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Enzyme sensors for environmental analysis

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Abstract

Biosensors are occasionally used to monitor pollutants in aquatic environment such as river or drinking water. Numerous important biosensors using enzymes have been fabricated for environmental monitoring purposes. Since immobilization of biomaterials such as enzymes are very important in the fabrication of biosensors for environmental monitoring, many intelligent materials such as photorosslinking polymers have been developed recently. This review introduces several immobilization materials and enzyme sensors for environmental monitoring (e.g. phosphate sensors). © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

There is a strong demand for environmental monitoring technologies today due to the ever increasing environmental pollutions. Water can be polluted by toxicants (e.g. cyanide, heavy metals or pesticides) and/or eutrophicants (e.g. phosphate or nitrogen compounds). Eutrophication of water leads to overgrowth of plants and toxic algae thereby making it unsuitable for drinking or industrial uses. Monitoring these contaminants in water is of extreme importance considering the impact that such polluted water would have on our everyday lives.

In situ monitoring of pollutants in water is highly desirable because it would allow us to monitor such pollutants in real-time and take immediate measures to remedy any unwanted situations. Biosensors are important class of sensors in this regard. Enzyme, immuno- and microbial sensors intended for environmental monitoring have been developed and reviewed previously by Karube et al. [1]. Enzyme sensors have been the target of intense research and development recently and have proven to be both rapid and highly selective. In this review, the author will emphasize the recent developments in the field of enzyme sensors and relevant technologies as applied to environmental monitoring.

2. Biosensor

A biosensor is a detection system composed of a biological sensing element (e.g. enzyme)

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and a transducer (e.g. electrode). The biosensor measures the change in the concentration of a co-reactant which reacts with the analyte or a co-product which is produced with the analyte of a biological reaction (e.g. enzyme reaction). When an electrode is used as a transducer in a biosensor, the electrode converts the change in concentration of a product of a biological reaction into an electric signal.

Biosensors have replaced conventional methods, which are often complicated, time-consuming, expensive and not suitable for in situ monitoring. Whereas stability and reproducibility have been sometimes problematic for biosensors due to the inherent instability of biomaterials used as sensing elements, several intelligent immobilization methods that help to overcome these difficulties emerged.

3. Immobilization of biomaterials

The first biosensor was an enzyme sensor (i.e. glucose sensor) reported by Clark in 1962 [2]. His biosensor measured the product of glucose oxidation by glucose oxidase using an electrode which was a remarkable achievement even though the enzyme was not immobilized on the electrode.

Updark and Hicks have developed an improved enzyme sensor using enzyme immobilization [2]. Their sensor combined the membrane-immobilized glucose oxidase with an oxygen electrode, and oxygen measurements were carried out before and after the enzyme reaction. Their report showed the importance of biomaterial immobilization to enhance the stability a biosensors.

Typical immobilization carriers (e.g. beads or membranes) are made from glass, alginate or artificial resins. Roughly classified, there are two types of immobilization methods, chemical (e.g. covalent bond) and physical (e.g. adsorption). Several resins such as photocrosslinking polymer have been also used as gel-entrapping methods. Fukui et al. [3] have developed a novel and convenient method for preparing gel-entrapped enzyme or microbial cells by using photocrosslinkable resins (e.g. PEGM and PVA). Since this method is convenient and does low damage to biomaterials, it is sometimes used in biosensors.

Immobilization matrices which confer higher stability of biomaterials, and thus the sensor response, attracted greater attention and some interesting studies have been reported in the last few years. Plasma polymerization technique has been applied to chemically modify PET and PP surfaces to yield anchoring functional groups (e.g. C=O group). Ganapathy et al. [4] used this technique to immobilize α-chymotrypsin in 1998 and found that the immobilized enzyme was more stable than in solution allowing them to reuse the enzyme up to 20 times. Another interesting example is the bio-skin membrane, which is a natural polymer produced by microorganisms. It was applied to catalase immobilization because of its low effect on the enzyme activity [5]. Half-life time of the immobilized catalase was more than 26 days, whereas that of the free enzyme was only a few days. It is expected that these emerging immobilization matrices and techniques will be heavily utilized in future biosensors.

4. Enzyme sensors for environmental analysis

Numerous enzyme sensors have been developed since the first enzyme sensor, and a considerable sum of these are used in environmental monitoring. The last few years have seen improvements in inorganic phosphate sensors using enzymes and it is expected that some of these sensors will be used in the field in the near future. Few examples of phosphate biosensors are described below.

4.1. Phosphate sensor

Many enzyme sensors for inorganic phosphate have been fabricated since the report by

Guilbalut et al., which described the first phosphate sensor [6,7]. The enzyme reactions exploited in phosphate sensors are listed in Table 1. Several enzyme reactions can sometimes be combined. The first sensor by Guilbalut et al. [6,7] used two enzyme reactions catalyzed by alkaline phosphatase and glucose oxidase. The enzymes were mixed with gultaraldehyde and placed on a platinum electrode and the sensor detected the change in dissolved oxygen concentration before and after the enzyme reactions. Because the phosphate ion detection in aquatic environment demands measurement of concentrations as low as ppb (μM) levels, highly sensitive phosphate sensors have been developed since the first sensor.

Guilbault and Cserfalvi [7] suggested that phosphate could be detected by using an enzyme reaction catalyzed by phosphorylase A, and Wollenberger and Scheller [7,8] constructed a sensor based on the phosphorylase reaction that could measure mM concentrations of phosphate using an oxygen electrode.

A combination of nucleoside phosphorylase and xanthine oxidase was found to be more suited for rapid detection than the phosphorylase based sensor. Amplification by orthophosphate recycling was achieved using an enzyme reaction catalyzed by alkaline phosphatase. Wollenberger et al. also constructed a sensor using these three enzymes (i.e. phosphorylase, xanthine oxidase and alkaline phosphatase), which lowered the detection limit by 10-fold (10^{-2} μM) compared to that without alkaline phosphatase (10^{-1} μM) [7,9].

More recently, a combination of maltose phosphorylase and acid phosphatase has been used for phosphate ion detection. These sensors also showed low detection limits down to 10^{-1} μM [7].

Flow injection analysis (FIA) is a useful technique often used in flow type biosensors.

Table 1
Enzyme reactions used in phosphate sensors

First sensor

β-glucose 6-phosphate + H_2O → $^{\text{alkaline phosphatase}}$ β-glucose + orthophosphate

β-glucose + O_2 → $^{\text{gulcose oxidase}}$ D-glucono-1,5-lactone + H_2O_2

Sensitive sensor

glycogen + orthophosphate → $^{\text{phosphorylase A}}$ α-glucose-1-phosphate

Highly sensitive sensor (Amplification sensor)

inosine + orthophosphate → $^{\text{nucleoside phosphorylase}}$ ribose-1-phosphate + hypoxanthine

hypoxanthine + $2H_2O$ + $2O_2$ → $^{\text{xanthine oxidase}}$ uric acid + $2H_2O_2$

ribose-1-phosphate + H_2O → $^{\text{alkaline phosphatase}}$ ribose + orthophosphate

Highly sensitive and practical use sensor

pyruvate + orthophosphatase + O_2 → $^{\text{pyruvate oxidase}}$ acetylphosphatase + H_2O_2 + CO_2

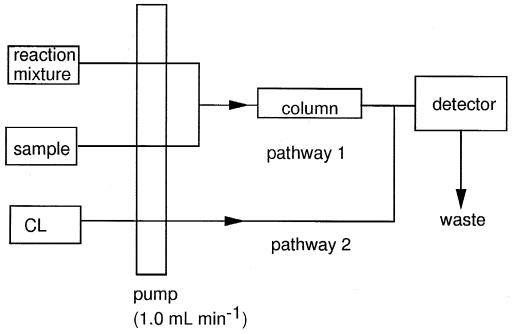

Fig. 1. FIA phosphate sensor. The system is based on two sequencial reactions: the first reaction catalyzed by pyruvate oxidase followed by luminol chemiluminescence reaction. The ''reaction mixture'' consists of pyrvate, thiaminpyrophosphate and flavin adenine dinucleotide in pH 7 HEPES buffer. ''CL'' is chemiluminescence reagent mixture (i.e. luminol). The reaction mixture is mixed with a phosphate sample before it is run through the column with immobilized pyruvate oxidase. The hydrogen peroxide (H_2O_2) produced in the reaction is detected by luminol chemiluminescence reaction.

As biosensors using FIA yield very rapid (usually within 5 min) and accurate measurements, FIA-based biosensors have been applied to various fields including environmental monitoring.

Nakamura et al. [10] and Ikebukuro et al. [11] investigated FIA systems based on the combination of the pyruvate oxidase reaction and chemiluminescence reaction (Fig. 1), which was reported to be a highly sensitive, automatic phosphate sensor system suitable for in-situ monitoring of reservoir waters for drinking. Their system uses a column-stuffed chitin-chitosan beads with immobilized pyruvate oxidase. Pyruvate oxidase catalyzes the reaction between pyruvate and phosphate, which produces acetylphosphate and hydrogen peroxide. Hydrogen peroxide is used in the well-known luminol chemiluminescence reaction. The detection limit of this sensor was 0.16 μM (5 ppb), which is below the maximum level allowed in drinking water in Japan. This sensor system is expected to be in field operation in the near future.

4.2. Organophosphate sensor

Many biosensors, such as pesticides, have been developed to detect toxicants in environment. Organophosphates and carbamates used as insecticides (e.g. paraoxon) are quite toxic but are widely used in modern agriculture. Jeanty and Marty [12], Vilatte et al. [13] and Bernabei et al. [14] reported both organophosphates and carbamate detection systems that were based on acetylcholinesterase (AchE) inhibition. AchE is an enzyme that catalyzes the hydrolysis reaction of acetylthiocholine to thiocholine which is inhibited by organophosphates (or carbamates). The degree of inhibition correlates with the change in the concentration of organophosphates.

Marty's sensor system [12] is composed of three electrodes; a platinum electrode with immobilized AchE (working electrode), a saturated calomel electrode as reference electrode. AchE was immobilized on the working electrode by photocrosslinking with PVA. They fabricated a reversible type sensor using oximes. Since the inhibition of AchE is achieved by the formation of a stable covalent enzyme–inhibitor complex, AchE could be reactivated by oximes such as pyridine-2-aldoxime methiodide. Using the oximes allowed the continuous use of the sensor. This sensor was able to measure 1 nM (ppb level) of paraoxon, which has the highest toxicity among organophosphates.

Other typical enzyme sensors developed for environmental monitoring include sensors for

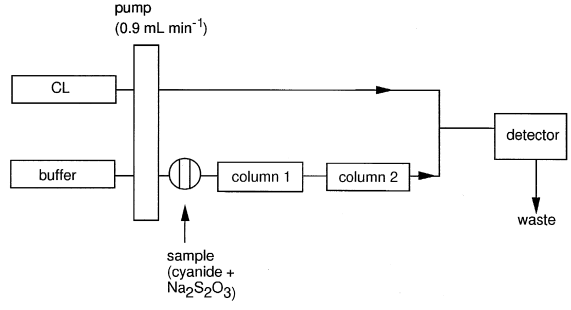

Fig. 2. Cyanide sensor using two enzyme reactions. Rhodanase and sulfite oxidase were individually immobilized in column 1 and 2. ''CL'' is chemiluminescence reagent mixture (i.e. luminol). A sample containing cyanide and thiosulfite ions is injected into phosphatebuffer solution (10 mM, pH 8.0), and run through the columns. The H_2O_2 produced in the reactions is detected by the same method as described in Fig. 1.

cyanide, nitrate or sulfite ions. Some of these sensors also employ FIA system. A cyanide sensor using FIA is shown in Fig. 2 [15]. Two enzymes, rhodanase and sulfite oxidase, were individually immobilized on modified agarose beads stuffed in columns which are connected sequentially. Rhodanase catalyzes the reaction of cyanide and thiosulfite ions to produce sulfite ions in the first column, and sulfite oxidase in the second column catalyzes sulfite oxidation to produce hydrogen peroxide. As in the case of phosphate sensor described above, the hydrogen peroxide is detected by luminol chemiluminescence reaction. The detection limit of the sensor was 10^{-2} μM.

Besides environmental monitoring purposes, there are numerous enzyme sensors that have been applied to other important fields such as medical diagnosis. For example, glucose sensors are used for diabetes treatment and represents one of the few but most commercially successful example of enzyme sensors.

Immobilization of biomaterials allowed biosensors to be used in the real world and further improvement in immobilization techniques has been and will be of intense interest. Another important, albeit relatively new, strategy for enhancing the stability of biosensors is to entirely or partially replace the biomaterials with synthetic, artificial sensing elements which mimic the functions of enzymes or antibodies. Both of these two approaches to enhance stability are being pursued today. With the vigorous research in biosensors for environmental monitoring and other fields conducted in laboratories around the world, it is only modest to expect that more examples of practically feasible sensors will be developed in the coming years.

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