ISSN : 0974 - 7435 Volume 8 Issue 2

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 8(2), 2013 [255-259]

Mediated microbial biosensor using *Bacillus subtilis* for wastewater BOD measurement

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ABSTRACT

In order to get some efficient microbial membrane, a *Bacillus subtilis* (BA1304) was isolated from wastewater, and was identified by the MicroStation System, and was used as the sensitive material for the BOD sensor (BODs) after being immobilized. Three immobilized mediators: polyethylene, sodium alginate and polyacrylamide were tested for choosing proper cell carriers. Furthermore, four temperature grads (25°C, 30°C, 35°C and 37°C) were studied to find the optimal working conditions. The result showed that the polyethylene was proven to be the most efficient mediator to the electrode in the presence of excess glucose/glutamic acid (GGA). The effective culture temperature was 35°C for Bacillus subtilis by testing values in standard BOD solutions, which were estimated using the BODs with deviation less than 5%. The BOD values were determined using the sensor and compared favorably with those determined by the conventional BOD5 method.

KEYWORDS

Biosensor;
Bacillus subtilis;
Biochemical oxygen demand;
Temperature;
Immobilized mediator.

INTRODUCTION

Biochemical oxygen demand (BOD) is an important indicator of organic pollution in water. The standard 5-day BOD determination of pollution is labor-intensive, involving specific sample incubation and often requires 5 days to produce a final result that is not always reproducible 1. Therefore, many kinds of microbial biosensors developed, some have been used with success for environmental monitoring These sensors are based on a combination of microorganisms and an electronic signal-transducing element, which has a number of advantages, such as high stability and short response time 2.

BOD biosensors require microorganisms of low selectivity and high biooxidation activity for a wide range of organics, so that they can be used to monitor process effluent and wastewaters from different sources. Therefore, it is vital to select suitable microorganism and optimal working conditions for a biosensor 1, 3. Until now, many assimilative microorganisms have been applied and reported including: Bacillut subtilis, Trichosporon cutaneum, Pseudomonas sp. and Hansenula anomala etc. 4, 6, 7. In the present study, efficient microorganism was isolated to make synthetic membrane, and a dissolved oxygen electrode was used as a transducer for the oxygen measurement. The influence of immobilized mediators and temperature were determined.

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EXPERIMENTAL

Microoganisms

The bacterials used for experiment were isolated from activated sludge collected from aeration tank of Liede wastewater treatment plant, Guangzhou, China. The pure culture was obtained by 3 times streak plate method, and named BA1304. The pure culture was cultured at 140 rpm (35°C) for 20h. The cells were harvested by centrifugation (4000 rpm, 10 min), washed three times by saline solution (0.8 w/v NaCl) and stored at 4°C for use. The bacterial BA1304 was identified by Biolog System (Microlog 3) and analyzed its utilization on carbon source.

Mediator screening

Calculated amounts (wet cells 10 mg) of the pure culture broth were mixed with three immobilized mediators (20 mg): polyethylene, sodium alginate and polyacrylamide and dropped on the cellulose nitrate membrane, and then adsorbed onto the membrane by suction. A porous cellulose nitrate membrane (20mm diameter, 0.25um pore size) was carefully sandwiched in a syringe filter holder connected to an aspirator. The prepared biofilms were immerge in 0.005mol/L Na2HPO4-KH2PO4 buffer (pH 6.9) and stored at 4°C.

Temperature optimization

Four temperature grads: 25°C, 30°C, 35°C and 37°C were employed to find the optimal temperature for BA1304. In order to stabilize the BOD sensor system and measure the response of the immobilized microbial membrane, 100ml of Na2HPO4-KH2PO4 buffer (pH 6.9) was placed in a thermo stated cell at different working temperature under constant and moderate stirring. The current change was observed after addition of the samples until a steady state was reached.

Biosensor testing

An microbial membrane was used for BOD sensor as previously described 2, 4. When the current output of the sensor reached a steady state, a sample of BOD test solution (Standard glucose-glutonic acid: GGA) was added to the 5.0-ml phosphate buffer solution. This caused the current output to decrease and a new steady-state current was established. The difference between the background steady-state current and the steady-state current after the addition of the BOD test sample provided the signal response (Δi) of the microbial BOD sensor. A GGA mixture was used as the standard BOD solution in this work. The GGA solution has been used widely as a calibration solution. It was operated continuously by feeding the wastewater through the injection port of the anode compartment at a rate of 0.35 mL/min using a peristaltic pump. The BOD sensor measurements were made using steady state method. The steady state indicates the consumption of oxygen by microorganism which diffuses from a sample solution to the membrane at equilibrium. In steady state method, the difference of current between the two steady states reflects the respiration rate of substrate and was used for BOD sensor estimation. The measuring time was 8 min followed by 10–15min recovery time. The conventional BOD5 method was used as the standard method for comparison.

RESULTS

Culture identification

The pure culture of BA1304was identified as $Gram^+$ germ after Gram stained. It showed that the strain had red-purple mycelium, indicating that BA1304 fit for BUG +B agar plating medium (BUG agar plate + 0.05%~0.07% sodium thioglycollate). Figure 1 and Figure 2 were the readings of BA1304 on GN2 micropore identification plate after 4-6 h and 16-24 h incubation. In turn, carbon source utilization of BA1304 was analyzed with the readings (TABLE 1, red grid means sufficient utilization). Compared with database, the utilization of carbon source showed that the ultimate matching score between BA1304and Bacillus subtilis was 95%. Base on this result, BA1304 was identified as *Bacillus subtilis.*

Immobilized mediator selection

The stability of three mediators was investigated by 16 times continually testing standard GGA solution (2000 mg/L) 4-6 h everyday. Figure 3 showed that during 16 times continually testing, the data in polyacrylamide treatment fluctuated greatly comparing with

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	253	-6 +++	271	87	14	-5	51	16	219	149 ---	51
B	16	-5	347	216	609	-10	30 +++	-15	289	83	482	3
C	26	5	430	509	294	330	112 ---	50	-2	3	80	405
D	378	-7	402	290	56	49	69	361	30	274	1	509
E	-16	505	416	9	18	22	-3	-8	19	-17	-2	16
F	4	16	85	13	294	134	102	-28	235	32	46	19
G	24	90	169	73	127	343	-6	9	272	-14	69	269
H	118	57	204	90	246	25	71	22	26	13	7	89

Figure 1 : The result of BA1304 on GN2 MicroPlate after 4-6h incubation

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	41	-30	364	79	-16	5	33	30	166	34	62
B	8	-17	299	211	323	-16	9	-13	164	62	388	35
C	-15	-16	381	375	156	236	45	20	-10	-22	66	258
D	327	-18	340	257	34	-7	37	307	18	145	11	409
E	-9	334	335	-6	21	20	-14	-18	8	-16	-12	3
F	2	12	68	-9	183	100	62	-9	207	-3	40	-7
G	3	21	168	-13	122	180	-2	-10	181	-4	93	255
H	119	62	179	98	193	-11	16	-14	-3	-2	-4	117

Figure 2 : The result of BA1304 on GN2 MicroPlate after 16-24h incubation

TABLE 1 : The carbon source of BA1304 after different incubation time

incubation time	carbon source (according to utilization sequence)
4－6h	Sucrose, α-D-Glucose, Maltose, Maltotriose, Dextrin, Turanose, D-Trehalose, D-fructose
16－24h	D-fructose, α-methyl-D-glucoside, Maltotriose, D-Trehalose, α-D-Glucose, Dextrin, β- methyl -D- glucoside, L-Trehalose

other 2 mediators. Its relative deviation (16.2%) was much higher than the national permissible value (8%), indicating that polyacrylamide is not suitable for precision request of BOD test. When compared average values between polyethylene (1998.1+10.3mg/L) and sodium alginate (2054+34.6mg/L), the data of polyethylene treatment was kept in the range of guarantee value of national standard and lower deviation (<4%). This result indicated that polyethylene can be used as immobilized mediators for BA1304.

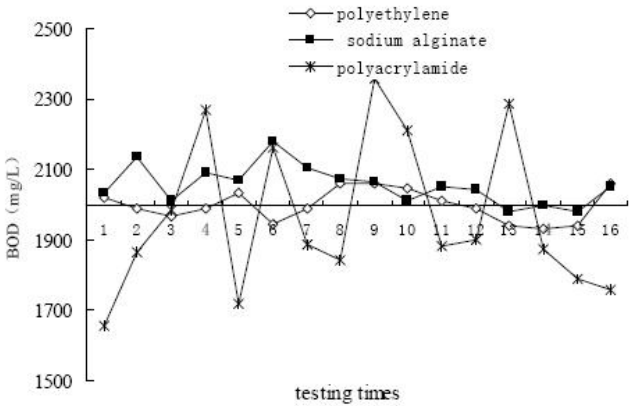

Figure 3 : The Testing results of different immobilized mediators using BA1304 in glucose-glutonic acid solution

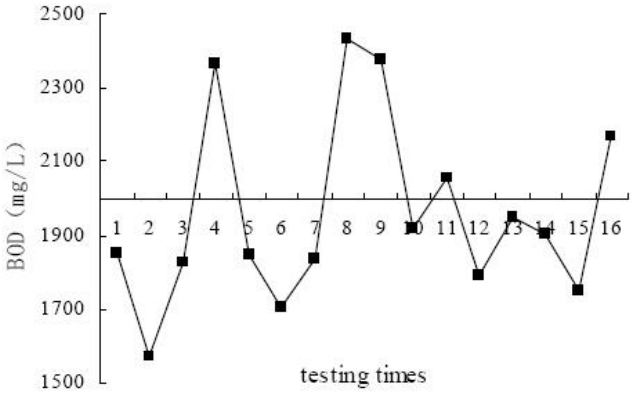

Figure 4 : The testing results of BA1304at 25°C in glucose-glutonic acid solution

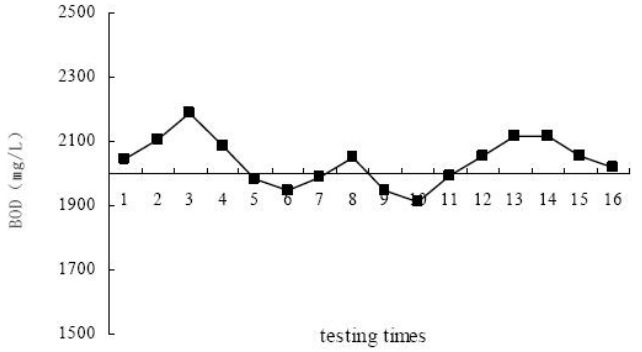

Figure 5 : The testing results of BA1304at 30°C in glucose-glutonic acid solution

Optimal temperature selection

Temperature was considered as a key factor that influences the precision of BOD testing. The effect of temperature on the steady-state response of the BOD sensor membranes immobilized with polyethylene was determined 16 times in standard GGA solution (2000 mg/L), as shown in figure 4-figure 7. The BOD values were fluctuated greatly in 25°C (figure 4), indicating that this temperature can not sustain stable aerobic res-

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piration for BA1304, though in many previous studies, it was used for other microorganisms 3, 5. Compared with the result in 25°C, though the testing values in 37°C had better stability, the high standard deviation (+116.7) implied that its stability was still suspicious (figure 7 and TABLE 2). On the other hand, the data under 35°C showed that it could keep the better precision and stability than other three temperature treatments (figure 6). Further analysis indicted that the average value in 35°C (1998.2+45.3 mg/L) is more closed to the standard values, comparing with the result of 30°C treatment (2028.1+75.2 mg/L) (TABLE 2). Therefore, among the 4 temperature grads, 35°C is the best working temperature for BA1304, which can sustain the BOD sensor performed well without significant deviation in standard GGA solution.

TABLE 2 : Comparison of testing results with different temperature treatments

Temperature	Average (mg/L)	Standard value (mg/L)	Standard deviation	Relative error(%)
25℃	1951.1	2000	253.3	2.4%
30℃	2028.1	2000	75.2	1.4%
35℃	1998.2	2000	45.3	0.9%
37℃	2039.5	2000	116.7	2.0%

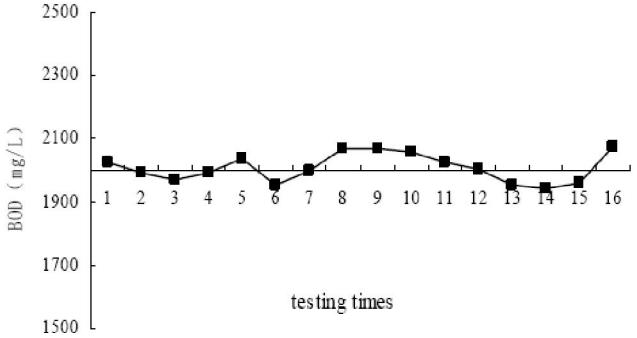

Figure 6 : The testing results of BA1304at 35°C in glucose-glutonic acid solution

Real sample testing

Different type of wastewater samples were used to test by the BOD biosensor and BOD5 standard method. Although the BOD values using biosensor gave less than those of conventional BOD5 standard method the results of two methods had no significant difference (TABLE 3), indicating that there was good agreement between biosensor made with BA1304 and BOD5 method.

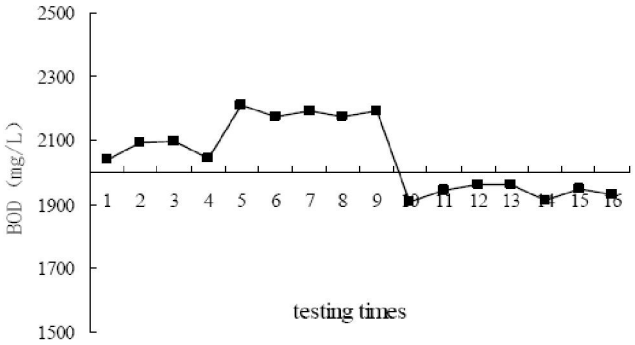

Figure 7 : The testing results of BA1304at 37°C in glucose-glutonic acid solution

TABLE 3 : Testing results of typical water samples

Sample	Mean testing value (mg/L)	BOD (mg/L)	relative deviation (%)
National standard sample	105.8	107.5	1.9
brewery wastewater	1089.1	1048.7	4.5
River water	82.2	78.8	2.5
Catering wastewater	405.0	425.3	5.0
Waste water from chemical factory	250.1	261.2	4.2

DISCUSSION AND CONCLUSIONS

In this study, an Bacillus subtilis (BA1304) isolated from sludge was used as biological sensing element. The microbial membrane made of it showed good repeatability, long-term stability, and good agreement with BOD5 standard method. Although the sensitivity showed some variability depending upon the real wastewater samples, this was probably due to the incomplete metabolism of certain substrates owing to the short reaction times and the assimilability of the substrate by the microorganisms.

The working condition experiments showed that the introduction of polyethylene as cell carrier in form of membrane was the most efficient method among several mediator treatments. However, more studies should be conducted to determine whether this result is a species-specific reaction or not. In many previous studies, 25°C or 30°C was considered to be proper temperature for biodegrading organic substances, but our result showed that the optimal working temperature for Bacillus subtilis is 35°C. it is probably due to the higher temperature demand for Bacillus subtilis. The result im-

plied that the sensor will be very reliably on how the operating conditions are for the cells in the electrodes. This will be of special importance when the biosensor signal is to be used for feed control to the reactor.

ACKNOWLEDGMENTS

This study was supported by the Science Foundation of Chinese Ministry of Construction (Grant No. 05-K2-6) and National Natural Science Foundation of China (Grant No. 31170509)

REFERENCES AND NOTES

[1] J.Liu, G.Olsson, B.Mattiasson; Biosensors and Bioelectronics, **20**, 562 **(2004)**.
[2] J.Li, Y.Li, G.Zhang; Key Engineering Materials, **467**, 316 **(2011)**.
[3] I.Karub, T.Matsunaga; Biolechnology and Bioengineering, **19**, 1535 **(1977)**.
[4] J.Li, H.Wu, L.L.Li; Biotechnology, **136**, 643 **(2008)**.
[5] S.Rastogi, A.Kumar, N.K.Mehra, S.D.Makhijani, A.Manoharan, V.Gangal, R.Kumar; Biosens. Bioelectron, **18**, 23 **(2003)**.
[6] M.M.Ghangrekar, V.B.Shinde; Bioresource Technology, **98**, 2879, November **(2007)**.
[7] M.Lehmann, C.Chan, A.Lo, M.Lung, K.Tag, G.Kunze, K.Riedel, B.Gruendig, R.Renneberg; Biosens. Bioelectron, **14**, 295 **(1999)**.