Talanta 109 (2013) 147–151

Contents lists available at SciVerse ScienceDirect

Talanta

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

Short communication

Field application of a biofilm reactor based BOD prototype in Taihu Lake, China

Changyu Liu, Shaojun Dong*

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

ARTICLE INFO

Article history:
Received 15 November 2012
Received in revised form
28 January 2013
Accepted 1 February 2013
Available online 19 February 2013

Keywords:
Tubular biofilm reactor
Biochemical oxygen demand (BOD)
Field application
Online analyzer
Automated monitoring
Taihu Lake

ABSTRACT

A tubular biofilm reactor (BFR) based online biochemical oxygen demand prototype was applied in Taihu Lake, China. Municipal tap water was used instead of conventional phosphate buffer as blank solution to avoid phosphate pollution. The background organic compounds in municipal tap water were taken into account and they were validated to result in negative deviation to accuracy. The microbial endogenous respiration was experimentally validated to be sensitive to salt ionic strength, and municipal tap water as blank was thought to generate positive deviation to accuracy. The system was continuously operated over 2 months without man intervention, and the automated monitoring data agreed well with that of the conventional BOD_5 methods. The BFR resisted the frequent measurements with samples of high turbidity, and the BOD monitoring data indicated the index of biodegradable organic compounds of Taihu Lake was accorded with the second class described in the environmental quality standard of surface water. Analyzed together with permanganate index on site, Taihu Lake was revealed to be of good capacity of self cleaning. Importantly, field application study of new BOD method made it more objective in evaluating its applicability, and could provide practical information and useful improvements in the process of commercializing.

1. Introduction

With the urban construction, economic construction and development and population growth, the water pollution problem has intensified and become a hot topic [1]. Recent years, the huge outbreaks of algae in China's inland lakes and coastal area have seriously imperiled local residents. Water pollution problem has become a bottleneck constraining economic growth in China. In order to rapidly gain the information of water qualities, protect water source and prevent pollution, it is imperative to establish a general and efficient system for water quality monitoring [2]. Normally, the water quality parameters such as pH, dissolved oxygen (DO), oxygen demand (OD), total organic carbon (TOC), conductivity, nitrogen and phosphorus related parameters are the most commonly being monitored [3]. These monitor units are integrated with peripheral sampling, washing and filtering system, forming an automated water quality monitoring station [3]. Among these parameters, OD usually includes chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Further in surface water quality evaluation, permanganate index, namely COD_{Mn}, is extensively applied [4]. The difference between COD_{Mn} and BOD is essentially determined by "oxidant" used in the two methods: permanganate or microorganisms [5]. Undoubtedly, BOD determination has been the most preferred mean for environmental estimation because it simulates the biodegradation process of the aggregate organic pollutants in natural conditions, providing useful information for biological and environmental impact assessment.

BOD is a necessary item in the monitoring of surface water quality [4]. The standard method for estimating biological oxygen demand is 5-day BOD assay (BOD_5) [6]. Obviously, the 5-day incubation is a fatal disadvantage in routine monitoring, resulting hysteretic feedback [7]. Rapid BOD methods based on biosensors [8–10], mediators [11,12] and biofuel cells [13,14] have been broadly lighted in the past thirty years, and some of them were commercialized. BOD-2000 analyzer utilizes a typical biosensor based BOD method developed by Nisshin Denki and Central Kagaku Co. Ltd., Japan [15]. The primary element is a biosensor, which consists of a microbial film coupled with an oxygen sensor. The output current of this oxygen sensor decreased when sample solution with biodegradable organic compounds flew through this biosensor on account of the increased microbial respiration rate. This is also the common operation principle of the biosensor based BOD analyzer and it is regarded as one of the simplest BOD determination principles to date. An online bioreactor based BOD analyzer BIOX-1010 was put forward by STIP Isco GmbH, Germany [16]. The samples were diluted and pumped into the bioreactor until a constant microbial respiration rate was obtained, and BOD was calculated according to the dilution multiples. The use of huge amount of complex microorganisms in the bioreactor brought promising in biodegradation efficiency [17].

* Corresponding author. Tel.: +86 431 8526 2101.
E-mail address: dongsj@ciac.jl.cn (S. Dong).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.02.006

However, the consumption of fresh water of 720 L/day made it impractical in application. Moreover, the HABS series BOD analyzer using the mediator-less microbial fuel cell was developed by KORBI Co. Ltd., Korea [18]. Electrochemically active bacteria produced electrons during the digestion of organic contaminants in wastewater. Produced electrons were transferred from the electrochemically active bacteria to the electrodes, generating an electric current. The current generated from a microbial fuel cell was directly proportional to the amount of organic waste supplied. It was then converted to measure the BOD value. However, without rigorous maintenance, all microbial fuel cells were instable and have a limited lifespan [5]. These listed BOD analyzers well represented the development directions and great progresses of rapid BOD methods. Even so, rapid BOD analyzers were seldom integrated in automated water quality monitor station to the best of our knowledge. In most cases, rapid BOD analyzers were not widespread, and this parameter was ignored. Occasionally, rapid BOD analyzers were provided but unused. The absences of BOD parameter in estimation water quality were mainly because of the unauthentic results and complicated maintenances of the BOD analyzer [19].

Recently, we developed a tubular biofilm reactor (BFR) based reagent-free BOD determination method [20,21]. This method embraced the advantages of simplicity as biosensor based BOD method and high biodegradation capacity as biofuel cell based BOD method. Municipal tap water was used as blank to avoid secondary pollution caused by phosphate buffer [22]. Long term operational and storage stabilities were studied with standard solutions in laboratory. Excellent performances, coupled with simplicity in device, convenience in operation and minimal maintenance, made it promising in field application. Herein, field application study based on the tubular BFR-BOD system was carried out in Taihu Lake, China. As a significant drinking water source, the water quality of Taihu Lake attracted the governmental and public attentions, especially since the breakout of blue-green algae bloom in 2007. Additionally, Taihu Lake is a characteristic inland lake in China and has been widely studied in many aspects. In this study, some practical problems in field applications, such as the effects of the trace amount organic compound and salt ionic strength to measurements, were discussed.

2. Experimental section

2.1. Standard and blank solutions

An artificial wastewater (AWW) was prepared according to previous report [23]. It contains 4.25 mg L^{-1} nitrohumic acid, 4.18 mg L^{-1} tannic acid, 2.43 mg L^{-1} sodium lignisulfonate, 4.70 mg L^{-1} gum arabic, and 0.94 mg L^{-1} sodium lauryl sulfate. The experimental BOD_5 of AWW was 3.7 mg L^{-1}. An artificial tap water was prepared by dissolving 200 mg Na_2SO_4, 200 mg $CaCl_2$, 200 mg KCl, 10 mg KNO_3, and 300 mg $MgSO_4 \cdot 7H_2O$ with deionized water, and diluted until its conductivity was around 340 μS cm^{-1}. The municipal tap water was collected and stored at room temperature (~20 °C) ahead.

2.2. Preparation of biofilm reactor

The BFR (φ=2.0 mm and L=105 cm) was prepared in according to our previous studies [20]. Basically, the glass tube was treated with HF/NH_4F (1.7%/2.3%, w/w) solution, followed by thorough washing with water to obtain a rough inner surface. Air-saturated Taihu Lake sample with added nutrients was continually pumped through the etched tube at a flow rate of 0.5 mL min^{-1} at a constant temperature of 30 °C. The status of biofilm formation was estimated by measuring the current responses of a DO probe to an injected AWW solution at intervals. The gradually decreased current signal with increased cultivation time indicates the progressive biofilm formation process. The cultivation process was terminated when no further decrease in current signal was observed from the injections of the AWW solution in two consecutive time intervals. The resultant BFR was filled in municipal tap water and stored at room temperature before use.

2.3. System operation

The BOD online prototype was developed by Changchun Institute of Applied Chemistry (Chinese Academy of Sciences) and fabricated by Jilin Grand Analysis Co., Ltd. based on our previous studies [20,21]. DO probe with an Au working electrode (φ=0.8 mm) covered by the Teflon membrane (Orbisphere 2956A) was used and all current signal measurements were performed under a constant applied potential of −700 mV vs. Ag/AgCl (0.1 M KCl), controlled by an integrated electrochemical platform. The BFR was placed in a thermostatic chamber at a constant temperature of 30 °C, as well as the tap water and sample containers. The taper water and sample alternately flowed through the BFR at a flow rate of 2.0 mL min^{-1}, and DO of the effluent from BFR was continuously monitored. When instructed to measure, the tap water in store was transferred to the container for air-saturation and heating. Five minutes later, the air-saturated tap water was pumped through the BFR, providing a steady-state current (i_b) of the DO probe. This process lasted for 35 min, during which the sample (standard or real sample) was injected into the sample container for DO equilibrium and heating. The air-saturated sample solution was then continuously injected into the BFR by switching a triple valve while the oxygen depletion of the effluent was simultaneously indicated by the current signal decrease, achieving a new steady-state current (i_s) in 10 min. The steady-state current change ($\Delta i = i_b - i_s$) was calculated and used as the analytical signal for BOD quantification. The BFR was stored with last sample in it until next measurement.

To apply the present method, several measurement instruments were utilized to measure the quality of Taihu Lake samples and municipal tap water. The COD_{Mn} values were obtained by using a COD_{Mn} analyzer (COD-203A, Hach). Conductivity was measured using a conductivity meter (FE30K, Mettler). DO was measured using a DO meter (Model 58, YSI), and pH was measured using a pH meter (FE20K, Mettler). The BOD_5 assay was carried out according to the standard method [6].

2.4. Field application site

The application site was located in Nanquan Waterworks (Wuxi, China), namely Wuxi ShaZhu automated water quality monitoring station (E120°13′46.4″, N31°23′57.8″)(Fig. 1). The BOD monitoring unit was experimentally installed in the monitoring station. The water intake extended to the middle of the lake. The samples were roughly filtered by sand filtration for this unit only. The general characteristics of the inflow sample are described in Table 1. Some of these water quality indexes can be freely obtained via Internet supported by National Environment Monitoring Station [24].

3. Results and discussion

3.1. Effect of organic compounds in municipal tap water

Municipal tap water has been successfully used in our BFR based BOD system instead of PBS over the past two years [22].

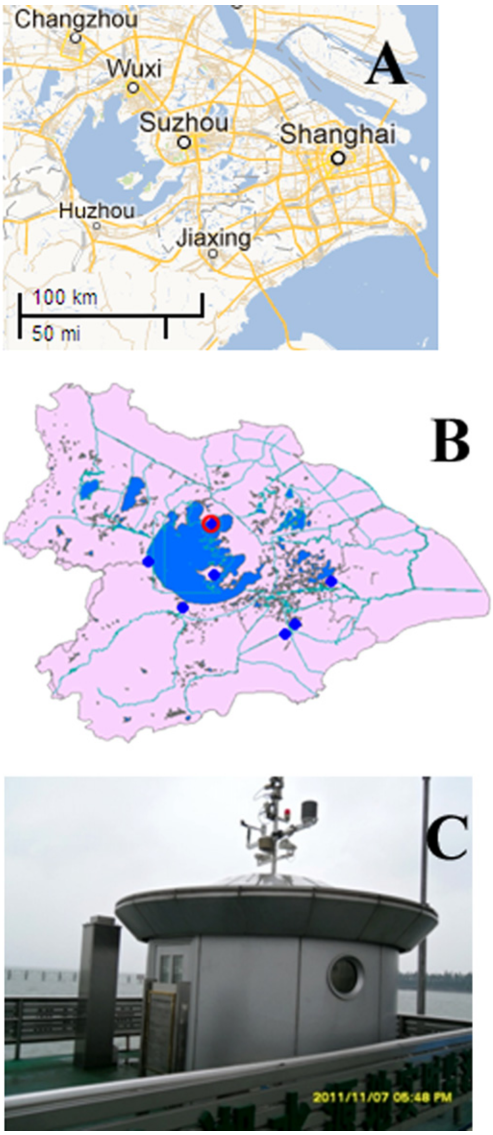

Fig. 1. Geographic location of Taihu Lake (A) and the automated water quality monitoring station (marked in the red circle) (B) and the photograph of the automated water quality monitoring station (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Typical parameters of Taihu Lake sample in a month (Sept. 2012).

Parameter	Average	Range
Temperature °C	24.2	17.6–30.4
pH	7.4	7.0–7.9
DO mg L^{-1}	7.44	5.98–8.82
NH_4–N mg L^{-1}	0.35	0.18–0.84
BOD_5 mg L^{-1}	1.8	1.5–2.1
Conductivity μS cm^{-1}	531	504–546
COD_{Mn} mg L^{-1}	4.8	3.1–5.5

The system maintains a good stability and sensitivity in the laboratorial measurements to standards and real samples. Normally, the effect of trace amount of organic compounds in municipal tap water was deducted because of dilution in the analysis of standard and high BOD samples. Both the standard and real samples embraced almost the same background as municipal tap water after multiple dilutions. However, the trace organic compounds in municipal tap water could be an issue decreasing the measured BOD values in the determination of low BOD samples because of free from sample dilution. Thus, the conventional parameters of local tap water were analyzed before it was used. The pH normally stabilized at 7.2, and the COD_{Mn} is normally less than 2.0 mg L^{-1}, which implied a sustainable concentration of organic compounds in municipal tap water. The further BOD5 experiments showed that the municipal tap water consumed at most 0.6 mg L^{-1} oxygen more than deionized water in 5-day incubation ($n=7$). In order to further verify the organic compounds influence, the artificial tap water was used as blank solution, and municipal tap water was used as sample to be measured repeatedly by BFR. The obtained responses were 13.4 ± 0.9 nA averagely ($n=7$). The results indicated that municipal tap water consumed more DO than artificial tap water. In other words, the trace amount of organic compounds in municipal tap water brought a negative deviation once municipal tap water was used as blank for BOD determination. Even so, municipal tap water was undoubtedly an eligible blank solution in field application to avoid secondary pollution by way of positive calibration, and the calibration constant (α) caused by trace organic compounds in the municipal tap water was -13.4 nA in the form of current response. In fact, BOD is never such a strict parameter, which is determined by the experimental error of BOD_5 and systematic error of analyzer itself [19].

3.2. *Effect of ionic strength*

The BFR was validated to be salt ionic strength insensitive when PBS concentrations ranged from 5 to 100 mM in our previous studies [20]. The microbial bioactivity and endogenous respiration were regarded to achieve a steady state in this PBS range. In these methods, it was necessary to add concentrated PBS into the sample in order to enhance the salt ionic strength, as well as decreasing the background differences between PBS blank and sample solution. On the other hand, it could be seen from the slopes (analytical current vs. BOD_5 values) that sensitivity decreased a little when municipal tap water was used compared to 5 mM PBS in our previous studies [20,22]. This indicated municipal tap water inhibited the bioactivity of the BFR though it was capable of providing the necessary conditions in maintaining the bioactivity and cell osmotic press. These evidences revealed that accurate measurements were closely related to proper salt ionic strength in the background and minimum background differences between blank and sample solutions. Conductivity was introduced in estimating the salt ionic strength, and it normally stabilized at 341 μS cm^{-1} for the municipal tap water, which was far less than that of 4 mM PBS (725 μS cm^{-1}) and Taihu Lake water (531 μS cm^{-1} averagely). Thus, influence to accuracy derived from background differences between local tap water and Taihu Lake water should be studied. Municipal tap water was used as a blank solution, and various concentrations of PBS (1–10 stepped by 1 mM) were tested as samples repeatedly. The obtained analytical signals were described in Fig. 2. In Fig. 2, the results implied that accelerated microbial endogenous respiration occurred with increasing PBS concentration, and vice versa. This indicated that the salt ionic strength affected the microbial endogenous respiration/bioactivity when the ion concentration was low. On the other hand, it can be seen that the analytical signal close to zero were obtained at PBS concentration of 4 mM. It seemed that 4 mM PBS and municipal tap water embraced equivalent effect in maintaining microbial endogenous respiration/bioactivity. However, it was known as mentioned above that the salt ionic strength of municipal tap water was far less than that of 4 mM PBS. Superficially, salt ionic strength was not the only influencing factor in this experiment. In fact, municipal tap water contained trace amount of organic compounds besides inorganic ions as described before.

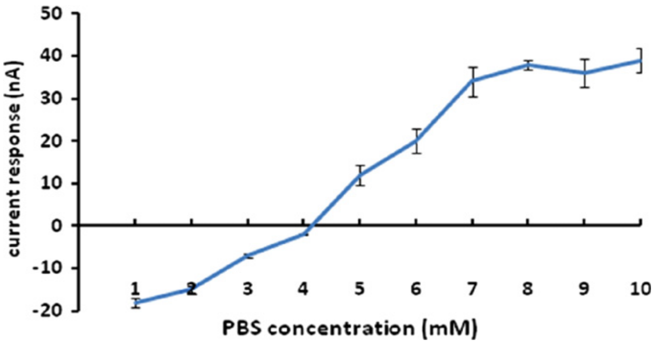

Fig. 2. Current responses to a series of PBS solutions (municipal tap water as blank). The error bars represent standard deviations of three successive measurements. Temperature: 30 °C, tubular BFR length: 105 cm.

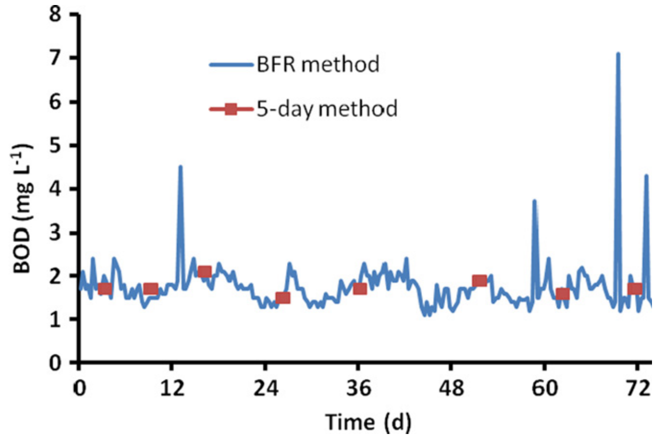

Fig. 3. BFR-BOD profile and BOD_5 results over 70 days.

The excess amount of ions in PBS compared with that of municipal tap water promoted the microbial respiration equivalently as organic compounds in municipal tap water acted. Additionally, the deviation factor was studied by comparing analytical responses changed vs. conductivities, and the degree was calculated to be 0.049 nA cm μS^{-1}. In our cases, the conductivity of municipal tap water is far less than that of Taihu Lake water (341 vs. 531 $\mu S\,cm^{-1}$ averagely, respectively). Therefore, the calibration constant (β) caused by salt ionic strength in the municipal tap water based BOD method was +9.5 nA in the form of current response in Taihu Lake BOD monitoring. In conclusion, salt ionic strength was thought to be one of the influencing factors in low BOD monitoring, and local tap water can be used as blank in Taihu Lake BOD monitoring by way of negative calibration.

3.3. System calibration and BOD quantification

AWW solution was prepared using the main refractory organics in river waters as reported, and it was used as the calibration solution for Taihu Lake BOD monitoring. Thus, AWW solution and municipal tap water were used as the standard and blank solutions in this study, respectively. The BOD online prototype was calibrated by two points, i.e. zero point and 3.7 mg L^{-1} AWW point on account of the low BOD level in Taihu Lake. According to the analytical rules, municipal tap water was used to wash the BFR to provide a steady-state baseline current before zero point solution (municipal tap water) or AWW solution was injected into the BFR for calibration. The mean and standard deviation of zero point measurements were calculated to be 0.9 ± 1.7 nA (n=20), which indicated the online prototype embraced a good accuracy and reproducibility. AWW calibration was conducted as well and the mean and standard deviation were calculated to be 85.8 ± 5.3 nA (n=20). Therefore, the sensitivity could be calculated according to the following formula

$$\text{Sensitivity (nA L mg}^{-1}) = \frac{(r_{\text{AWW}} - r_{\text{zero}}) - \alpha - \beta}{C_{\text{AWW}}} \quad (1)$$

In the equation, r_{AWW} and r_{Zero} represented the average analytical response to AWW and blank solution, respectively. CAWW represented the BOD_5 concentration of AWW. The sensitivity was hence calculated to be 24.0 nA L mg^{-1}. The obtained sensitivity was used for Taihu Lake BOD quantification.

3.4. Field application in Taihu Lake

After being calibrated, the BOD prototype was integrated in the automated water quality monitoring station. The analytical cycle was set at 4 h, and the other parameters and the analytical procedures were the same as those described above. Stock solution of municipal tap water was supplied every week. The conventional BOD_5 test was carried out irregularly. The capability and applicability of the online BOD prototype were studied over 70 days. The overall performances of both methods were illustrated in Fig. 3. In Fig. 3, it could be concluded from both the rapid and conventional methods that the oxygen demand of biodegradable organic compounds in Taihu Lake was less than 3 mg L^{-1} in this period. The occasionally increased responses were derived from sample supply problem, which needs improvements next. The consistent results showed that the municipal tap water based BOD prototype was an alternative method for Taihu Lake BOD monitoring. Moreover, such a result showed that the BOD in Taihu Lake was accorded with the second class (class II) described in the environmental quality standard of surface water. The COD_{Mn} results were averagely 4.8 mg L^{-1} in this duration, and the comparative studies of BOD and COD_{Mn} indicated that the water body was of a fine self cleaning capacity. High turbidity and wide distribution of algae are still deep-rooted problems in Taihu Lake, and the inner surface of the BFR was covered with a bed of sludge from the 4th month, which indicated by the gradually decreased sensitivity, and accordingly, the prototype needed to be recalibrated. Taihu Lake is a shallow lake, and the average depth is only about 2 m. Sludge in the lakebed was readily disturbed in windy days. The aforementioned problem was thought to be originated from the frequent sludgy sample measurements and, hence, a more delicate filter system might be needed. The BFR was over blown as occasion requires and re-cultivated over night. The analytical responses to AWW recovered easily and the BFR was competent after a few days' domestication.

4. Conclusions

Water pollution, especially drinking water source pollution, is becoming increasingly serious, which is closely related to local people's lives. Comprehensive estimation of the water quality is a useful means in pollution alerting and environmental protection. BOD is a must be monitored item, but rapid BOD analyzers are of extreme deficiency in automated water quality monitoring compared with numerous laboratorial methods. Field application studies coupled with new BOD methods are imperative in promoting the development of BOD analyzers. A tubular BFR based prototype was applied in Taihu Lake, China in this study. Municipal tap water was used as blank solution to avoid secondary pollution, and the application feasibility and potential problem were studied. The trace amount of organic compounds in municipal tap water brought a negative deviation of 0.6 mg L^{-1} to accuracy. The experiments indicated that inorganic ions in low concentrations influenced the microbial endogenous respiration, and local tap water based prototype would result in a positive deviation of 0.4 mg L^{-1} in Taihu Lake BOD monitoring. These deviations were considered and calibrated in the terminal BOD quantification. The continuously automated monitoring showed that the biodegradable organic compounds in Taihu Lake maintained at a relatively low level. The sample supplying and self filtering system remained some problems to be solved in our following studies.

Acknowledgments

We acknowledge financial supports from the National Natural Science Foundation of China (No. 20820102037 and 20935003), and the National Basic Research Program of China (No. 2010CB933603).

References

[1] T.P. Ouyang, Z.Y. Zhu, Y.Q. Kuang, J. Environ. Monit. 7 (2005) 664–669.
[2] V. Vandenberghe, P.L.M. Goethals, A. Van Griensven, J. Meirlaen, N. De Pauw, P. Vanrolleghem, W. Bauwens, Environ. Monit. Assess. 108 (2005) 85–98.
[3] Monitoring and Analysis Methods for Water and Wastewater, 4th Bureau of National Environmental Protection, Chinese Environmental Science Press, Beijing, 2002.
[4] National Standard-Environmental Quality Standard for Surface Water, Bureau of National Environmental Protection, Chinese Environmental Science Press, Beijing, 2002.
[5] N. Ph. Namour, Jaffrezic-Renault, Trends in Anal. Chem. 29 (2010) 848–857.
[6] Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC, 1997.
[7] Y. Lei, W. Chen, A. Mulchandani, Anal. Chimica Acta 568 (2006) 200–210.
[8] I. Karube, T. Matsunaga, S. Mitsuta, S. Suziki, Biotechnol. Bioeng. 102 (2009) 660–672.
[9] S. Velling, A. Mashirin, K. Hellat, T. Tenno, J. Environ. Monit. 13 (2011) 95–100.
[10] C.Y. Liu, C. Ma, D.B. Yu, J.B. Jia, L. Liu, B.L. Zhang, S.J. Dong, Biosen. Bioelectron. 26 (2011) 2074–2079.
[11] M. Celina Bonetto, N.J. Sacco, A.H. Ohlsson, E. Cortón, Talanta 85 (2011) 455–462.
[12] L. Liu, L. Deng, D.M. Yong, S.J. Dong, Talanta 84 (2011) 895–899.
[13] M. Kim, S.M. Youn, S.H. Shin, J.G. Jang, S.H. Han, M.S. Hyun, G.M. Gadd, H.J. Kim, J. Environ. Monit. 5 (2003) 640–643.
[14] I.S. Chang, J.K. Jang, G.C. Gil, M. Kim, H.J. Kim, B.W. Cho, B.H. Kim, Biosens. Bioelectron. 19 (2004) 607–613.
[15] R. Iranpour, B. Straub, T. Jugo, J. Environ. Eng. 123 (1997) 154–159.
[16] 〈http://www.envitech.co.uk/〉.
[17] R. Iranpour, M. Zermeno, Water Environ. Res. 80 (2008) 298–307.
[18] 〈http://www.korbi.com/eng/index.asp〉.
[19] J. Liu, B. Mattiasson, Water Res. 36 (2002) 3786–3802.
[20] C.Y. Liu, H.J. Zhao, L.J. Zhong, C. Liu, J.B. Jia, X.L. Xu, L. Liu, S.J. Dong, Biosen. Bioelectron. 34 (2012) 77–82.
[21] C.Y. Liu, J.B. Jia, S.J. Dong, Biosen. Bioelectron. (2012).
[22] C.Y. Liu, H.J. Zhao, G. Shan, J.B. Jia, L.M. Zhao, D.M. Yong, S.J. Dong, Biosen. Bioelectron. http://dx.doi.org/10.1016/j.bios.2013.01.041,in press.
[23] Y.C. Kim, K.H. Lee, S. Sasaki, K. Hashimoto, K. Ikebukuro, I. Karube, Anal. Chem. 72 (2000) 3379–3382.
[24] 〈http://58.68.130.147/#〉.