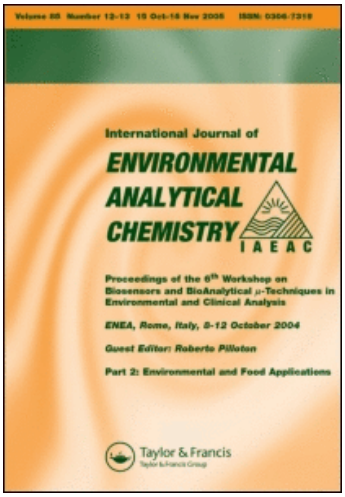

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Comparison of BOD optical fiber biosensors based on different microorganisms immobilized in ormosil matrixes

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COMPARISON OF BOD OPTICAL FIBER BIOSENSORS BASED ON DIFFERENT MICROORGANISMS IMMOBILIZED IN ORMOSIL MATRIXES

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Fiber-optical microbial sensors for determination of biochemical oxygen demand (BOD) are described. Sensing films consisting of layers of an oxygen-sensitive fluorescent material and two different kinds of seawater microorganisms immobilized in poly(vinyl alcohol) sol–gel matrix were investigated. Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) perchlorate was used as the oxygen fluorescent quenching indicator. After preconditioning, the BOD biosensors could consistently perform well for up to one month. For films of domestic bacilli and films of sieved bacteria from seawater, the linear fluctuant coefficients (R^2) in the range of 4–200 mg/L were 0.9975 and 0.9783 when a glucose/glutamate BOD standard was applied. The relative error of standard deviations for the two microorganism-immobilized BOD sensing films were within 4% and 2% of the mean value, respectively. The effects of temperature, pH and sodium chloride concentration on the two microbial films were also studied. For low biochemical oxygen demand, a film of sieved bacteria from seawater had superior sensitivity and is expected to be developed further.

Keywords: BOD biosensor; Microorganisms; Sol–gel; Ormosils

INTRODUCTION

The authorized method for the determination of BOD (biochemical oxygen demand) is BOD_5, which must last five days under specified standard incubation and is not suitable for *in situ* determination [1,2]. Since Karube *et al.* [3] developed a rapid and reliable biosensor for BOD determination in 1977, biosensor development has been driven by the imperative need for simple, rapid and continuous *in situ* monitoring techniques in environmental areas. BOD biosensors have been developed by applying several treatments and a variety of microorganisms [4–7], while some of the considerations are focused on fiber-optic sensors [4].

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Silicones, Teflon, plasticized poly(vinyl chloride), cellulose and poly(vinyl acetate) are suitable polymer materials for immobilization, and the sol–gel process is a new and attractive technology. Silicate glasses obtained by the sol–gel method are promising host matrixes for entrapping microorganism owing to their chemical inertness, thermal stability and transparency, enabling their use as fiber-optic transducers. Microencapsulation in the pores of sol–gel matrixes can isolate the microorganisms, while maximally maintaining their activity. Almost all reports of biomolecular immobilization have described the employment of a silica-based sol–gel. Chen *et al.* [8] reported an effective and useful BOD sensor, in which a sol–gel acted as the immobilizing material to immobilize yeast in the sol–gel host matrix on an oxygen electrode. Aerobic nonpathogenic and survivable microorganisms have many advantages in biosensor applications such as tolerance of changes in pH or temperature, easy isolation from natural sources, exemption from extensive sample preparation and facile regeneration via the regrowing of the cells.

In our work, ormosil–PVA was used as a matrix to immobilize two different seawater bacteria, sieved bacteria from seawater and domestic bacilli. The microbial film was laid over an oxygen film and the complex film was placed in a sample cell. We compared the two different microbial films in terms of response time, reproducibility, linear range, temperature, pH and sodium chloride concentration. The results proved that the sieved bacteria from seawater are a more appropriate biological recognition element than domestic bacilli for the estimation of low values of BOD.

EXPERIMENTAL

Apparatus

A home-made optochemical BOD sensor as shown in Fig. 1 was employed for acquiring the change of fluorescence intensity. The sensing film was placed in the middle of

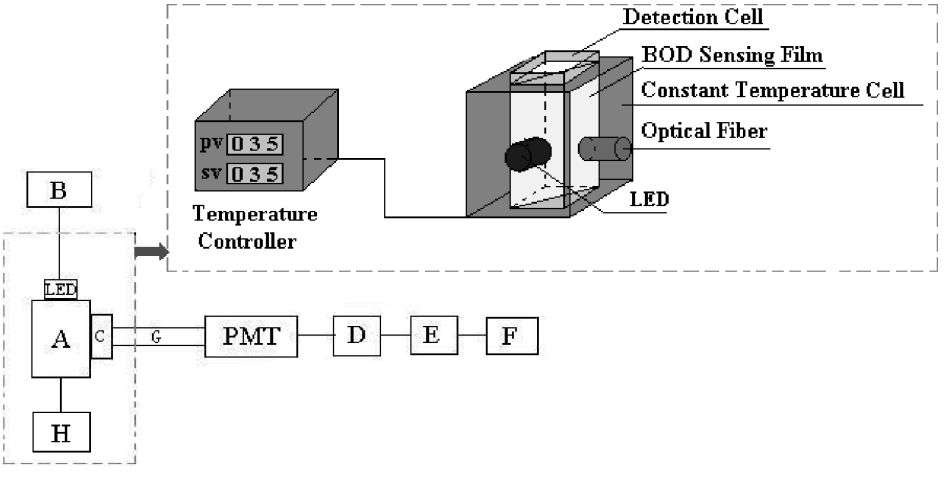

FIGURE 1 System of fiber-optical BOD microbial sensor. A, sample cell; B, power supply; C, optical window; D, GD-1glimmer meter; E, Echrom98 Chromatogram Processor; F, computer; G; optic fiber; H, temperature controller; LED, light-emitting diode; PMT, photomultiplier tube.

the detection cell, whose temperature was kept constant by a temperature controller with a precision of ±0.2°C. An excitation light, wavelength 465 nm from a blue LED, was directed onto the sensing layer at an angle of, typically, 45°. The emission fluorescence was filtered by a cut-off filter with a half band width of 10 nm at 580 nm, and transferred by an optical fiber to a GD-1glimmer measure (Xi'an Reike Electronic Instrument Co., Ltd.) equipped with a R928 PMT (Hamamatsu, Japan). The experimental results were then processed by an Echrom98 chromatogram workstation (Dalian Elite Scientific Instruments Co., Ltd.).

Chemicals and Standard Solution

Tetramethoxysilane (TMOS) and poly(vinyl acetate) (PVA) were purchased from Aldrich (Milwaukee, WI, USA). Dimethyldimethoxysilane (DiMe-DMOS) was obtained from Fluka AG (Buchs, Switzerland). All were used without further purification. The $[Ru(Ph_2phen)_3](ClO_4)_2$ (Ph_2phen = 4,7-diphenyl-1,10-phenanthroline), acting as the oxygen-sensing indicator, was synthesized and purified in the laboratory of Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University. The 8% (w/w) PVA solution was prepared by dissolving PVA in hot twice-distilled deionized water. The buffer solution employed was 0.067 mol/L KH_2PO_4–Na_2HPO_4 buffer (pH 7.4). The standard BOD solution (1000 ± 185 mg/L, GGA) was prepared by adding 0.0750 g glucose and 0.0750 g L-glutamic acid into 100 mL phosphate buffer solution (pH 7.2). The seawater samples were taken from the area around Xiamen University and filtered using 10-μm isopore membrane filters (Millipore, USA) before determination. All other chemicals were of analytical grade and distilled water was used throughout.

Oxygen-sensing Film Preparation

In a typical experiment, ormosils were made for oxygen-sensing films by mixing TMOS of different molar ratios with organosilicon. 1.0 mL of TMOS and 1.6 mL of DiMe-DMOS were added to an open vial. After the mixture was magnetically stirred for approximately 1 min, 2.0 mL of 0.01 mol/L hydrochloric acid was added dropwise to the mixture. The whole solution was then immersed in a water bath at 60°C and stirred for 3 h. An emulsion was formed during this step. The emulsion was kept for 2 h, then 0.2 mL of $[Ru(dpp)_3]^{2+}$, concentration 1.5 g/L in THF, was added to the solution. The mixture was then vigorously stirred for 20 min to ensure thorough mixing. Films were prepared by pippetting 60 μL of the mixture onto a glass slide, which had been soaked in concentrated nitric acid for 12 h and washed with distilled water and ethanol. The resulting films were left undisturbed under ambient conditions for 12 h. Finally, individual films were thermally cured in an oven for another 12 h at 150°C and were ready for use after the films cooled to room temperature. The thickness of the films prepared in this way was estimated to be about 5 μm. Variation of film thickness can be achieved by pippetting different amounts of the mixture onto the glass slide.

Microorganisms and Incubation

Two kinds of microorganisms were selected and applied for BOD sensing films. Sieved bacteria were selected and obtained from the seawater around the Xiamen conurbation

according to its assimilation efficiency to BOD, and then distilled solely from incubation solution. The other kind were domestic bacilli limnetic bacteria and domesticated in seawater. The incubation solution for sieved bacteria from seawater contained 0.5% beef extract, 1% peptone and 0.1% glucose in seawater, while for domestic bacilli an incubation solution of 0.8% beef extract, 1% peptone and 0.5% amylum in seawater was used. The microorganisms were fostered under aerobic condition in a 250-mL conical flask and a rotating shaker at 35°C for 24 h, then enriched by centrifugation at 5000 rpm for 15 min, washed twice with phosphate buffer (pH = 7.4) and subsequently suspended in the same buffer.

Immobilization of Microbial Cells

An organically modified silicate (ormosil) was prepared by mixing TMOS, DiMe-DMOS (1 : 1.2, v/v) and 0.01 mol/L HCl in a 5-mL flat-bottomed flask for immobilization of microbial cells. The ormosil was stirred magnetically at 60°C for 1 h, then mixed with an equal volume of 8% (w/w) aqueous solution of PVA and the microbial suspensions. The sol mixture was spread onto an optical oxygen-sensing film produced by immobilizing the $[Ru(Ph_2phen)_3](ClO_4)_2$ in sol–gel [9]. The area of BOD sensing film was kept within 25 mm × 25 mm, and the thickness was estimated to be 0.20 mm. The obtained microbial films were later dried at room temperature for 24 h and stored in 100 mg/L GGA solution at 4°C before use.

Experimental Procedure

BOD measurement was performed in a batch mode in which the BOD sensing film was placed in a black and airtight detection cell and the determined sample solution was kept undisturbed in all measurements. The initial steady-state output of the sensor represented the endogenous respiration state of the immobilized microorganisms. A typical response of the BOD sensing film is presented in Fig. 2. As shown by 'a' in Fig. 2, when the sample solution (15 mL) was added to the cell, consumption of

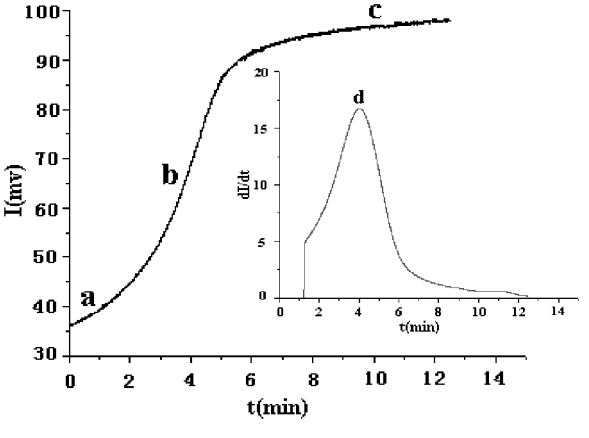

FIGURE 2 Typical response of the BOD sensor when exposed to a standard BOD solution. Experimental conditions: constant temperature, 35°C; pH of buffer, 7.4; concentration of NaCl, 3.2%; concentration of BOD, 30 mg/L. Film with immobilized sieved bacteria from seawater was used.

the dissolved oxygen surrounding the microbial film started and the output of the sensor increased gradually accordingly. After a while, the microorganisms stepped into an active respiration state, decomposing various organic substances, leading to rapid consumption of the oxygen surrounding the microbial film and resulting in a rapid increase of the output of the fluorescence intensity ('b' in Fig. 2). When the consumption and diffusion rates of BOD on the microbial film were again equal, the immobilized microorganisms regained the steady endogenous respiration state and the output gradually reached a steady state ('c' in Fig. 2). The results showed that there was a linear relationship between the maximum rate of change of fluorescence intensity (dI/dt) and BOD values ('d' in Fig. 2).

RESULTS AND DISCUSSION

Effect of Quantities of PVA and Immobilized Microbial Cells

PVA is a nontoxic and hydrophilic material to the immobilized microbial cells and is capable of maintaining the maximum microbial activity. It readily dilates in water and may flake away from the oxygen-sensing film, but mixing with ormosil can overcome this shortcoming. The PVA content has a great effect on the physical characteristics of the microorganism films. The presence of DiMe-DMOS in the ormosil gives the matrix two methyl groups, resulting in poor affinity for water so that it does not undergo hydrolysis through the typical sol–gel process. The Si-CH_3 groups have replaced the surface Si-OH groups and consequently enhanced the surface hydrophobicity, which increases as a function of DiMe-DMOS content in the film, and this accounts for the enhanced response and the flexibility of the microbial sensing layer. However, further improving the hydrophobicity will not guarantee sufficient solubility for PVA in the ormosil matrix and may increase the response time. Under our preparation conditions, the optimized volume ratio of DiMe-DMOS to TMOS was 1.2 to 1. Based on the experimental results, as the content of PVA in the BOD sensing film increased, the response intensity of microbial film rose and the response time reduced accordingly. However, when the percentage of PVA reached 10%, the film began to dilate and flake away from the oxygen film, so a content of 8% PVA in ormosil was selected.

One would wish to have a microbial layer as thick as possible in order to achieve a large signal change even at low BOD. As expected, with an increase in quantity of immobilized microbial cells the sensor response went up and the response time decreased. However, increasing the thickness of the microbial ormosil–PVA layer to above 0.2 mm caused an increase in the response time, due to the diffusion of GGA and the limitation of dissolved oxygen by the thickness of films

Fluorescent Response and Linearity of BOD Sensing Film

Calibration curves were established for the two microbial films using diluted GGA solutions. Linear relationships were observed between the fluorescent intensities of BOD sensing films and BOD concentrations in the range 0.5 mg/L to 200 mg/L BOD. Taking into account the application for seawater pollution, the linearity curves of BOD at lower concentration (< 10 mg/L) are shown in Fig. 3. The detection limits of

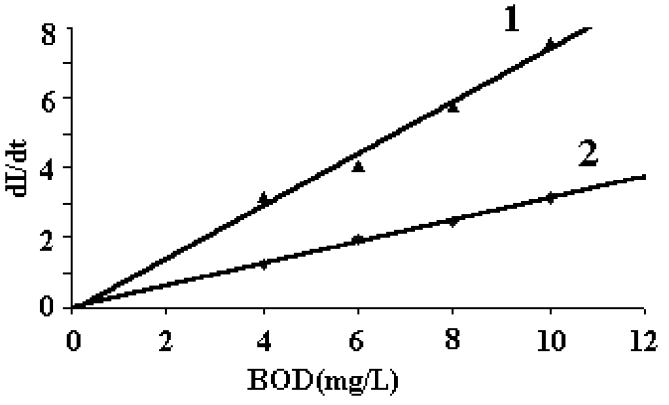

FIGURE 3 Linear responses of BOD sensing films to different BOD concentration: 1, Film immobilized with sieved bacteria from seawater (film 1); 2, Film immobilized with domestic bacillus (film 2); Experimental conditions: the same as for Fig. 2.

BOD for the two kinds of BOD sensing films were both 1 mg/L, and the response time of the sensors depended on the BOD level. For the film of domestic bacilli, it took between 30 and 14 min to reach the maximum rate of change in the fluorescence intensity (dI/dt), while the film of sieved bacteria from seawater took between 23 and 32 min, with a BOD concentration between 4 mg/L and 10 mg/L. The sensing film containing sieved bacteria from seawater was more sensitive than the film with domestic bacilli, the curve slopes being 0.7510 and 0.3115, respectively. For a BOD of 100 mg/L at 35°C and pH 7.4, for film 1 and film 2, the relative error of standard deviation for the two films made in different batches was 4% and 2% of the mean value of six replicates, and the reproducible responses for the same film could be obtained within $\pm 2.54\%$ and $\pm 0.95\%$ ($n = 6$) for film 1 and film 2, respectively.

Effect of pH

The physiological state of the microorganisms, in particular their respiratory activity, strongly depends on the pH of the solution. To determine the optimum conditions for measurements using sieved bacteria from seawater and domestic bacilli, the influences of pH on BOD response were investigated for BOD of 100 mg/L in phosphate buffer at 35°C.

The effect of pH was investigated from pH 5.6 to 9.0. As shown in Fig. 4, the fluorescent response increased rapidly and reached a maximum at pH 7.4, then decreased, while the response time decreased to pH 7~8 and then rose rapidly. Though there were some differences between the two kinds of BOD sensing films, the optimum pH for both was 7.4 when considering response signal and response time together. As seawater bacteria, sieved bacteria from seawater and domestic bacilli were conditioned to living in seawater with a pH around 7~8. At such pH the respiration of bacteria was active and the consumption of organic substances was correspondingly rapid, but at lower or higher pH values the bacteria appeared inactivated. Therefore, in subsequent experiments, the pH of the samples was adjusted to 7.4 using phosphate buffer or seawater directly.

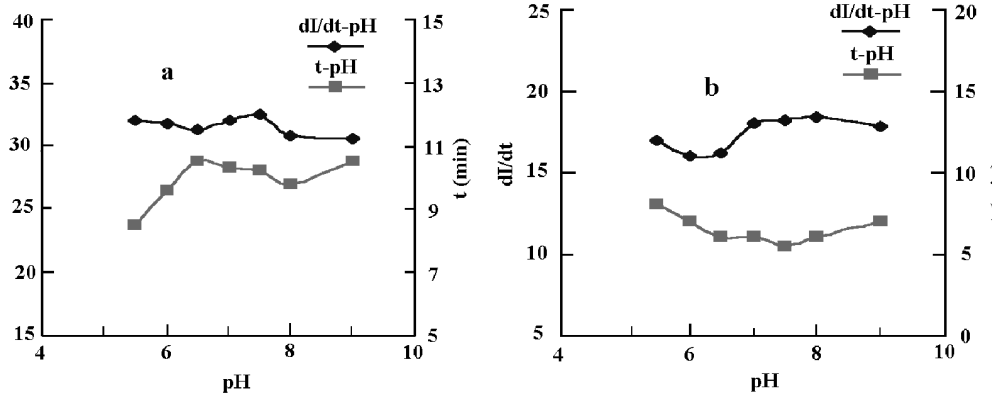

FIGURE 4 Effect of pH on the sensor response, (a) film 1; (b) film 2. Experimental conditions: as for Fig. 2; GGA concentration, 50 mg/L.

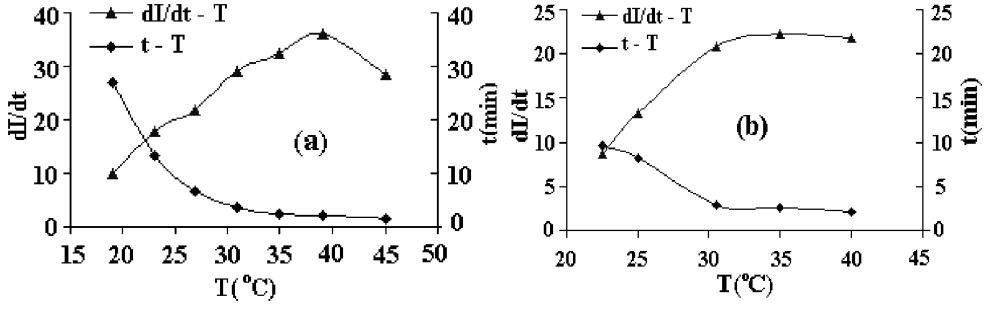

FIGURE 5 Effect of temperature on the sensor response (C_{GGA}: 200 mg/L in seawater), (a) film 1; (b) film 2. Experimental conditions: as for Fig. 2; GGA concentration, 50 mg/L.

Effect of Measurement Temperature

Temperature is one of the most important factors and exerts a great influence on microorganism metabolism. An appropriate temperature can promote the respiration of bacteria and enhance activation, while, conversely an inappropriate temperature will change the bacteria's conformation and metabolism and may even cause protein solidification, resulting in death of the bacteria.

For the film of sieved bacteria from seawater, the optimum temperature was studied in the range 19°C to 45°C and found to be 39°C (Fig. 5a), above which the fluorescence intensity decreased rapidly, probably because of inactivation of the microorganism due to heating. The response time decreased rapidly to 35°C, after which only a slow decrease of response time was obtained. As a result, in order to prolong the lifetime of the bacteria, a temperature of 35°C was selected in studying the films of sieved bacteria from seawater.

For the film of domestic bacilli, the optimum temperature was studied in the range 22.5°C to 40°C. As shown in Fig. 5b, the response intensity and time both remained constant above 30°C, which indicated that the film of domestic bacilli was stable in a certain range of temperature compared with the film of sieved bacteria from seawater. In this case, an optimum temperature of 35°C was chosen for the experiment, which was also the incubation temperature for the two kinds of microorganisms.

Effect of Chloride Anion and Other Coexisting Ions

Since chloride anion is the most commonly existing substance in seawater, normally with a concentration of 3.2%, the influence of chloride ion on the BOD sensor response has to be examined. Most biosensors with immobilized limnetic microorganisms are influenced negatively by chloride anion and the response decreases rapidly with increase of chloride ion concentration [8].

In this study, the influence of chloride anion on the response of the two sensors was studied from 0% to 6% in phosphate buffer (pH 7.4) at 35°C and a BOD of 100 mg/L. For the film of immobilized sieved bacteria from seawater, the maximum fluorescent response appeared at about 1%, while for the film of domestic bacilli, it was at about 3%, and the response times also reached minima at 1% and 3%, respectively (Fig. 6). This means that the two kinds of BOD sensing films are able to withstand the inhibition by high salt concentrations of microorganism respiration. Moreover, in a suitable concentration of chloride anion, the response of the biosensor could reach a maximum value with the least response time. Comparing the two sensing films, the film of domestic bacilli has better performances than those of the film of sieved bacteria from seawater since the maximum permissible concentration of chloride anion was similar to that in seawater.

The effect of coexisting compounds is another essential factor for BOD sensing film. Our results showed that there were no obvious changes when the determination solution was saturated with nitrogen, ammonia or hydrogen sulfide. Similar work was performed to test the effect of most metal ions at a concentration of 100 mg/L on the fluorescent intensities. Except for silver and lead cations, the prepared BOD sensing films were almost independent of those ions owing to their highly hydrophobic surface (Table I).

Stability and Service Time

Generally, the BOD sensing films needed reactivation after various times of storage. Typically, the sensing films were stored in GGA solutions at 4°C when not used. The stability of the sensors was determined after they had been immersed in the GGA solution (BOD of 100 mg/L) at pH 7.4 and 35°C. The reactivation concentration of GGA has no obvious effects on the responses of the films. The response times of the films for 4 mg/L BOD ranged from 28 to 32 min for film 1 and 27 to 30 min

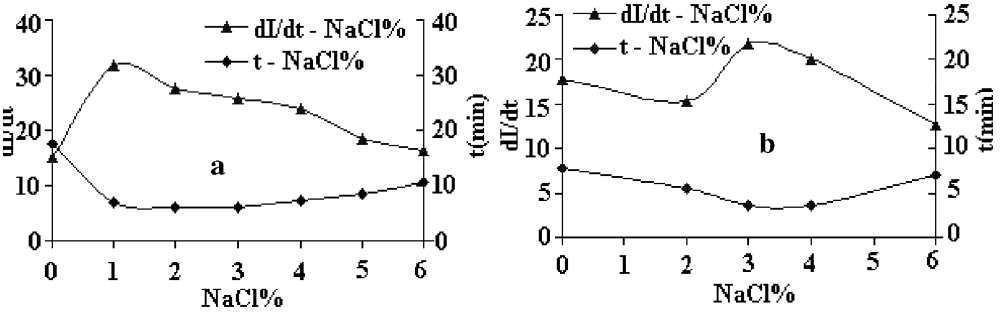

FIGURE 6 Effect of chloride ion concentration on the sensor response, (a) film 1; (b) film 2. Experimental conditions: as for Fig. 2; GGA concentration, 50 mg/L.

TABLE I Effect of some coexisting metal ions on the response of BOD sensing film with immobilized sieved bacteria from seawater

M^{n+}	$\Delta I/I_0 \times 100\%$	M^{n+}	$\Delta I/I_0 \times 100\%$	M^{n+}	$\Delta I/I_0 \times 100\%$	M^{n+}	$\Delta I/I_0 \times 100\%$	M^{n+}	$\Delta I/I_0 \times 100\%$
Al(III)	+7.78	Fe(III)	−6.98	Mg(II)	−3.68	Ba(II)	+6.97	Ca(II)	+3.85
Cu(II)	−1.45	Zn(II)	+3.95	As(III)	−4.47	Bi(III)	+0.85	Mn(II)	+6.99
Sr(II)	+3.73	W(VI)	+4.26	V(V)	+3.05	Ni(II)	−6.13	Sn(II)	−3.51
Co(II)	+0.45	Cd(II)	+5.03	Mo(VI)	+0.93	Se(VI)	−6.65	Li(I)	+7.53
Cr(III)	−1.60	Ti(IV)	−5.88	Pb(II)	−14.44	Ag(I)	−81.10		

The concentration of each ion is 100 mg/L, the concentration of BOD is 20 mg/L, in 0.067 mol/L KH_2PO_4–Na_2HPO_4 buffer (pH 7.4).
I_0: Initial fluorescent intensity, I: fluorescent intensity after coexisting substance added, $\Delta I = I - I_0$.

TABLE II Comparison of two kinds of BOD sensing films

Parameter	*Film of sieved bacteria from seawater (film 1)*	*Film of domestic bacilli (film 2)*
R^2	0.9783	0.9977
Working linear range (mg/L)	0.5–200	0.5–250
Response time (min)[a]	32–2	30–2
Curve slope	0.75	0.31
RSD ($n = 6$)	4%	2%
Optimum pH	7.4	7.4
Optimum temperature (°C)	39	30
Optimum NaCl% concentration	1%	3%

[a]Concentration of BOD ranges from 10 to 4 mg/L.

for film 2 when the reactivation concentration of GGA changed from 50 mg/L to 200 mg/L. After one month's storage, the initial fluorescence response of the films decreased by 10% compared with newly made films, but the response to BOD remained the same if the sensing film was reactivated well. Experimental results demonstrated that BOD sensing films could be stored for up to nine months without significant deterioration. However, the films had to be reactivated for one day in GGA solutions (BOD of 100 mg/L) before use, in order to achieve the best sensitivity, stability and reproducibility.

The characteristics of BOD sensing films with two kinds of immobilized microbial cells were compared under the same experimental conditions. As summarized in Table II, in terms of sensitivity and reproducibility, the film of sieved bacteria from seawater was superior to the film of domestic bacilli, but its R^2 and response time were inferior to the film of domestic bacilli. The effects of pH on the response of the sensing films were similar, with a maximum response at pH 7.4 which is close to the pH of seawater. The maximum permissible concentrations of chloride anion for the two films were 1% and 3%, respectively. In the estimation of low BOD, sieved bacteria from seawater were preferable in respect of sensitivity and further development is anticipated.

Determination of BOD in Seawater

The BOD sensing films with immobilized sieved bacteria from seawater were used in the determination of low concentrations of BOD in seawater. Seawater samples from

TABLE III Results of BOD determination of seawater samples by film 1 ($n = 5$)

Seawater[a]	*pH*	*Nominal value*[b] (mg L^{-1})	*Found* (mg L^{-1})
1[c]	7.89	6.3 ± 0.3	5.9 ± 0.6
2[d]	7.90	5.6 ± 0.3	5.2 ± 0.4
3[e]	8.03	4.4 ± 0.2	4.1 ± 0.5
4[f]	7.95	3.8 ± 0.2	3.5 ± 0.3

[a]Samples from living areas around Xiamen University.
[b]Detection according to the National Standard Method of China (GB 3097-1997).
[c]Refluent seawater, sampling date, Oct 16, 2003.
[d]Refluent seawater, sampling date, Oct 17, 2003.
[e]Tide seawater, sampling date, Oct 18, 2003.
[f]Tide seawater, sampling date, Oct 19, 2003.

different sampling sites were filtered through a membrane filter with a pore size of 10 μm. Before testing, the water sample was pre-heated to 35°C, and then 15 mL processed water sample was added to the detection cell and analyzed. The BOD detection results are listed in Table III.

There are obvious differences of BOD concentration between refluent and tidal seawater around the Xiamen University area. The BOD concentration of refluent seawater is higher than that of tidal seawater, which indicated that the increase of the BOD value was caused by wastewater from the living area. Furthermore, about a 7% subtractive error of BOD concentrations between the BOD microbial film method (film 1) and the standard BOD_5 method could be found in Table III. Two possible explanations may be considered. The first is the limitation of using GGA solution, only containing glucose and glutamic acid, which is much simpler than real samples [5]. Using GGA as a standard solution for the BOD sensor succeeded in achieving determination of high BOD, but was less sensitive to samples with lower BOD. The other is the limitation of using a single kind of immobilized microbe for the BOD sensing film. Since the BOD sensing film with a single kind of immobilized microbe is incapable of assimilating a wide range of organic substrates [9,10], mixed-culture BOD sensors, utilizing a variety of microbes, should be considered, so that a BOD sensing film could be applied for assimilation of a broad range of organic compounds, with consequent increased sensitivity.

CONCLUSION

Ormosil–PVA material has first been used to immobilize two kinds of microorganisms in the preparation of BOD biosensors. The concentrations of PVA and immobilized microbial cells in the sensing films have been shown to have obvious effects on the characteristics of the films. The stored sensing films can be employed for BOD measurements after reactivation for one day, even if they were kept for up to nine months at 4°C. Sensing films with different microbes immobilized have their own optimum pH, measurement temperature and concentration of sodium chloride for optimum response. From their sensitivity, response time and reproducibility, the film immobilizing sieved bacteria from seawater has superior characteristics in the determination of low BOD compared with those of the film immobilizing domestic bacilli.

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References

[1] American Public Health Association, *Standard Methods*, 17th Edn. American Public Health Association, Washington DC (1989).
[2] Japanese Industrial Standards Committee, *Testing Methods for Industrial Wastewater*, JIS K0102, P47. Japanese Industrial Standards Committee, Tokyo (1989).
[3] I. Karube, T. Matsunage, S. Mitsuda and S. Suzuki, *Biotechnol. Bioeng.*, **19**, 1535–1547 (1977).
[4] C. Preininger, I. Klimant and O.S. Wolfbeis, *Anal. Chem*, **66**, 1841–1846 (1994).
[5] G.J. Chee, Y. Nomura and I. Karube, *Anal. Chim. Acta*, **379**, 185–1911 (1999).
[6] K. Riedel, R. Renneberg, M. Kuhn and F. Scheller, *Appl. Microbiol. Biotechnol.*, **28**, 316–318 (1988).
[7] Y. Sakai, N. Abe, S. Takeuchi and F. Takahashi, *J. Fermen. Bioeng.*, **80**, 300–303 (1995).
[8] D.D. Chen, Y.B. Cao, B.H. Liu and J.L. Kong, *Anal. Bioanal. Chem.*, **372**, 737–739 (2002).
[9] Y.Q. Jiang, X. Chen and K.Y. Wong, *Chem. Res. Chinese University*, **17**(4), 374–379 (2001).
[10] T.C. Tan and C.H. Wu, *Sens. Actuators B*, **54**, 252–60 (1999).