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Development of photocatalytic biosensor for the evaluation of biochemical oxygen demand

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Abstract

The photocatalytic biosensor of flow system using semiconductor TiO_2 was developed to evaluate biochemical oxygen demand (BOD) levels in river water. Photocatalysis of sample was carried out in a photoreactor with TiO_2 and a 6 W black-light blue fluorescent tube as light source. Sample from a photoreactor outlet was measured by an oxygen electrode with a biofilm. The sensor response of photocatalytic biosensor was between 5 and 10 min depending on concentration of biochemical in the samples. At BOD of 1 mg l^{-1}, the sensor response increased 1.33-fold in comparison with that without photocatalysis. The degradation of tannic acid and humic acid with photocatalysis were 51.8 and 38.4%, respectively. Gum arabic and linear alkylbenzene sulfonate (LAS) were degraded a little, but gave the responses of more than double to the sensor. Free radicals yielded by photocatalysis in a photoreactor did not affect the sensor response because their lifetime is extremely short. Fairly good correlation ($r = 0.983$) between the sensor method and the conventional method was obtained for test samples. This biosensor using photocatalytic pretreatment improved the sensitivity.

Keywords: Photocatalysis; TiO_2; BOD sensor; Biosensor; Biofilm; *Pseudomonas putida*; Artificial wastewater

1. Introduction

Many countries have enforced many policies for the environmental protection and the environmental conservation, nevertheless environmental pollution become gradually serious. Particularly, rivers, the major drinking water sources are getting polluted. Biochemical oxygen demand (BOD) was one of the most typical methods determining pollutants in river. Since BOD sensor is first reported in 1977 (Karube et al., 1977), many research groups have developed BOD sensors that use various microorganisms and devices involving mediators (Morris et al., 2001; Liu and Mattiasson, 2002). In the present, the BOD_5 method is adopted (APHA, 1986), and this test method takes 5 days until results are obtained. Therefore our laboratory has developed a biosensor for the determination of BOD in river water (Chee et al., 1999, 2000). A standard solution used for this biosensor was artificial wastewater (AWW) instead of solution containing equal mass concentration of glucose and glutamic acid (GGA). AWW generally consisted of humic acid, lignin, tannic acid, gum arabic and LAS, which were recalcitrant organic compounds in river water (Murakami et al., 1978; Tanaka et al., 1994). This biosensor, however, often showed low values compared with the conventional BOD_5 in the evaluation of river waters. The results would show that recalcitrant organic compounds in river are uneasily assimilable to the immobilized microorganism in such a short measuring times.

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To overcome this problem, a pretreatment by photocatalytic oxidation was introduced in the sensor of batch system (Chee et al., 2001). Semiconductor photocatalysis has received considerable attention in recent years as an alternative for treating water polluted with recalcitrant or hazardous organic compounds (Hoffmann et al., 1995; Rajeshwar, 1995; Lu and Chen, 1997; Hasegawa et al., 1998; Bandala et al., 2002). Photocatalysis would have many potential applications for treating water containing organics, for removing oil from water, for degrading pollutants from air. Titanium dioxide was a widely applied photocatalyst which had been used for water treatment (Prousek, 1996; Rodriguez et al., 1996; Bolduc and Anderson, 1997; Vidal, 1998; Goutailler et al., 2002), predominantly for the oxidative destruction of uneasily biodegradable organic compounds (Kutsuna et al., 1993; Shama and Drott, 1997). The photocatalytic degradation of humic acid in acidic medium was better than that in basic one (Bekbölet and Özkösemen, 1996). The photodecomposition of Kraft lignin was investigated using titanium dioxide by Kobayakawa et al. (1989). The photodecomposition of lignin leads to a decrease of pH. Machado et al. (2000) assessed the role of hydroxyl radicals on photocatalyzed degradation of lignin.

In semiconductor photocatalysis for aquatic environment containing organic compounds, the overall process can be summarized by the following reaction equation (Prousek, 1996):

$$\text{organic compounds} + O_2 + \text{semiconductor}(h\nu \geq E\text{bg}) \rightarrow CO_2 + H_2O + \text{mineral acids}$$

Band gap illumination ($\lambda < 380$ nm) of a semiconductor particle causes electronic transitions from the valence band to the conduction band, leaving holes in the former. Then produced electrons and holes either migrate to the particle surface and become involved in redox reactions or they recombine and simply liberate heat. Conduction band electrons are consumed in reactions that reduce oxidants, while holes are filled via oxidation reactions. Hydroxyl radicals generated by the oxidation of aqueous solution at the valence band of titanium dioxide. In the valence band organic compounds are degraded to the compounds of lower molecular weight by hydroxyl radicals (Kamat, 1993; Hoffmann et al., 1995). Degraded organic compounds would be faster assimilable into microorganism immobilized in the biofilm. This will promise the increasing of the sensitivity of the sensor. In this work we have developed a photocatalytic biosensor using titanium dioxide for monitoring samples continuously by flow system, and used it in analysis of an environmental samples.

2. Experimental

2.1. Materials

Nitrohumic acid, EDTA, Scopoletin and Quinine were purchased from Wako Co. Ltd. (Japan), and TanniVer3, buffer solution sulfate and detergents reagent powder pillows from HACH (USA). Gum arabic was purchased from Sigma, and methylene blue from TCI Co. Ltd. (Japan). The other organic compounds used in this study were of reagent-grade.

TiO_2 (ST-A01, Ø 2 mm) granule was purchased from Ishihara Sangyo Co. (Japan), a 6 W black-light fluorescent tube from Ushio Co. (Japan), and an oxygen probe from Able Co. (Japan).

2.2. Cell cultures and preparation of biofilm

Pseudomonas putida SG10 was isolated and identified from the sewage disposal plant, as described in a previous paper (Chee et al., 1999). The composition of the medium growing bacterium was as follows per liter: nutrient broth, 2000 mg; nitrohumic acid, 84.92 mg; gum arabic, 93.90 mg; sodium ligninsulfonate (NaLS), 48.54 mg; tannic acid, 83.50 mg; linear alkylbenzene sulfonate (LAS), 18.84 mg and pH 7.0. *P. putida* SG10 was grown under aerobic conditions in a rotating shaker at 30 °C for 24 h in the medium described above. The cells were centrifuged and washed at 7000 rpm for 10 min, and were subsequently resuspended in 10 mM phosphate buffer (pH 7.0).

The biofilm for the BOD sensor was prepared using an aspirator connected to a syringe filter holder. A porous cellulose nitrate membrane (20 mm diameter, 0.45 μm pore size, Advantec, Japan) was carefully sandwiched in a syringe filter holder. Calculated amounts (wet cells 40 mg, $OD_{660} = 1.7$) of the pure culture broth were dropped on the cellulose nitrate membrane. The remnant culture broth was stored at 4 °C in the refrigerator before preparing a new membrane.

2.3. Construction of the photocatalytic BOD sensor

The photocatalytic BOD sensor was shown schematically in Fig. 1. The photocatalytic BOD sensor consists of a photoreactor with black-light fluorescent tube, an oxygen electrode with a biofilm, a digital meter (Model. TR6840, TakedaRiken, Japan) and an electronic recorder (Model. EPR-200A, TOA Electronics, Japan). An inner diameter of a photoreactor was 22 mm and an outer diameter 34 mm and a length 205 mm. Out-wall of a photoreactor was a stainless steel, and in-wall a quartz glass of thickness 1 mm. Plastic gum of doughnut was used to fix both ends of between the stainless and the quartz glass. A 15 mm diameter 6 W black-light blue fluorescent tube was inserted into in-wall of a quartz glass, and then both ends were fixed by an O-ring. Amount of TiO_2 in a photoreactor was 47 g.

The biofilm was placed on the top of the Teflon gas membrane covering an oxygen electrode, and then was tightly secured using 200 mesh nylon and an O-ring. An oxygen electrode was linked to both a digital multimeter and an electronic recorder. Also this electrode with a biofilm was connected to a detection reactor. The void volume of the detection reactor was 3 ml. The electrode with a biofilm and the detection reactor were installed in a temperature-controlled chamber at