Talanta 81 (2010) 1170–1175

Contents lists available at ScienceDirect

Talanta

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

A new mediator method for BOD measurement under non-deaerated condition

Ling Liu, Li Shang, Chang Liu, Changyu Liu, Bailin Zhang, Shaojun Dong*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Remming Street 5625, Changchun, 130022, China

ARTICLE INFO

Article history:
Received 29 October 2009
Received in revised form 28 January 2010
Accepted 28 January 2010
Available online 6 February 2010

Keywords:
Biochemical oxygen demand (BOD)
Mediator method
Ferricyanide
Non-deaerated condition
E. coli

ABSTRACT

Monitoring biochemical oxygen demand (BOD) by mediator method (BOD_{Med}) has been developed for recent years and deaerated condition was generally adopted to avoid the effect of oxygen, but the deaerated condition was unfavorable in practical applications. Herein, we first proposed another way to explore non-deaerated BOD_{Med} (called NDA-BOD_{Med}) method utilizing ferricyanide, which was reduced by *Escherichia coli* upon catalyzing organic substrate to produce ferrocyanide. We attempted to explain the feasibility of NDA-BOD_{Med} by the two aspects. Firstly, the obtained biodegradation efficiencies of the bacteria under the deaerated and non-deaerated conditions were similar, and the concentration of O_2 (0.25 mM at 8 mg/L O_2) is 1–2 order of magnitude lower than that of mediator commonly used (55 mM ferricyanide), so the effect of O_2 to measurements could be neglected. Secondly, the relationship between the artificial and the natural electron acceptor was investigated, and it was found that the oxygen consumption in the NDA-BOD_{Med} measurement was mainly contributed to endogenous values. Furthermore, the performance of present NDA-BOD_{Med} was reported, and this method was optimized for measuring the low-concentration samples, synthetic wastewater and real polluted wastewater. The NDA-BOD_{Med} provides a simple and efficient way in rapid BOD determinations, especially advantageous for in situ monitoring of water system.

1. Introduction

Biochemical oxygen demand (BOD) is an important parameter of environmental indicator and 5-day biochemical oxygen demand assay (BOD_5) is an international legislated standard test for BOD monitoring [1]. The BOD_5 assay is based on the biodegradation of organic substrate under the aerobic oxidation to measure the consumed dissolved oxygen in 5 days, which requires the complicated procedures, skilled analysts and is time-consuming. It is obvious that the BOD_5 test is not suitable for rapid feedback information in practice. To date, many rapid biosensors for BOD monitoring have been developed and those biosensors mostly depended on the dissolved oxygen as the terminal electron acceptor for biodegradation of organic substrates. However, the variations of the dissolved oxygen level in the sample solution may cause fluctuations in the result, and the accessorial instrument needed to be equipped to supply air [2,3]. On the other hand, studies suggest that the oxygen-type BOD biosensor is limited by the concentration of oxygen in water (8.7 mg/L at 25 °C) and that sample dilution would further decrease the accuracy of BOD measurement [4]. Thus, the oxygen-type BOD biosensor is greatly restricted. In recent years, a rapid method by using synthetic electron acceptor instead of oxygen for BOD measurement was developed [5–8]. The synthetic electron acceptor shuttles electrons from the bacterial electron transport chain to the electrode, so it is called a 'mediator' [9]. With the high solubility of synthetic electron acceptor, the rapid mediator BOD (BOD_{Med}) method overcame the problem of limitation from oxygen, and the concentration of the mediator could be controlled during the process of preparing solution [10].

So far, the BOD_{Med} method has been successfully attempted by several groups and the enteric bacteria *Escherichia coli* combined ferricyanide has been widely adopted, because its respiratory pathway has been well investigated [11]. The metabolic processes of microbe are dependent on a series of electron transport between living and non-living system [12]. The large polymer molecules (e.g. carbohydrates) and their constitutive blocks (e.g. hexoses) would have the cleavage for producing the simple molecules, and ultimately enter into a common pathway (e.g. tricarboxylic acid cycle) and be oxidized to CO_2 and H_2O. In these processes, microorganisms metabolized the organic substances under the aerobic condition and the electrons were transported through a way by electron transport chain to the oxygen [13]. Obviously, the process for accepting electrons would be complicated, if both artificial and natural electron acceptors coexisted. So it is easy to understand that removing dissolved oxygen from the reaction samples was required for BOD_{Med} method.

For a long time, the concept that the oxygen and the mediator competed for accepting electrons seemed reasonable, so the deaer-

* Corresponding author. Tel.: +86 431 85262101; fax: +86 431 85689711.
E-mail addresses: dongsj@ciac.jl.cn, dengliu@ciac.jl.cn (S. Dong).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.01.062

ated condition for BOD_{Med} method was indispensable and widely adopted. Here, a question has been arisen that the deaerated operation would disturb the sample solution to bring a fluctuation to response of the result, and it was not suitable for monitoring the real polluted water (such as the sewage treatment plant and the river, etc.). The problem about deaerated condition for BOD_{Med} method being necessary or not was remained to argue all along. The lack of such information represents a doubtless conclusion for explaining the related problem. Previous work indicated that ferricyanide was a preferable electron acceptor to oxygen in mediator method [14]. Similarly, others reported that ferricyanide was preferentially reduced to ferrocyanide when ferricyanide was present in the reaction medium [15]. However, Yoshida et al. found that the redox proteins in the respiratory chain of *P. fluorescens* were equally able to use either oxygen or ferricyanide as the electron acceptor during the anabolic process of the organic substances [16]. Ramsay and Turner [17] and Kalab and Skladal [18] reported that, as an electron acceptor in the respiratory chain of aerobic bacteria, the relationship of mediator and oxygen was competitive. It is known that the NADH dehydrogenases act as ferricyanide reductases, which are terminal components of the electron transport chain and couple the extracellular reduction of ferricyanide to the intracellular oxidation of NADH [19]. The main question, such as the mediator accepting electron pathway and the order for accepting electron of the two acceptors, had not been clear yet.

The environmental monitoring requires the increasing development of on-line, rapid and inexpensive assays [20,21]. The objective of the present work was to explore the non-deaerated method (called NDA-BOD_{Med}) instead of BOD_{Med} method. Comparing to the conventional BOD_5 method, the BOD_{Med} method increased the rate of the biochemical reaction and allowed for biodegradative conversion efficiencies similar to the conventional method in a short time [22]. The present NDA-BOD_{Med} method focused on resolving the practical problem and offered a better means for engineering practice as compared to the BOD_{Med} method.

2. Experimental

2.1. Materials and instrumentation

The BOD^{198} standard glucose-glutamic acid (GGA) solution with a BOD value of 198 ± 31 mg O/L was prepared according to APHA method contains 150 mg/L glucose and 150 mg/L glutamic acid [1,5]. Solution with other concentrations was prepared by appropriate dilution of the BOD^{198} standard solution with deionized water. The composition of organization for economic corporation and development (OECD) synthetic wastewater was as follows (g/L): peptone, 16; meat extract, 11; urea, 3; NaCl, 0.7; $CaCl_2{\cdot}2H_2O$, 0.4; $MgSO_4{\cdot}7H_2O$, 0.2; K_2HPO_4, 2.8. The BOD_5 value of this solution is defined as 14,000 mg O/L [23]. All chemicals used in this study were of analytical reagent grade and all solutions were prepared with deionized water being sterilized.

The dissolved oxygen measurement was completed by commercial oxygen meter Thermo Orion 86A (Orion, America). Spectrophotometric measurements were conducted using a Cary 500 UV–vis-NIR spectrometer (Varian, America). Electrochemical responses were measured with a CHI 832 electrochemical analyzer (CHI Co., Shanghai, China). The setup was conducted in single-potential time-base mode.

2.2. Electrochemical measurements

The platinum array microelectrode (2×2 microdiscs of 25 μm diameter each) was used as working electrode, and another Pt was used as the counter electrode. A potential of −450 mV versus Ag/AgCl reference electrode was applied to the Pt-working electrode throughout all measurements in 0.1 M potassium chloride (KCl) as the supporting electrolytes. The working electrodes were pre-treated by polishing in 0.05 μm alumina slurry on a polishing cloth. A 5 mM ferrocyanide was used to evaluate the performance of the electrode.

2.3. Microorganism and culture conditions

E. coli strain DH5α was maintained on Luria-Bertani (LB) agar plates at 4 °C. The bacteria were cultured aerobically at 37 °C for 11 h in a LB medium with shaking at 180 rpm. The culture was harvested by centrifugation at 4000 rpm for 10 min at room temperature. The cells were washed thrice in phosphate buffer solution (PBS, 0.12 M Na_2HPO_4/0.08 M K_2HPO_4/0.1 KCl, pH 7) and re-suspended in the same solution. The cell concentrations were adjusted to the desired optical density measured at 600 nm (OD_{600}) using the spectrometer.

2.4. BOD_{Med} measurement under deaerated condition

All solutions were pre-purged for 15 min using oxygen-free nitrogen at 37 °C in a water bath. The 15.00 mL sample mixture for incubation was prepared with the following volumes: 2.50 mL potassium ferricyanide, 5.00 mL test organic sample and 7.50 mL of *E. coli* at the desired absorbance value. Endogenous control solutions were prepared by adding PBS in place of the test organic substrate. Generally, microbe OD_{600} 5 and ferricyanide 55 mM were adopted in the present work. To terminate the reaction, the samples were centrifuged with 10,000 rpm for 3 min. The supernatant solution was then used for analysis of microbially produced ferrocyanide. The results were obtained by the mediate method with direct accurate electron transfer number.

2.5. NDA-BOD_{Med} measurement

Before incubation, all solutions were incubated at 37 °C for 15 min, and then following the same procedure for preparation of sample mixtures as that in Section 2.3. After mixing, the sample solution (containing of microbial cells, potassium ferricyanide, and organic substrate) was encapsulated into 1.5 mL eppendorf tube and plugged.

2.6. Measurement of real polluted wastewater

The final concentrations of 55 mM ferricyanide and the OD_{600} 5 *E. coli* were used for measuring the real polluted wastewater from a sewage treatment plant. The sample solutions were incubated at 37 °C under both deaerated and non-deaerated conditions for an optimized time (1 h for the present work).

2.7. Calculation of results

The ratios of the response of endogenous to the response of total (R_e/R_t) were obtained by calculating the proportion between the response signal of endogenous and of the total response. The biodegradation efficiency values were obtained according to Morris [25].

2.8. The method for plate culture count

The final concentrations of 55 mM ferricyanide and the OD_{600} 5 microbe were adopted, and 100 mg O/L GGA solution was used for test sample. The same concentration of microbe without mediator was used for control test. PBS was adopted to replace organic

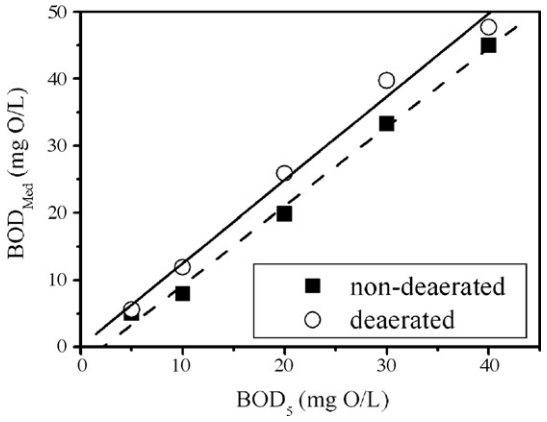

Fig. 1. The measurement of GGA solutions with BOD_{Med} method by *E. coli* under the deaerated and non-deaerated conditions.

substrate for the blank test. The incubation time for BOD_{Med} experiment was 1 h. After the BOD_{Med} experiment, the incubated sample solutions were centrifuged for collecting bacterial cells and resuspended in the PBS (pH 7) with same volume. 1 mL of bacterial solutions diluted by PBS were mixed into germination medium (LB medium with 2% agar, w/v) and incubated in the nutritional plate at 37 °C at 12 and 24 h for counting the colony forming units (CFU).

3. Results and discussion

3.1. Response obtained by the NDA-BOD_{Med} method

As a part of the preliminary investigation, we examined the possibility of obtaining response signal with the NDA-BOD_{Med} method. As shown in Fig. 1, GGA solutions with different concentrations were measured by the BOD_{Med} method under the deaerated and the non-deaerated conditions, and the BOD values were calculated directly by the electrons accepted by ferricyanide. As expected, the responses obtained from the two conditions were quite close. The slopes of the curves under the deaerated and non-deaerated conditions were 1.24 and 1.18, respectively. This result indicated that the reaction of the microbe and mediator at the non-deaerated condition was feasible and the biodegradation efficiencies of *E. coli* under both conditions were similar. However, the measured values from the deaerated condition were higher than that from non-deaerated case. This may be possibly caused by the competition between O_2 and ferricyanide for electron transfer and reoxidation of the produced ferrocyanide when O_2 existed. But the effect of O_2 on results could be neglected, because the concentration of O_2 (0.25 mM at 8 mg/L O_2) is 1–2 orders of magnitude lower than that of the mediator (55 mM ferricyanide) used. It is obvious that the NDA-BOD_{Med} was a feasible means for measuring the standard GGA solution. By using the NDA-BOD_{Med} method, the equipments of air-supply (for oxygen-type BOD sensor) and oxygen exclusion (for BOD_{Med} method) were not needed, resulting in quite a simple operation.

3.2. Relationship between natural and artificial electron acceptor

The endogenous metabolism was an important factor affecting the results of the BOD_{Med} method [24]. Fig. 2A reveals the dissolved oxygen consumption by NDA-BOD_{Med} method and the residual dissolved oxygen after incubation with microbe and organic substrate. Here, the concentration OD_{600} 5 *E. coli* and 100 mg O/L GGA solution were employed, and the dissolved oxygen was monitored by Orion 862A meter. The blank control (without both of mediator and organic substrate), the endogenous control (without organic substrate) and the assay (with both of mediator and

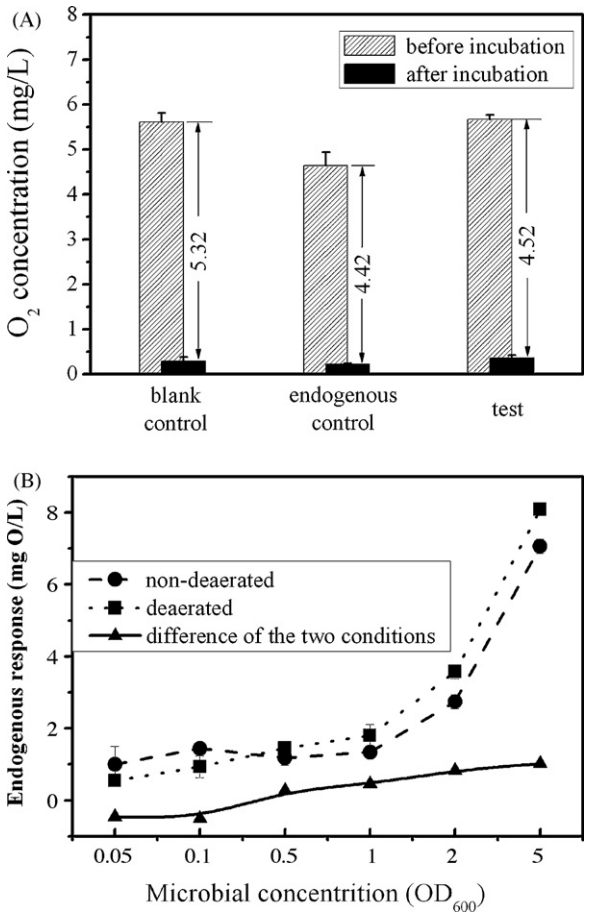

Fig. 2. (A) The oxygen consumption in the NDA-BOD_{Med} method. A high concentration OD_{600} 5 of *E. coli* and 55 mM $K_3[Fe(CN)_6]$ were adopted. The blank control was without $K_3[Fe(CN)_6]$ and organic substrate, the endogenous control was without organic substrate, and the test sample contained 100 mg/L GGA. (B) Endogenous responses were obtained with BOD_{Med} method by monitoring the produced ferrocyanide under deaerated (diamond) and non-deaerated (dot) conditions (without organic substrate). The triangle was the difference by subtracting non-deaerated from deaerated response.

organic substrate) experiments were conducted. The oxygen consumption after incubation was 5.32 and 4.42 mg/L for blank control and endogenous control, respectively. This result indicated that the dissolved oxygen consumption has not been affected by the mediator. Furthermore, the residual dissolved oxygen measured for all samples was lower than 0.36 mg O/L (the detection limit of instrument). Obviously, the oxygen consumption was mainly contributed to endogenous metabolism, so the dissolved oxygen would not influence the result of NDA-BOD_{Med} method.

The endogenous responses (without organic substrate) of the microbe with different concentrations measured by BOD_{Med} method were shown in Fig. 2B. The results were obtained by the ferrocyanide produced in the endogenous metabolism process under the deaerated and the non-deaerated conditions. As shown in Fig. 2B, when the final microbial concentrations were lower than OD_{600} 1, the endogenous responses were below 1.8 mg O/L for both conditions. The difference of endogenous responses between the two conditions was lower than 1.0 mg/L. This reveals the endogenous response of non-deaerated condition did not carry significant disparity to that of deaerated condition. So the non-deaerated condition could be acceptable in BOD_{Med} method.

The ratios of the response of endogenous to the response of total (R_e/R_t) under the deaerated, non-deaerated and aerated conditions were further studied. As shown in Table 1, the GGA solutions with

Table 1
Ratio of endogenous response to total response (R_e/R_t) in BOD_{Med} method at different conditions.

Condition	Electron acceptor	R_e/R_t (%)[d]		
		100[e]	15[f]	5[g]
Deaerated[a]	Ferricyanide	13 ± 1	57 ± 2	79 ± 5
Non-deaerated[b]	Ferricyanide and limited oxygen	11 ± 2	43 ± 2	77 ± 6
Aerated[c]	Ferricyanide and timely supplemented oxygen	15 ± 2	48 ± 1	82 ± 2

The superscripts letters [a,b,c] indicate that the experiments were conducted by purging nitrogen gas for deaerated condition and supplying air for aerated condition; [d] indicates 55 mM ferricyanide and OD_{600} 5 microbe; [e,f,g] indicate mg O L^{-1} GGA solutions.

three concentrations (the corresponded BOD_5 values were 5, 15, and 100 mg O/L) were adopted for the measurement of R_e/R_t values under different conditions. Obviously, the significant disparity of R_e/R_t was obtained at different concentrations of the organic substrates, and the insignificant disparity was obtained at different experimental conditions for same concentrations. This result indicated that the difference between single electron acceptor and two electron acceptors coexisted was not in evidence. It was noteworthy that the R_e/R_t reached to ~82%, when the concentration of GGA solution was 5 mg O/L. It indicates that the measurement of low-concentration organic substrate was not ideal and it was needed to further investigate.

3.3. Performance of the NDA-BOD_{Med} method

As shown in Fig. 3, responses of organic substrate GGA with BOD_5 value in the range of 5–400 mg O/L are obtained, and a linear correlation from 5 to 100 mg O/L ($y = 2.80 + 0.90x$, $R = 0.9938$) was shown. When the concentration is higher than BOD_5 100 mg O/L, another linear range between 100 and 400 mg O/L with lower slop (shown in insert) was seen. The results indicated that the bacterial degradation efficiency was gradually decreased with increasing the concentration of organic substrate, which may be caused by amount of the mediator was lacked, and the ability of microbe for transporting electrons was limited.

As shown in Table 2, biodegradation efficiencies of the microbe in NDA-BOD_{Med} were calculated according to the literature [25]. It is known that the average conversion rate of the standard

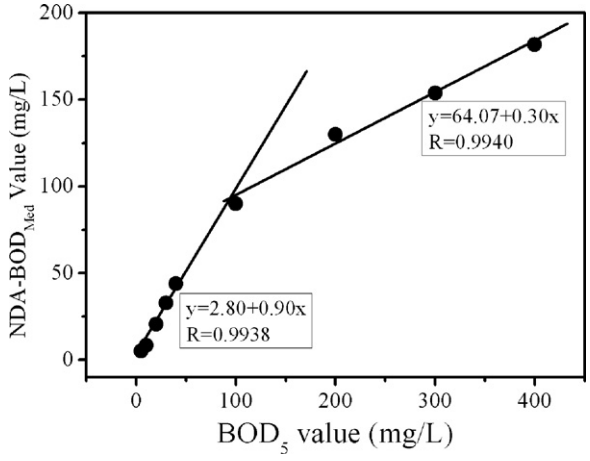

Fig. 3. Measurement of GGA solutions with the BOD_5 values in the range of 5–400 mg O/L. Insert was the linear curve of GGA solutions in the range of 100–400 mg O/L.

Table 2
Biodegradation efficiencies of *E. coli* in the NDA-BOD_{Med} method.

GGA solution (mg O/L)[a]	Biodegradation efficiencies (%)			
	30 min	1 h	2 h	3 h
50	37	61	66	77
100	23	60	66	77
200	16	43	55	62
300	14	34	42	54
400	12	30	34	45

[a] 55 mM ferricyanide and OD_{600} 5.

substrate in 5-day BOD5 test was about 60% [4]. In the present NDA-BOD_{Med} method when GGA solution was lower then 100 mg O/L, the biodegradation efficiency of *E. coli* achieved 60% in 1 h and 77% in 3 h. However, the biodegradation efficiency could not achieve 60% even after 3 h when GGA solution was higher then 300 mg O/L. The result indicated that the biodegradation efficiency of microbe by NDA-BOD_{Med} method is quite high, and it could reach to the BOD_5 level rapidly for measuring a certain concentration range (below 200 mg O/L) of organic substrates within 3 h.

3.4. Optimizing the NDA-BOD_{Med} method

In fact, measuring of low-concentration organic substrate was difficult, because the endogenous responses were excessive. For example, the R_e/R_t could be reached to 82%, when the microbe concentration was OD_{600} 5 and the GGA solution was lower than 5 mg O/L (see Table 1) in 3 h. So the NDA-BOD_{Med} method is needed to be optimized. As shown in Table 3, the matched microbe (concentrations were showed by OD_{600}) and $K_3[Fe(CN)_6]$ with different concentrations were adopted for measuring the organic substrate from 0.5 to 550 mg O/L GGA solutions. Based on the results, matched component of 55 mM ferricyanide to OD_{600} 5 microbe was suitable for measuring the sample with the concentration below 100 mg O/L,

Table 3
Optimizing the NDA-BOD_{Med} method.

GGA solution (mg O/L)	The NDA-BOD_{Med} value (mg O/L)			
	a	*b*	*c*	*d*
550.0		91.5		
400.0	181.8			
300.0	153.8			
275.0		68.6		
200.0	129.8			
137.5		50.8		
100.0	90.1			
73.3			35.2	
68.8		33.6		
40.0	44.0			12.2
36.7			26.5	
30.0	32.9			
20.6		14.8		
20.0	20.6			6.3
18.3			17.9	
15.0				
10.0	8.4			5.1
9.2			8.7	
6.9		4.9		
5.0				4.2
2.8			1.6	
2.5				3.4
1.5				1.8
0.9			0.7	
0.5				1.1

a denotes 55 mM ferricyanide and OD_{600} 5 microbe; *b* denotes 41.25 mM ferricyanide and OD_{600} 2.5 microbe; *c* denotes 27.5 mM ferricyanide and OD_{600} 2.5 microbe; *d* denotes 11 mM ferricyanide and OD_{600} 1 microbe.

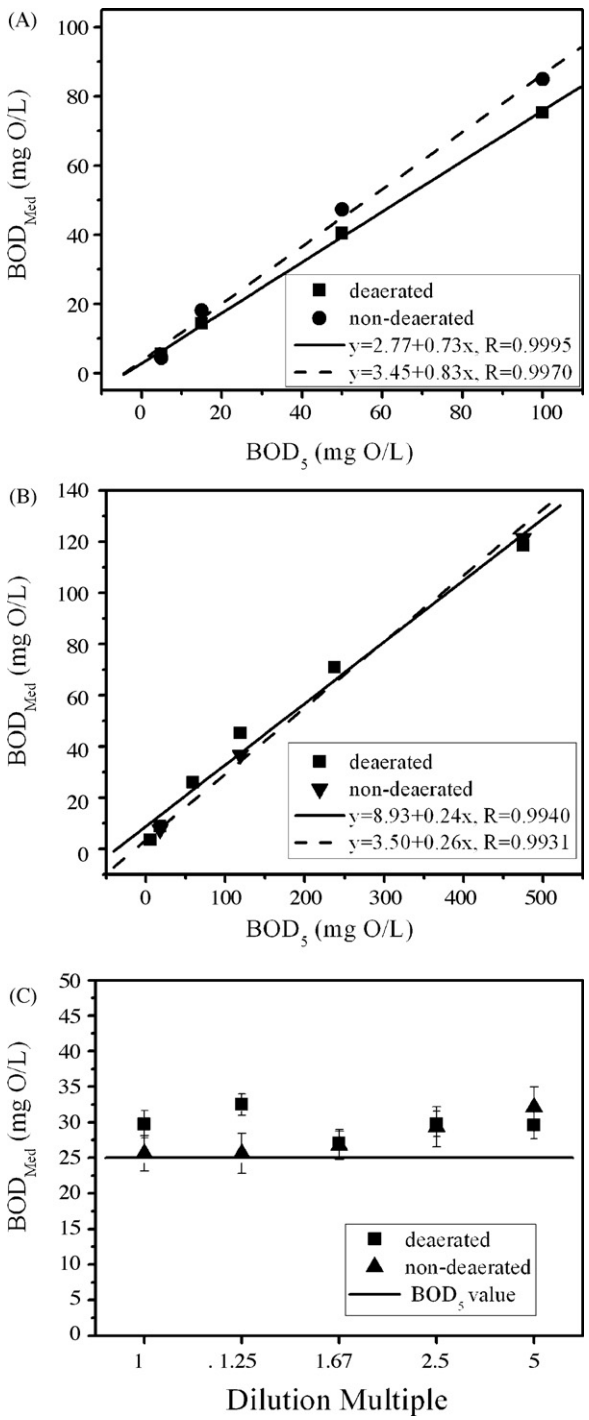

Fig. 4. Measurement of the glucose, OECD solution and real polluted wastewater. The real polluted wastewater was from a sewage treatment plant with a BOD_5 value 25 mg O/L, and the BOD_{Med} vales were obtained under the deaerated (diamonds) and non-deaerated (triangle) conditions.

27.5 mM to OD_{600} 2.5 microbe for sample below 18.3 mg O/L, and 11 mM to OD_{600} 1 microbe for sample below 5 mg O/L, respectively. Furthermore, there were not ideal results for the six measuring responses in the matched component of 47.25 mM to OD_{600} 2.5 microbe (as shown in Table 3 *b*), which means that the ratio of the microbe (OD_{600}) and the $K_3[Fe(CN)_6]$ (mM) with 1:11 was suitable for the NDA-BOD_{Med} measurement. The analysis of the results explained that the low-concentration substrate might suitable to be measured by decreasing the concentration of microbe and mediator.

3.5. Responses to typical organic substances and real polluted wastewater

Herein, for confirming the potential application of NDA-BOD_{Med} method, various kinds of typical samples were measured. As shown in Fig. 4A–C, the measurements of glucose, OECD solution and real polluted wastewater were conducted under both deaerated and non-deaerated conditions. All the calibration curves showed good linearity with linear regression coefficients of 0.26 and 0.24 (R was 0.9940 and 0.9931) for OECD solutions, and that of 0.73 and 0.83 (R was 0.9995 and 0.9970) for glucose solutions under the deaerated and the non-deaerated conditions. It was seen that the linear regression coefficient of glucose was close to that of GGA solutions (0.90, shown in Fig. 3), and whereas the linear regression coefficient of OECD (Fig. 4B) was ~29% of GGA. Obviously, *E. coli* has different biodegradation efficiencies for these organic substrates. The OECD solution included large polymer molecules such as peptone and meat extract, and the biodegradation of such substrates were more difficult than that of glucose and GGA. Most importantly, a wide spectrum of organic substrates for biodegradation was obtained in the NDA-BOD_{Med} method. The result indicated that, the NDA-BOD_{Med} method just provided a good linearity in a certain concentration range of organic substrate and the BOD values could be calibrated by mathematic method (such as the standard curve approach). Indeed, the selection of a more appropriate standard solution for wastewater measurements of different biodegradation levels should lead to even more accurate BOD values.

The measurements of real polluted wastewater with different dilutions were carried out by the BOD_{Med} method under the non-deaerated and deaerated conditions. As shown in Fig. 4C, the BOD_{Med} values were compared with BOD_5 values under the two conditions. In the conventional oxygen-type BOD biosensor, extensive dilution of samples was necessarily adopted. So the gradient dilution of sample was adopted in the present strategy for demonstrating that the measurement of real polluted wastewater did not need dilution. For dilution ratio 1 and 5 of samples, the relative standard deviations (RSD) by NDA-BOD_{Med} method with BOD_5 values were 2.5% and 28.6%, respectively. A large RSD for the five-fold dilution sample was obtained. It was noted that the result by NDA-BOD_{Med} method for non-diluted sample was more close to the BOD_5 method. Usually, the RSD of ±15% for BOD measurement was acceptable. So the present NDA-BOD_{Med} method could be used for measuring real polluted wastewater without sample dilution.

3.6. Affect the growth of E. coli

The effect of mediator to microorganisms was evaluated by plate culture count. Fig. 5 reveals the colony forming units (CFU), which

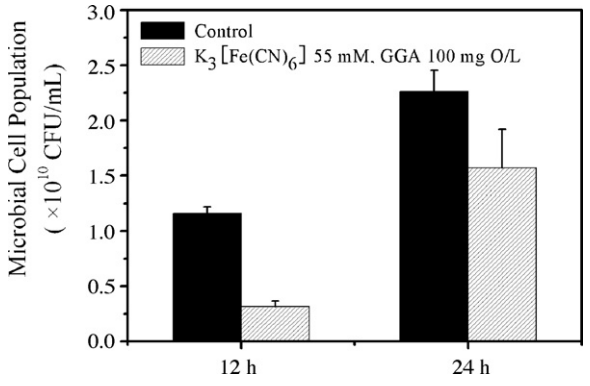

Fig. 5. Colony forming units (CFU) was counted after incubation for 12 and 24 h, the samples were incubated by 55 mM ferricyanide for 1 h.

was counted after cultivation for 12 and 24 h. After 12 h, the bacterial cells were 0.315×10^{10} and 1.16×10^{10} CFU/mL for the sample solution (incubating for 1 h with 55 mM ferricyanide) and the blank (without mediator), respectively. The CFU of the sample solution was 27% of the blank after 12 h, and the percentage increased up to 70% after 24 h. We speculated the possible reason for the result was that the negative effect of mediator to microbe could be recovered along with the culture extended. The result was significant for that the immobilized microorganisms could be applied in practical application and the mediator type BOD biosensor could be developed in future.

4. Conclusion remarks

We had explored a novel NDA-BOD_{Med} method for measurement of BOD and explained the feasibility of NDA-BOD_{Med} method. The biodegradation level by the present method could be reached to the conventional BOD_5 method in 1 hour. The relationship between the artificial and the natural electron acceptor was investigated, and it was found that the oxygen consumption in the NDA-BOD_{Med} measurement was mainly contributed to endogenous values. The negative effect of mediator to microbe could be recovered along with the culture extended. This method does not require both of air-supply and deaerated equipment for rapid measuring BOD value of real polluted wastewater. The present strategy offered a simple way for resolving the problems for the fluctuations of the response resulted from the deaeration, and simplifying the constitution of BOD_{Med} instrument. The NDA-BOD_{Med} method was proposed can offset the weakness in BOD_{Med} method and is more useful in practical application for environmental analysis.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20820102037), The National High Technical Development Project Foundation (863 project, 2007AA061501) and The Special Funds for Major State Basic Research Projects of China (973 project, 2010CB933600).

References

[1] Standard Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association, Washington, DC, 1997.
[2] Y. Lei, W. Chen, A. Mulchandani, Anal. Chim. Acta 568 (2006) 200.
[3] Z. Du, H. Li, T. Gu, Biotechnol. Adv. 25 (2007) 464.
[4] K. Catterall, H. Zhao, N. Pasco, R. John, Anal. Chem. 75 (2003) 2584.
[5] K. Catterall, K. Morris, C. Gladman, H. Zhao, N. Pasco, R. John, Talanta 55 (2001) 1187.
[6] A. Tizzard, J. Webber, R. Gooneratne, R. John, J. Hay, N. Pasco, Anal. Chim. Acta 522 (2004) 197.
[7] C. Liu, T. Sun, Y. Zhai, S. Dong, Talanta 78 (2009) 613.
[8] H. Nakamura, K. Suzuki, H. Ishikuro, S. Kinoshita, R. Koizumi, S. Okuma, M. Gotoh, I. Karube, Talanta 72 (2007) 210.
[9] N. Yoshida, J. Hoashi, T. Morita, S.J. McNiven, K. Yano, A. Yoshida, H. Nakamurab, I. Karube, Analyst 126 (2001) 1751.
[10] N. Maximova, O. Dahl, Chem. Soc. Rev. 36 (2007) 1323.
[11] P. Ertl, B. Unterladstaetter, K. Bayer, S.R. Mikkelsen, Anal. Chem. 72 (2000) 4949.
[12] K. Morris, H. Zhao, R. John, Aust. J. Chem. 58 (2005) 237.
[13] N. Pasco, J. Hay, A. Scott, J. Webber, Aust. J. Chem. 58 (2005) 288.
[14] H. Chen, T. Yea, B. Qiu, G. Chen, X. Chen, Anal. Chim. Acta 612 (2008) 75.
[15] S.P. Trosok, B.T. Driscoll, J.H.T. Luong, Appl. Microbial Biotechnol. 56 (2001) 550.
[16] N. Yoshida, K. Yano, T. Morita, S.J. McNiven, H. Nakamura, I. Karube, Analyst 125 (2000) 2280.
[17] G. Ramsay, A.P.F. Turner, Anal. Chim. Acta 215 (1988) 61.
[18] T. Kalab, P. Skladal, Electroanalysis 6 (1994) 1004.
[19] N. Pasco, K. Baronian, C. Jeffries, J. Webber, J. Hay, Biosens. Bioelectron. 20 (2004) 524.
[20] K.R. Rogers, Anal. Chim. Acta 568 (2006) 222.
[21] B. Heffernan, C.D. Murphy, E. Syron, E. Casey, Environ. Sci. Technol. 43 (2009) 6776.
[22] K. Morris, K. Catterall, H. Zhao, N. Pasco, R. John, Anal. Chim. Acta 442 (2001) 129.
[23] Organization for Economic Corporation and Development (OECD), OECD Guidel. Testing Chem. 209 (1984) 1.
[24] N. Pasco, K. Baronian, C. Jeffries, J. Hay, Appl. Microbial. Biotechnol. 53 (2000) 613.
[25] K. Morris, Ph.D. dissertation, Griffith University, Australia, 2005, p. 191.