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A modified optical microbial biosensor for detection of Methane using Gold nanoparticle and Methanotrophic bacteria

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Abstract

An optical microbial biosensor was described for the detection of methane. Whole cells of Methylobacterium extorquens were immobilized and were used as biocomponent along with optic system. The immobilized microbial biocomponent was disposable, cost-effective and showed high reproducibility and uniformity. The detection of methane by the use of disposable microbial biocomponent with optical biosensor was simple, single step and direct measurement of very low quantity of the sample. To improve the biosensor performance, cell was modified with nanoparticles. Comparing the results for cell without and with nanoparticles shows that the absorbance was improved from 0.329 to 0.3625 nm. Analysing the experimental results indicates that the biosensing system response depends linearly on methane concentration.

Introduction

It is well known that alkanes and specially methane, even at very low concentrations, are chemical compounds very dangerous for human safety and environment. Low-cost chemical sensors are then needed for monitoring methane leakages.

Several measuring techniques were previously used to develop methane sensors. Metal oxide thin films have been traditionally used as gas sensing materials. These sensors operate on the principle that the surface conduction of the semiconductor sensors varies in relation to the adsorption of the ambient gas [1], [2], [3] and [4]. In order to make them selective, the concept was based on the different catalytic activity of platinum and palladium towards the oxidation of methane, at around 400 °C. The detection limit attained is about 0.5% of methane [5]. Catalytic gas sensors were also developed for methane detection. They consist of narrow diameter Pt wire coils surrounded by a catalyst supported on an inert porous refractory material such as alumina. The detection limit of this type of sensor is 0.1% of methane [6].

The major shortcomings with these methods are that they are usually expensive, hard to maintain the equipments, and unfavorable for rapid screening of large-scale test or applied in situ [7,8].

Using biosensors to conduct the chemical detection offer an alternative as a result of rapidly screening, lower cost, and ability to measure bioavailability [9-10]. A biosensor is an analytical device which converts a biological response into an electrical signal (Figure 1). A biosensor comprises two main components, biological sensing element and signal transducer element, which can identify and generate detectable signals.

Depending on the method of signal transduction, biosensors can also be divided into different groups: electrochemical, optical, thermometric, piezoelectric, or magnetic. Optical biosensors are the most commonly reported class of biosensors. The detection typically relies on an enzyme system that catalytically converts analytes into products that can be oxidized or reduced at a working electrode, maintained at a specific potential. The main advantage of this optical transducer is the low cost and the use of biodegradable electrodes. An optical biosensor is a compact analytical device, having biological sensing element, integrated or connected to, an optical transducer system. The detection of specific binding of the analyte of interest to the complementary optical biorecognition element is immobilized on a suitable optical substrate. The basic objective of optical biosensor is to produce an electronic signal which is proportional in magnitude or frequency corresponding to the concentration of a specific analyte or group of analytes, to which the biosensing element binds.

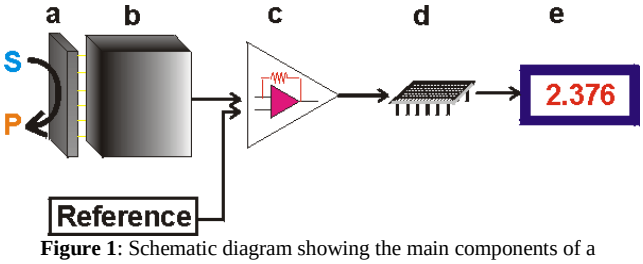

Figure 1: Schematic diagram showing the main components of a biosensor.

The biocatalyst (a) converts the substrate to product. This reaction is determined by the transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e).

One of many challenges in biosensor development is the efficient signal capture of the biological recognition event (transduction). Such transducers translate the interaction of the analyte with the biological element into electrochemical, electrochemiluminescent, magnetic, gravimetric, or optical

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signals. In order to increase sensitivities and to lower detection limits down to even individual molecules, nanomaterials are promising candidates due to the possibility to immobilize an enhanced quantity of bioreceptor units at reduced volumes and even to act itself as transduction element. Among such nanomaterials, gold nanoparticles, semi-conductor quantum dots, polymer nanoparticles, carbon nanotubes, nanodiamonds, and graphene are intensively studied [11].Within the group of noble metal nanoparticles, gold nanoparticles are mostly used for biosensor application [12] due to their biocompatibility, their optical and electronic properties, and their relatively simple production and modification [13]. Particular interesting is the optical behavior of gold surfaces where irradiation with light of one specific wavelength causes an oscillation of the electrons in the conduction band, called resonant surface plasmons. Gold nanoparticles have also demonstrated their advantages in bioanalysis using SPR transduction. This method is usually based on the change of the dielectric constant of propagating surface plasmons' environment of gold films where the detection of the analyte can be recorded in different ways like the changes of the angle, intensity, or phase of the reflected light [14-15]. Gold nanoparticles have also shown their ability to form a powerful transduction platform for single molecule detection. Besides the outstanding optical properties, gold nanoparticles also have the ability to transfer electrons between a wide range of electroactive biological species and the electrode. This principle is principally used for redox enzyme biosensing where the bioreceptor unit catalyzes the oxidation or reduction of the analyte. These outstanding properties of gold nanoparticles made them promising candidates not only for bioanalytics but also for many other research fields. The particular properties of such gold nanoparticles can be tuned and adjusted. Whatever the desired application, almost any desired shape or size can be obtained using the appropriate synthesis technique. These different morphologies result in different optical, catalytic, and electronic behavior of these gold nanoparticles [16].

In this present study, the Methylobacterium Extorquens (Methanotrophic bacteria) was used in the optical biosensor modified with gold nanoparticles. The biosensor is easy-to-use and inexpensive for gas screening in environment samples and could be complementary to physicochemical methods.

Experimental

Materials and methods

Polyvinyl alcohol (a degree of polymerization of 1750 ± 50, average m.w. 30,153,160), Sodium alginate and boric and the buffer solution of 25 mM monosodium dihydrogen phosphate–disodium hydrogen phosphate at pH 7.0. 99.99% (v/v) methane gas were purchased from Merck & Co. All reagents were of analytical-reagent grade or above and used without further purification. All solutions were prepared with deionized (DI) water.

Cell culture and morphological character

Methylobacterium extorquens were collected from water samples and were cultured under methane–air atmosphere (20% (v/v) methane and 80% (v/v) air) at 30 °C for about 7 days in a medium (pH 7.2) containing: 0.5 g KH_2PO_4, 0.5 g Na_2HPO_4, 0.4 g NaCl, 1.0 g KNO_3, 0.5 g NH_4Cl, 1.0 g $MgSO_4{\cdot}7H_2O$, 0.2 g $CaCl_2$, 4.0 mg $FeSO_4{\cdot}7H_2O$, 4.0 mg $CuSO_4{\cdot}5H_2O$, 4.0 mg $MnSO_4{\cdot}H_2O$, 4.0 mg $ZnSO_4{\cdot}7H_2O$, and 0.24 mg $NaMoO_4{\cdot}2H_2O$ per liter of distilled water. Methanol was provided as a single carbon source for substrate growth. Methylobacterium extorquens was grown in a 500-mL suction flask sealed with a rubber stopper. Enrichment was applied using the same cultivation method. The bacteria were collected by centrifugation at 6000 rpm for 5 min and washed twice with a 25.0-mM pH 7.0 phosphate buffer. The strain, designated ME16, was selected for identification with reference to Bergey's Manual of Determinative Bacteriology.

Immobilization of methane-oxidizing bacteria by PVA–boric acid method

Immobilization of biomaterials is the hardest difficulty in the process of fabrication of optical biosensor. Material losses are observed during the process of immobilization of biomolecules on solid substrate. 11.1% (w/v) of PVA solution was prepared by adding 1.0 g of PVA in 9.0 mL of DI water and heated to a temperature of around 80 °C to dissolve PVA. 1.0 mL of 10% (w/v) sodium alginate solution in water was added to 9.0-mL of 11.1% (w/v) PVA solution (the final concentrations were about 10% (w/v) PVA and 1% (w/v) sodium alginate). The PVA–alginate mixture was gently stirred for 45 min, cooled to room temperature (20–25 °C), and then followed by addition of about 40 mg of wet cell and mixed well. The mixture was dropped into a gently stirred 4% (w/v) boric acid solution containing 2% (w/v) $CaCl_2$ to form spherical beads. These beads were kept in the boric acid solution for 24 h at 4 °C to gel. The beads were then removed and washed with distilled water. The average diameter of the beads is about 3.0 mm.

To immobilize gold nanoparticles on cell, 0.75 gr nano powder was dispersed on cell surface uniquely and then dried in Avon for 3hr at 30 ^{o}C.

Transducer

The transducer used was a SF2000 miniature optical fiber spectrophotometer from Ocean Optics Inc. Duiven, The Netherlands. The heart of the SF2000 miniature optical fiber spectrophotometer is preconfigured to a 360–1000 nm-wavelength range with a 200 μm entrance and detector collection lens (for increased light throughput). The data acquisition and visualization software OOIBase32™ was provided with the instrument. The 32-bit PCI analog to digital converter card (ADC) required to interface the equipment with the computer was also supplied along with the instrument (model ADC 2000 PCI). Absorbance is linearly related to the concentration of the substance. The software calculates absorbance using the following equation:

$$A_\lambda = -\log\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right) \quad (1)$$

where S is the sample intensity at wavelength λ, D the dark intensity at wavelength λ, R is the reference intensity at wavelength λ. Figure 2 shows the schematic of the experimental setup.

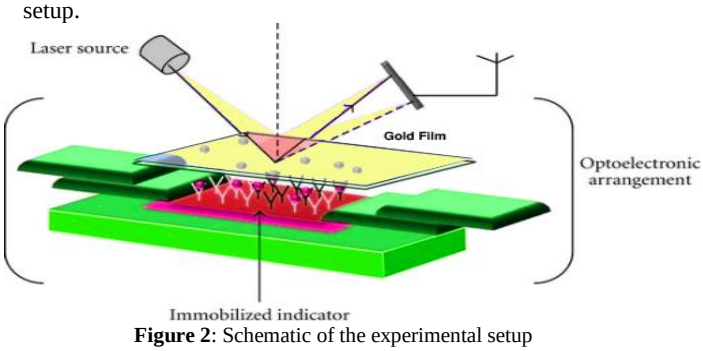

Figure 2: Schematic of the experimental setup

Results and Discussion

Experiments without nanoparticle

At first step, experimental setup was tested with air. The results have been listed in table 1. It shows that the cells grown having absorbance 0.297 at λ_{opt} 300 nm have maximum activity. The

cells grown under these conditions were used for the subsequent experiments.

To investigate the biosensor performance in methane detection, 4cc methane gas was released over the cell and the absorbance was recorded. The results show that absorbance 0.329 at λ_{opt} 300 nm have maximum activity. Comparing the results for air and methane indicates the effect of gas on Methanotrophic bacteria in which gas presence increases absorbance from 0.297 to 0329 nm.

Table 1: Experimental results without nanoparticles

Experiment	Absorbance (nm)
Bacteria+Cell+Air	0.297
Bacteria+Cell+Methane	0.329

Figure 3 shows the effect of gas concentration on process performance. As seen, increases gas concentration improves the process performance by increasing the absorbance.

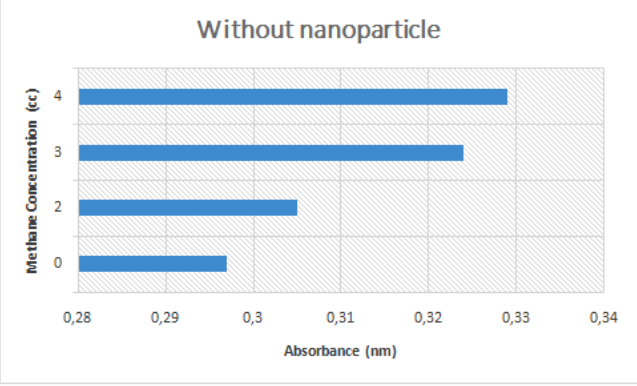

Figure 3: Effect of methane concentration an absorbance

Experiments with nanoparticle

Table 2 shows the comparison between the cell with and without nanoparticle. Based on the table, adding nanoparticle to the cell leads to increasing light absorption capacity. In other words, nano particles make the results of the biosensor more accurate. The cells with nanoparticle having absorbance 0.3625 at λopt 300 nm possess maximum activity.

Table 2. Experimental results with nanoparticles

Experiment	Absorbance (nm)
Bacteria+Cell+Air+nanoparticle	0.319
Bacteria+Cell+Methane+nanoparticle	0.3625

Correlation of biosensor response

Regressions are commonly used in biology to determine the causal relationship between two variables. This analysis is most commonly used in morphological studies, where the allometric relationship between two morphological variables is of fundamental interest. Comparing scaling parameters (i.e. slopes) between groups can be used by biologist to assess different growth patterns or the development of different forms or shapes between groups.

In order to access the gas concentration effect due to naturally occurring compound in real world samples, the optical biosensor response obtained with methane biodegradation bacteria were plotted against the gas concentration for cell with and without nanoparticles. Coefficient correlation, linear regression line and goodness of fit were established in linear plots and were observed over a wide range of methane concentration ranging 0-4 cc in optical biosensor (Fig. 4). Regression colorations show that the optical biosensor response has a direct relation with gas concentration. The slope of the correlations represents the expected increment in the response per unit change in gas concentration.

To explore the difference between the cell with and without nanoparticles, we need to test whether the slopes regression lines are equal. Based on the statistical analysis p-Value is 0.007, and therefore, we can conclude that there is a significant difference between the cell without nanoparticle and the cell with gold nanoparticles. This means that adding nanoparticles to the cell has a significant effect on biosensor performance.

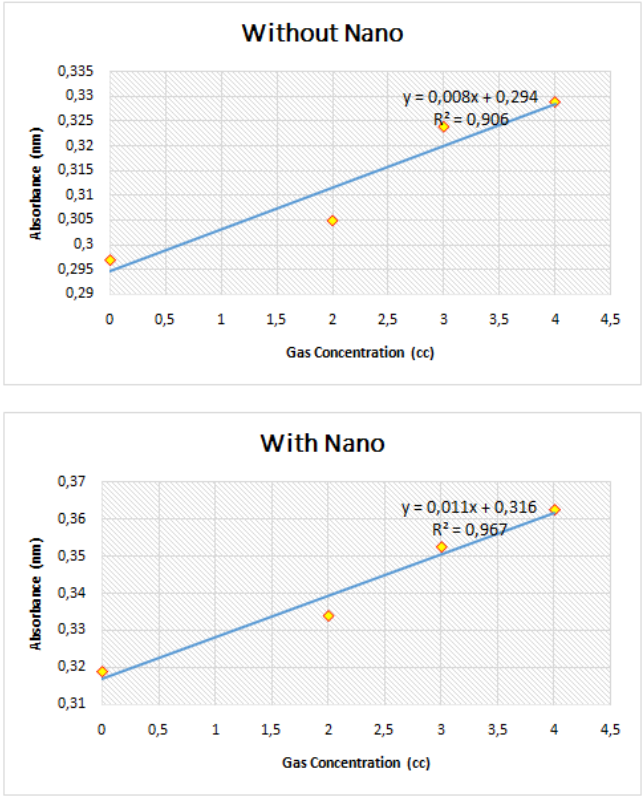

Figure 4: Correlation of the optical biosensor measurement with the gas concentration

Conclusions

This work has demonstrated the feasibility of fabricating a methane biosensing system based on immobilized methane-oxidizing bacteria in conjunction with an optical sensor. The system was modified with gold nanoparticles. The most promising features of our system are simple sensing design and ease of operation. This microbial biosensing system using co-immobilized Methylobacterium extorquens appears very attractive for rapid determination of methane. The results show that modifying the system with nanoparticles improves biosewnsor performance by increasing absorbance capacity from 0.329 to 0.3625 nm.

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