S were injected to the system. Eventually, the effect of bacteria's interference in the system was investigated through the volume. In this regard, two systems of 300 and 500 ml volume were used for the tests and mathematical explorations have been discussed in each system.

The main scope of the present study was to obtain an optimized biosensing system based on analyzing the effect of four different parameters affecting the response time of the system. In order to set up the system, a gas fed batch reactor was used, which is a stirred bilayer bioreactor. The bacteria was immobilized on two different surfaces of sodium alginate and agarose. The biosensor could effectively respond to low concentrations of hydrogen sulfide swiftly and with high specificity.

2. Experimental

2.1. Cell culture and media

The original strain of *T. thioparus* (PTCC 1668 taken from Ministry of science, Research and Technology of Iran) was used in all experiments. For long storage of the strain (up to 3 years), 850 μl of the media containing bacteria was put in 1.5 ml vials, and 150 μl Glycerol was added as the anti-frost solution. The solution was put in −86 °C ultra-low temperature freezer (Kaltis, Taiwan) for further use.

T. thioparus was grown in DSM 486 containing (in g/l): KH_2PO_4, 2.00; K_2HPO_4, 2.00; NH_4Cl, 0.4; $Na_2S_2O3 \cdot 5H_2O$, 5; $MgCl_2 \cdot 6H_2O$, 0.2; Na_2CO_3, 0.4; in addition to vitamin and trace metal solutions. The cultivation was done in 500 ml Erlenmeyer flasks for 1–3 days at 30 °C and 180 rpm in a shaking-incubator (Kuhner, Switzerland) and the pH was adjusted to 7. The cell growth curve of the bacteria was also plotted by the application of optical density method in 605 nm. Cell harvesting from the media was achieved by centrifugation in 4000*g* for 15 min at 4 °C. The resulting bacteria was washed 2–3 times by normal saline and was again centrifuged to remove the remaining media from the cell. The volume of the obtained cell suspension was about 50 ml H_2S.

2.2. Determination of the best time for cell harvesting

In order to find the best time for cell harvesting, bacteria was taken out from shaking-incubator after 56, 67, 96, 120 h. Cells were tested in the biosensor system, and the oxygen consumption rate curve was plotted for each sample. It was observed that cells which were harvested after 96 h, presented the best activity and also maximum rate of oxygen consumption in the system.

2.3. Design and operational parameters

The effect of four parameters, including cell concentration, immobilization bed, H_2S concentration and geometrical shape, on the response time of the system was investigated. Additionally, the biological activity and the mechanical strength of the immobilized beads were also analyzed by the reference methods (Yujian et al., 2006; Myung et al., 2007). The whole achieved data were optimized by the Respond Surface Method.

2.3.1. Cell concentration

The best response time for the biosensor mainly depends on the cell concentration owing to its high capability of oxygen consumption. Consequently, different concentrations of cell, designed by the Central composite design (CCD) method, were used to prepare the immobilized beads in the system. The concentration yielding the best result was acclaimed.

2.3.2. Immobilization on sodium alginate

Different concentrations of sodium alginate beads in the range of 2–4% (w/v) were prepared by the following method. Sodium alginate and calcium chloride of analytical grade (Merck) were used. To prepare 3% (w/v) beads, 3 g of sodium alginate was added to 100 cc diluted water. The solution was heated for 30 min up to the time when homogeneity appeared. The 0.2% (w/v) solution of calcium chloride was also prepared by addition of 1.5 g calcium chloride to 100 cc diluted water. Hereafter, sodium alginate was cooled down to ambient temperature. 5 ml of Cell suspension with specific concentration was added to 100 cc sodium alginate solution. In order to obtain similar beads in terms of size, the yielded mixture was put in a syringe pump (JMS Syringe Pump, USA), and it was gradually dropped into the calcium chloride solution to form immobilized sodium alginate beads.

2.3.3. Immobilization on agarose

The method of Parkash and Jaiswal was used for preparing agarose beads (Parkash and Jaisawal., 2011). 0.1 M Sodium acetate buffer with pH 5.5 was prepared by adding 28.82 ml of 1 M acetic acid (Merck) to 273.3 ml of sodium acetate and the volume was increased to 1 l. In order to form 2% (w/v) agarose beads, 0.2 g of agarose (Sigma-Aldrich) was added to 10 cc of sodium acetate buffer at 50 °C. Other concentrations between 1% and 3% (w/v) was also prepared by the same procedure. Afterward, 5 ml of cell suspension with a specific concentration was added to 100 cc (same as sodium alginate beads) of the agarose solution prepared in the previous step. The resulting solution was put into 100 cc syringe and injected into a plastic mesh with same sized cavities. The solution was then cooled down to ambient temperature for 3 h. Finally, the resulting beads were taken out of the mesh and preserved for 24 h at 4 °C in 25 mM sodium acetate solution before being used in the system.

2.3.4. Measuring dissolved H_2S concentration

Different concentrations of H_2S, ranging from 0.5 to 20 ppm, were injected to the system in order to acquire detection limit of biosensor. Theoretical and practical methods were both employed for measuring the concentration of dissolved H_2S. The variance between the results was less than 5%. The method of Duan et.al was used for theoretical approach as well as the titrimetric method which was used for the practical approach. In the theoretical approach the basis of calculation is on the equality of chemical potential between liquid and gas phase. The resulted equation can be seen below (Duan et al., 2007; Suleimenov, and Krupp 1994):