Biosensors and Bioelectronics 25 (2010) 1705–1709

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An innovative reactor-type biosensor for BOD rapid measurement

Jianlong Wang[a,*], Yixin Zhang[a], Yeyao Wang[b], Runhua Xu[c,d], Zhonghua Sun[d], Zhou Jie[e]

[a] Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, PR China
[b] China National Environmental Monitoring Center, Beijing 100012, PR China
[c] Beijing-Tsinghua Industrial R&D Institute, Tsinghua University, Beijing 100084, PR China
[d] Tsinghua-Tonghe Environmental Technology Limited Company, Yacheng 224051, PR China
[e] Bureau of Science and Technology, Yancheng Municipal Government, Yacheng 224002, PR China

ARTICLE INFO

Article history:
Received 19 November 2009
Received in revised form
11 December 2009
Accepted 14 December 2009
Available online 23 December 2009

Keywords:
BOD
Biosensor
Water pollution monitoring
Immobilized microbial cell

ABSTRACT

Biochemical oxygen demand (BOD) is one of the most important and widely used parameters for characterizing the organic pollution of water and wastewater. In this paper, a novel reactor-type biosensor for rapid measurement of BOD was developed, based on using immobilized microbial cell (IMC) beads as recognition bio-element in a completely mixed reactor which was used as determining chamber, replacing the traditionally used membrane as recognition bio-element. The IMC beads were freely suspended in the aqueous solution, so the mass transfer resistance for dissolved oxygen and organic compounds significantly reduced, and the quantity of the microbial cells used as recognition element can be easily adjusted, in comparison with the traditional membrane-type BOD biosensor, in which exists a unadjustable contradiction between the quantity of biomass and the thickness of the bio-membrane, thus limiting the stability and the detection limit. This novel kind of BOD biosensor significantly increased the sensitivity of the response, the detecting precision and prolonged the life time of the recognition element. The experimental data showed that the most appropriate temperature for biochemical reaction in the reactor was 30 °C, and the IMC beads could keep the bioactivity for about 70 d at the detecting frequency of 8 times every day. The standard deviation of repeatability and the reproducibility of responses were within ±6.4% and ±5.0%, respectively, which are within acceptable bias limits, and meet the requirement of BOD rapid measurement.

1. Introduction

Biochemical oxygen demand (BOD) is one of the most important and widely used parameters for characterizing the organic pollution of water and wastewater, which is estimated by determining the amount of oxygen required by aerobic microorganisms for degrading organic matters in wastewater. The BOD value represents the amount of biodegradable organic compounds. The standard method for measuring biodegradable organic levels in wastewater is the 5-day biochemical oxygen demand assay (BOD_5) described by the APHA (1998). The BOD test has its widest application in measuring wastes loadings to treatment plants and in evaluating the BOD removal efficiency of such treatment systems (APHA, 1998). The BOD value has been determined conventionally by taking a sample of water, aerating it well, placing it in a sealed bottle, incubating for a standard period of time at 20 ± 1 °C in the dark, and determining the oxygen consumption in the water at the end of incubation. Usually the incubation time is 5 days and BOD values based on this standard are called BOD_5 value.

However, the conventional BOD method requires not only 5 days, but also experiences and skills. Therefore, the method using a sensor is a very attractive alternative because it can conduct a measurement within several minutes. Different kinds of BOD sensors for rapid measurement of BOD have been developed in last three decades. Hudson et al. (2008) investigated the possibility of using fluorescence spectrometry as a surrogate for BOD test in water quality assessment. Seo et al. (2009) developed a flow injection analysis system with encapsulated high-density *Saccharomyces cerevisiae* cells for rapid determination of BOD. Nakamura et al. developed a series of new methods for BOD measurement, including a chemiluminescence BOD measuring method, a spectrophotometric BOD determination method using 2,6-dichlorophenolindophenol as the redox color indicator and the eukaryote *S. cerevisiae*, a double-mediator system by ferricyanide and menadione using the eukaryote *S. cerevisiae*, and a absorption-based highly sensitive and reproducible biochemical oxygen demand measurement method for seawater using salt-tolerant yeast *S. cerevisiae* ARIF KD-003 (Nakamura et al., 2007a,b,c, 2008).

* Corresponding author. Tel.: +86 1062784843; fax: +86 1062771150.
E-mail address: wangjl@tsinghua.edu.cn (J. Wang).

0956-5663/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2009.12.018

BOD biosensor generally consist of a bio-membrane (containing biological recognition element) and an oxygen electrode (transducer), it can offer the possibility of faster estimation of a BOD_5-related parameter. The bio-membrane contains an immobilized bioactive material to catalyze the biochemical reaction. The change in the response of the oxygen electrode is measured in terms of electric current. Karube et al. (1977) reported the first BOD sensor based on the principle of biosensor. Since then, BOD biosensor has been developed in last three decades (Liu et al., 2004a,b; Chen et al., 2008a,b; Kara et al., 2009). However, all these BOD biosensors developed were membrane-type, i.e., they tended to immobilize the microbial cells in a porous membrane such as nitrate cellulose, acetate cellulose membrane and the like. This membrane-type BOD biosensor has several disadvantages, such as (1) due to the utilization of the microbial membrane in the sensor, during the process of the organic compounds and dissolved oxygen permeating the microbial membrane, there exists certain mass transfer resistance, which will result in the decline of dissolved oxygen (DO) to great extent; (2) the biosensor is not stable and the reproducibility of the measurement results is low because of the small amount of biomass immobilized in membrane; (3) the requirement for DO electrode is high due to the decline of DO through membrane, the DO after permeating the membrane declined remarkably, which requires high sensitive oxygen electrode, even though it leads to the inaccuracy of the measuring results.

In recent year, our research group has been carrying on the study on the rapid measurement of BOD using a novel BOD sensor, based on the completely mixed determining chamber containing immobilized microbial cell (IMC) beads instead of bio-membrane element used in conventional BOD sensor. The reactor-type BOD biosensor invented by our group can overcome the drawbacks of the membrane-type BOD biosensor (Wang, 2002; Wang et al., 2003a,b; Ye et al., 2005; Chen et al., 2007).

Entrapment of cells in polymeric matrixes is widely used for cell immobilization. Many natural and synthetic polymers have been used. Polyvinyl alcohol (PVA) is a promising type of synthetic polymer, which is cheap and nontoxic to microorganisms. It is very suitable for microbial immobilization (Wang et al., 1995).

We fabricated the recognition component by using polyvinyl alcohol (PVA) to immobilize the microbial cells, which forms small-size microbial spherical particles. The PVA gel has large specific area, porous surface, and the particles suspended in the aqueous solution dispersedly, so the mass transfer resistance for dissolved oxygen and organic compounds was much smaller, compared with the traditional membrane-type BOD sensor. Therefore, this kind of biosensor could enhance the accuracy of the measuring results. On the other hand, it could also shorten the measuring time because the quantity of the microbial cells was much higher than that of membrane-type BOD sensor.

The objective of this study was to investigate the response characteristics of the novel BOD biosensor, including the effect of temperature, the life time of IMC beads, and the repeatability and the reproducibility of the sensor BOD measurements.

2. Experimental

2.1. Chemicals

PVA (polyvinyl alcohol), calcium chloride, glucose and glutamic acid were purchased from Beijing Chemical Reagents Company. Other reagents were commercially available analytical reagents of laboratory grade materials.

2.2. Microorganisms

Activated sludge was collected from Beijing Gaobeidian Sewage Treatment Plant and cultivated under aerobic conditions at 30 °C. The growth medium consisted of $CaCl_2{\cdot}2H_2O$, 1 mg; $MgSO_4{\cdot}7H_2O$, 19 mg; K_2HPO_4, 4.4 mg; NH_4HCO_3, 26.8 mg; $C_6H_{12}O_6$, 0.1031 g. The pH of the medium was maintained at 7.0 ± 0.2.

2.3. BOD_5 standard solution

The BOD standard solution (150 mg l^{-1} glucose and 150 mg l^{-1} glutamic acid) was prepared according to standard procedures (APHA, 1998). This solution has a known BOD value of 198 ± 31 mg BOD_5/l. Higher strength BOD standards were prepared by increasing the GGA loading and lower strength BOD standards were prepared by appropriate dilution with distilled water.

2.4. Preparation of immobilized microbial cell particles

Ten gram of polyvinyl alcohol (PVA, nominal degree of polymerization = 1750, approx. molecular weight 75,000–80,000) was dissolved in 50 ml of distilled water, dissolved in boiling water bath, and then cooled to 40 °C, then mixed thoroughly with 50 mL of cell suspension with concentration of ca. 4.0×10^7 cells ml^{-1}. The resulting mixture was dropped into saturated boric acid solution and kept for 1 h to form spherical beads. The formed gel beads with an average diameter of 3 mm were then soaked in 0.5 M sodium orthophosphate solution for 1 h. The particles were washed with physiological saline, then dried at 4 °C for 24 h and stored at 4 °C before use.

2.5. Reactor fabrication

An innovative reactor for biochemical reaction was developed to reduce the mass transfer resistance as well as enhance the response signals of the sensor. A DO probe (ORION 850A, America) was used as the transducer, which was plugged in the centre of the reactor. The immobilized microbial cell particles were placed in a particularly designed cage, which was connected with a lift. A heating system was placed in bottom of the reactor for heating the aqueous sample to desired temperature, and the reactor was made by glass, which is a kind of insulated material. The volume of the reactor was about 100 ml.

2.6. Instrumentation

The major flow chart of the instrument was shown in Fig. 1. It includes a sample preparation unit, a reactor unit, a control unit, a data acquisition unit, and a data calculating unit.

2.7. Experimental procedure

The sample was pumped into the reactor and aerated. When the current output of the oxygen electrode reached a steady state, the steady current output I_0 was taken as the baseline current. After that, the elevator descended, leading the cage to the solution. When the immobilized microbial cells contacted the water sample, they metabolized the biodegradable organic compounds of the sample and consumed the dissolved oxygen, resulting in the output current decreasing. When the current output reached a new steady state, the steady current I_1 was recorded. The elevator ascended, the detection process finished. The response was taken as difference between the steady state current ΔI ($I_0 - I_1$).

Glucose and glutamic acid (GGA) solution was used as standard solution for the calibration of BOD sensor, as described in our previous paper (Zhang et al., 2001). The measurement of the

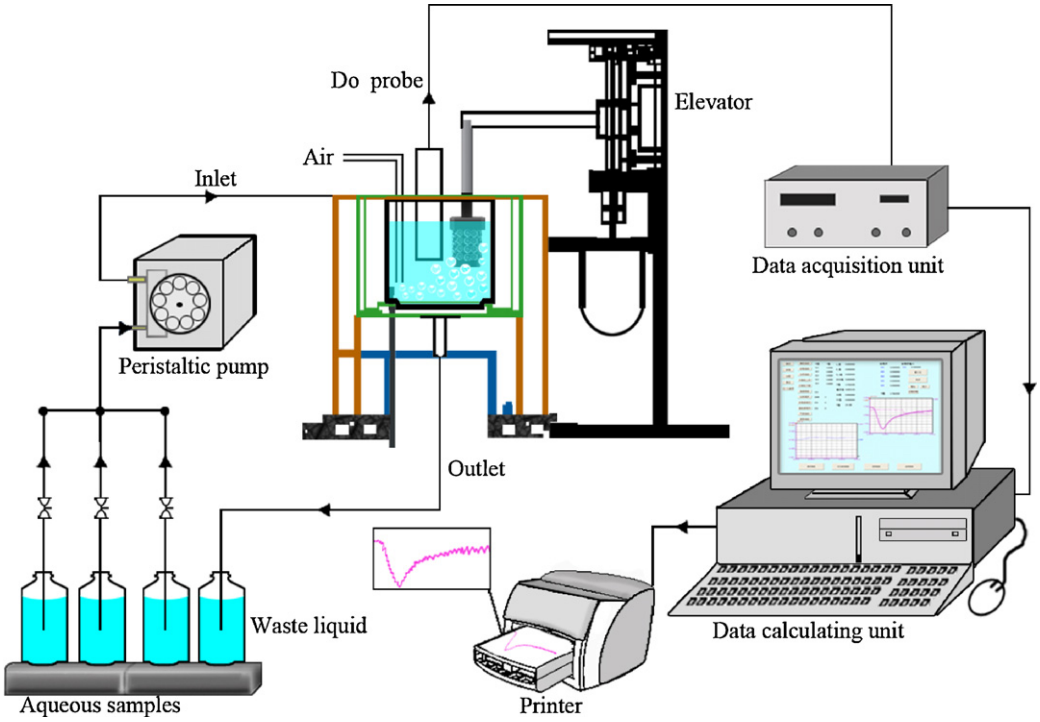

Fig. 1. Schematic process of the reactor-type BOD biosensor system.

conventional BOD_5 value was carried out by the standard method (APHA, 1998).

3. Results and discussion

3.1. Effect of temperature

Considering that the appropriate temperature for activated sludge from sewage treatment plant is about 20–30 °C, the temperature effect was examined by calibrating the sensor at 20, 25, 27, and 30 °C, using GGA solution.

The effect of temperature on the sensor response was shown in Fig. 2.

It can be seen that the temperature effect was consistent with the results reported by other researchers (Chee et al., 1999a, 1999b, 2000; Liu et al., 2000; Kim and Park, 2001; Kwok et al., 2005; Seo et al., 2009; Kara et al., 2009; Tan and Qian, 1997). Simultaneously, we could observe from Fig. 2 that the difference of detecting time was within than 2 min at 25, 27, and 30 °C. However, when it decreased to 20 °C, the detecting time was much longer, for example, the detecting time was only 15, 16, 16.8 min at 25, 27, and 30 °C, respectively, for 50 mg l^{-1} GGA solution. But to detect the same sample the detecting time was 20.5 min, prolonging nearly 36.7% compared to that at 30 °C. Therefore the optimum temperature was determined to be 30 °C.

3.2. Calibration of the sensor

The biosensor was calibrated under optimum conditions with standard solution of various BOD concentrations ranging from 5 to 100 mg l^{-1} prepared from stock GGA solution. The results were

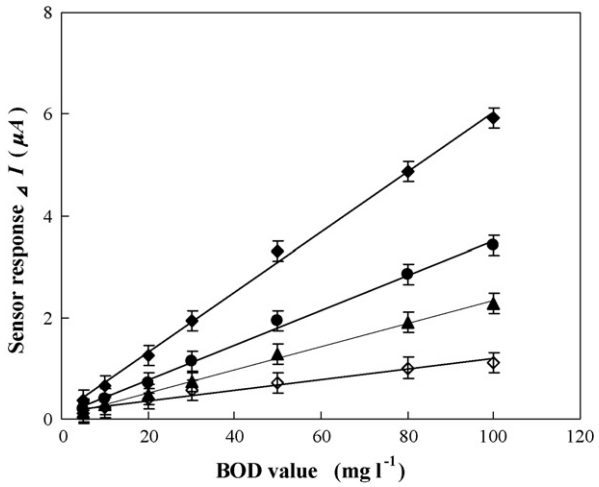

Fig. 2. Effect of temperature on biosensor response. (–♦–) 30 °C, (–●–) 27 °C, (–▲–) 25 °C, and (–◇–) 20 °C.

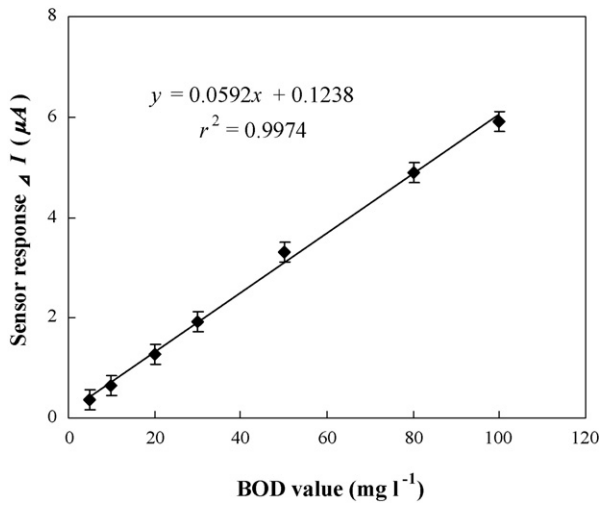

Fig. 3. The calibration curve of the biosensor.

Table 1
The repeatability comparison of the reactor-type and membrane-type BOD biosensor.

BOD value ($mg\,l^{-1}$)	Repeatability ($n=8$)					
	Average BODst ($mg\,l^{-1}$)		Standard deviation			
	Reactor-type	Membrane-type	Reactor-type		Membrane-type	
			$mg\,l^{-1}$	%	$mg\,l^{-1}$	%
20	20.97	18.53	±1.28	±6.4	±1.96	±9.8
30	30.68	27.02	±1.35	±4.5	±2.23	±7.4
40	41.23	38.46	±1.87	±4.6	±3.85	±9.6
50	51.50	46.51	±1.81	±3.6	±3.26	±6.5

illustrated in Fig. 3. It can be seen that the relationship of the sensor response and BOD_5 of the GGA solution is linear up to $100\,mg\,l^{-1}$ ($r^2 = 0.9974$).

The sensitivity of the biosensor was $0.0592\,\mu A/(BOD_5\,mg\,l^{-1})$ or about $0.035\,mg\,O_2\,l^{-1}/(BOD_5\,mg\,l^{-1})$ (Fig. 3), which was nearly 4 times higher than that of our previous researches (Zhang et al., 2001), using pure culture to fabricate the recognition bio-element. Such high sensitivity may possibly attribute to better metabolic capacity for a organic compounds of the recognition component.

3.3. Precision

The purpose of BOD sensor development is to obtain a fast alternative analytical method having at least an equally good performance (precision and bias) as the existing conventional test (Liu et al., 2004a). Precision covers random errors while bias covers systematic errors. The precision of the biosensor depends on two parameters: repeatability and reproducibility. Repeatability is the standard deviation of a single sensor tested by one operator under the same conditions, while reproducibility is the standard deviation of a series of sensors tested by more than one operator under different conditions. In order to compare the sensor precision with traditional membrane-type BOD biosensor, the repeatability of a commercialized BOD sensor (C1-Alpha-1 Type BOD Speedy Testing Instrument produced by Beijing Midwest Yuanda Technology Co. Ltd.) was tested under the same conditions.

The precision of two BOD biosensors has been studied using GGA standard solution in different concentrations (Table 1). As indicated in Table 1, for the reactor-type biosensor, the standard deviation of repeatability varied from ±3.6 to ±6.4%, while for the membrane-type biosensor, the standard deviations of repeatability varied from ±6.5 to ±9.8%. For $30\,mg\,l^{-1}$ GGA standard solution, the standard deviation of the reactor-type biosensor was 4.5%, lower than that of membrane-type biosensor, 7.4%. It was also lower than that reported by Liu et al. (2004a).

The standard deviation of the reactor-type biosensor reproducibility was also determined, the results was shown in Table 2.

As shown in Table 2, the reproducibility standard deviation was from ±3.45% to ±4.87%. Liu et al. (2004a,b) studied to apply the short-term BOD (BODst) as a parameter for on-line monitoring of biological treatment process, their results indicated that the standard deviations of sensor repeatability varied from ±2.5 to ±7.5%, whereas the standard deviations of sensor reproducibility was between ±1.2 and ±7.3% (Liu et al., 2004a,b). In comparison with their results, the reactor-type biosensor invented by our group seems to be more stable.

Table 2
The reproducibility of the reactor-type BOD biosensor.

BOD ($mg\,l^{-1}$)	Reproducibility ($n=8$)		
	Average BODst ($mg\,l^{-1}$)	Standard deviation	
		$mg\,l^{-1}$	%
20	19.76	±0.69	±3.45
30	31.47	±1.46	±4.87
40	42.33	±1.94	±4.85
50	54.72	±2.18	±4.36

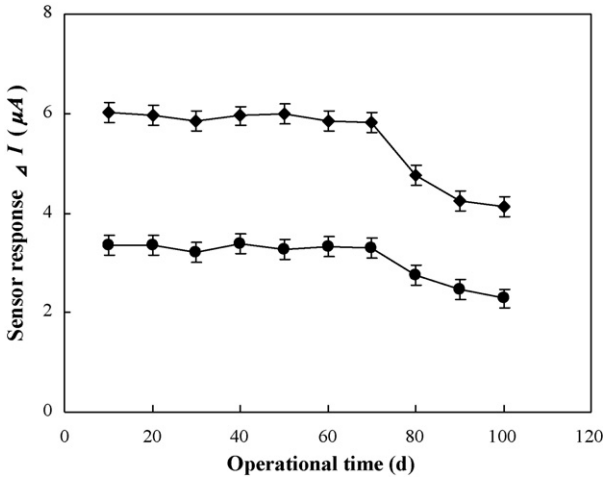

Fig. 4. The operational stability of the sensor response. (–♦–) 50 mg l^{-1}, (–●–) 100 mg l^{-1}.

3.4. The sensor stability

The operational stability of the sensor is primarily related to the stability of the immobilized microbial cell particles used as recognition element. Fig. 4 demonstrated that the sensor response was stable for 70 d at the detecting frequency of 8 times every day.

In traditional membrane-type BOD biosensor, the recognition bio-element was immobilized in a porous membrane. Usually, the life time of the membrane was about 24 h to 50 d (Tan and Wu, 1999; Yoshida et al., 2001; Jia et al., 2003; Li et al., 1994; Liu et al., 2004a,b; Kwok et al., 2005; Chen et al., 2008a,b). The life time of the IMC particles used as recognition element for the reactor-type BOD biosensor was longer, suggesting that the biosensor was more stable during the practical application.

3.5. Agreement between the BODst and BOD_5

The BOD sensor was used to analyze the municipal wastewater. As a comparison, BOD_5 values of the samples, meanwhile, were

Table 3
Comparison of sensor BOD and BOD_5 for municipal wastewater.

Sample No.	BOD sensor ($mg\,l^{-1}$)	BOD_5 ($mg\,l^{-1}$)	Difference (%)
1	24.1	26.9	10.4
2	85.4	90.0	5.1
3	129.6	135.0	4.0

estimated according to the American standard procedure (APHA, 1998). The results were listed in Table 3.

As shown in Table 3, there was a good agreement between the results of the sensor BOD measurement and conventional BOD_5 analysis.

4. Conclusions

The novel reactor-type BOD biosensor was more effective for BOD measurement, in which IMC beads were used as recognition element for detecting biodegradable organic compounds in samples. The IMC beads suspended in the reactor could decrease the mass transfer resistance for dissolved oxygen and organic compounds, which significantly increased the sensitivity of the sensor response and the detecting precision, it also prolonged the life time of the recognition element to about 70 d. The standard deviation of repeatability and reproducibility of the reactor-type BOD biosensor was between ±3.6 to ±6.4% and between ±3.45% to ±4.87%, respectively, indicating that it can meet the requirement of BOD rapid measurement.

Acknowledgements

This work was supported by the High-Tech Program (863) of China (Grant No. 2007AA06A404) and Key Industralization Project of Jiangsu Province (Grant No. BA2009100).

References

APHA, 1998. Standard Methods for the Examination of Waters and Waste-Waters, 20th ed. American Public Health Association, Washington, DC.
Chee, G.J., Nomura, Y., Karube, I., 1999a. Anal. Chim. Acta 379, 185–191.
Chee, G.J., Nomura, Y., Ikebukuro, K., Karube, I., 1999b. Anal. Chim. Acta 394, 65–71.
Chee, G.J., Nomura, Y., Ikebukuro, K., Karube, I., 2000. Biosens. Bioelectron. 15, 371–376.
Chen, H., Ye, T., Qiu, B., Chen, G., Chen, X., 2008a. Anal. Chim. Acta 612, 75–82.
Chen, J., Yu, Z., Sun, J., Jia, J., 2008b. Wat. Environ. Res. 80 (8), 699–702.
Chen, J.S., Zhang, L.S., Wang, J.L., 2007. Biomed. Environ. Sci. 20, 78–83.
Hudson, N., Baker, A., Ward, D., Reynolds, D.M., Brunsdon, C., Carliell-Marquet, C., Browning, S., 2008. Sci. Total Environ. 391, 149–158.
Jia, J.B., Tang, M.Y., Chen, X., Qi, L., Dong, S., 2003. Biosens. Bioelectron. 18, 1023–1029.
Kara, S., Keskinler, B., Erhan, E., 2009. Chem. Technol. Biotechnol. 84, 511–518.
Karube, I., Matsunaga, T., Mitsuda, S., Suzuki, S., 1977. Biotechnol. Bioeng. 19 (10), 1535–1547.
Kim, M.N., Park, K.H., 2001. Sens. Actuators B: Chem. 80, 9–14.
Kwok, N.Y., Dong, S., Lo, W., Wong, K.Y., 2005. Sens. Actuators B: Chem. 110, 289–298.
Li, F., Tan, T.C., Lee, Y.K., 1994. Biosens. Bioelectron. 9, 197–205.
Liu, J., Björnsson, L., Mattiasson, B., 2000. Biosens. Bioelectron. 14 (12), 883–893.
Liu, J., Olsson, G., Mattiasson, B., 2004a. Biosens. Bioelectron. 20, 562–570.
Liu, J., Olsson, G., Mattiasson, B., 2004b. Biosens. Bioelectron. 20, 571–578.
Nakamura, H., Abe, Y., Koizumi, R., Suzuki, K., Mogi, Y., Hirayama, T., Karube, I., 2007a. Anal. Chim. Acta. 602, 94–100.
Nakamura, H., Kobayashi, S., Hirata, Y., Suzuki, K., Mogi, Y., Karube, I., 2007b. Anal. Biochem. 369, 168–174.
Nakamura, H., Suzuki, K., Ishikuro, H., 2007c. Talanta 72, 210–216.
Nakamura, H., Mogi, Y., Hattori, H., Kita, Y., Hattori, D., Yoshimura, A., Karube, I., 2008. Anal. Chim. Acta 620, 127–133.
Seo, K.S., Choo, K.H., Chang, H.N., Park, J.K., 2009. Appl. Microbiol. Biotechnol. 83, 217–223.
Tan, T.C., Qian, Z., 1997. Sens. Actuators B: Chem. 40, 65–67.
Tan, T.C., Wu, C., 1999. Sens. Actuators B: Chem. 54, 252–260.
Wang, J.L., 2002. Microbial Immobilization Techniques and Water Pollution Control. Science Press, Beijing.
Wang, J.L., Hou, W.H., Qian, Y., 1995. Biotechnol. Tech. 9 (3), 203–208.
Wang, J.L., Wen, X.H., Shi, H.C., 2003, China Patent, 00132126.9.
Wang J.L., Zhang Y., Shi H.C., 2003, China Patent, 00132125.0.
Ye, Y.C., Wang, J.L., Pu, L.F., 2005. Chin. J. Anal. Chem. 33, 405–410.
Yoshida, N., Hoashi, J., Morita, T., McNiven, S.J., Nakamura, H., Karube, I., 2001. J. Biotechnol. 88, 269–275.
Zhang, Y., Wang, J.L., Li, H.Z., Shi, H.C., Zhu, J.R., 2001. Marine Sci. Bull. 3 (1), 69–73.