



Self-build packed-bed bioreactor for rapid and effective BOD estimation

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Abstract

This work demonstrated a simple, low-cost, rapid, and effective biochemical oxygen demand (BOD) estimation system based on a packed-bed bioreactor that can be easily self-built on-site at a particular wastewater treatment plant for continuous monitoring of the influent and effluent. The use of natural microbial consortium that were collected from the target wastewater and immobilized on a cheap porous carrier simply by adhesion resulted in an acceptable accuracy of over 95%. The newly developed semi-continuous operating mode with peak-type signals was shown to be able to continuously estimate BOD at a high flow rate to overcome the flow dependence of the oxygen electrode, limit clogging issues, enhance the response time, and lower the limit of detection. The resulting packed-bed bioreactors could work continuously for 22 h with a coefficient of variance (CoV) of only 1.8% or for 13 h a day for several days with a maximum CoV of 1.4% and their response was observed to be stable over 80 consecutive measurements. They exhibited stable responses at a wide pH range of 6.5–8.5, which is also the recommended range for aerobic wastewater treatment, emphasizing the greater ease of use of natural microorganisms for BOD estimation.

Keywords Packed-bed bioreactor · Biochemical oxygen demand · Biosensor · Semi-continuous operating

Introduction

Although becoming a global problem, water scarcity is particularly apparent and more severe in Asia, which is home to 60% of the world's population but contains only 36% of the world's freshwater resources (Gössling 2006). The countries, particularly those most vulnerable to climate change impacts such as Vietnam and other neighboring countries, are facing with enormous pressure on freshwater resources from competing demands of domestic, commercial, industrial, and agricultural use. Moreover, declining surface and ground water quality caused by discharging untreated or inadequately treated wastewater has become a global issue of concern. Therefore, developing cost-effective analytical techniques for fast determination and online monitoring of pollution

levels in wastewater has gained much interest in the recent years.

BOD, which stands for biochemical oxygen demand, is one of the most useful indicators of water quality or organic pollution level; however, prompt measurement of BOD is practically impossible. The traditional analytical method is very complex, labor intensive, and time-consuming (generally 5 days for determination of BOD₅) and the results may fluctuate with differences in the microbial diversity of the used inoculum. Although some improvements on the present devices have been achieved for this method to be more reliable and less complex, the required time to determine a real BOD₅ value cannot be shorter than 5 days (Jouanneau et al. 2014). Thus, a biosensor which can be used to predict BOD₅ value immediately has shown prospect of being an effective device for online monitoring of water pollution levels.

Works performed on the development of BOD biosensors have resulted in several technologies which can be classified by many aspects such as by a bio-receptor (yeast) (Arlyapov et al. 2013; Jia et al. 2003; Zhao et al. 2017), bioluminescent bacteria (Sakaguchi et al. 2003; Sakaguchi et al. 2007), other pure bacterial strains (Hu et al. 2017; Raud et al. 2012), artificial microbial mixture, natural microbial population, and activated sludge (Kumlanghan et al. 2008; Liu et al. 2013; Liu

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et al. 2012); by a biodegradation marker (dissolved oxygen) (Niyomdech et al. 2017; Zhao et al. 2017), a bioluminescence activity (Sakaguchi et al. 2003; Sakaguchi et al. 2007), a redox mediator (Wang et al. 2014; Zaitseva et al. 2017), and an electrical potential (Chouler et al. 2017; Pasternak et al. 2017); and by operating type (batch) (Wang et al. 2010), continuous flow through (Liu et al. 2012), and flow injection (Seo et al. 2009; Vanrolleghem et al. 1994)). In a recent review, Jouanneau et al. (2014) simply classified BOD biosensors into five groups: (i) biosensors based on bioluminescent bacteria, (ii) biosensors with redox mediators, (iii) microbial fuel cell (MFC) biosensor, (iv) biosensors with entrapped microorganisms, and (v) biosensors based on the bioreactor/chemostat technology although they are not always those upon this classification since some have been developed based on several technologies. Among them, the two former technologies have shown least potential for actual applications due to the complexity of the bioluminescence reaction and their low precision (Nakamura et al. 2007a; Nakamura et al. 2007b; Sakaguchi et al. 2003; Sakaguchi et al. 2007; Zaitseva et al. 2017). The MFC has been considered as a promising technology for converting organic matters in wastewater into energy and consequently shown outstanding potential to be a self-powered portable BOD biosensing device. However, this technology needs further development to become more suitable for real-time BOD monitoring due to its present long response and recovery times which are required to reach the steady-state current and recover from the applied disturbance. Moreover, obtaining the repeatable outputs regardless of the fluctuation of operating conditions such as pH, conductivity, and temperature of the wastewater is not straightforward.

Although the biosensors with entrapped microorganisms have been studied widely and shown high correlation with the traditional method, only few works have been performed with actual wastewater to assess the precision of the biosensors (Kumlanghan et al. 2008; Rastogi et al. 2003a; Rastogi et al. 2003b). In addition, the performance fluctuation of the biosensor due to the cellular growth inside the structures which were used to entrap or immobilize microorganisms results in a need of expensive replacement of bio-receptors. There are several available marketed systems based on this technology (Nomura et al. 2008); however, most of them are recognized as single cell-based microbial biosensors which may not contain all the required enzymes for a sequential set of degradation reactions. Thus, they seem not to be suited for prediction of BOD₅ value of some complicated wastewater sources.

It has been widely accepted that the bioreactor-based technologies are the most suitable ones for in situ real-time monitoring of BOD₅ in any respect (Bahadır and Sezgintürk 2015; Jouanneau et al. 2014; Nomura et al. 2008). All marketed versions employ natural microbial population and activated sludge as bio-receptors to fully utilize their wide detection

range (Endress+Hauser, 2018, LAR BioMonitor, n.d., RODTOX, n.d). However, as other marketed BOD biosensors, they are too expensive to be widely adopted for on-site and online monitoring of wastewater treatment plants, especially in developing countries (Reshetilov et al. 2013). For such application purposes, the existing microbial population in accordance with the particular type of wastewater would be the most efficient bio-receptor for the detection of its BOD. Thus, a cheap self-built BOD biosensor might be more appropriate than a costly universal one. Unfortunately, most of the promising biosensors that have possessed acceptable precision and stability are too complicated to self-build (Liu et al. 2016; Liu et al. 2013; Liu et al. 2012), while the simpler ones have shown either narrow range of detection (Seo et al. 2009) or low stability (Liu et al. 2000; Liu et al. 2004).

This paper aims to develop a simple and low-cost bioreactor-based device that would be easy to self-build for on-site prediction of BOD in both influent and effluent of a wastewater treatment plant. The natural microbial cells were immobilized on a cheap porous carrier by adhesion which is the simple, most eco-friendly, and cost-effective method, thus widely used in many industrial processes (Dzionic et al. 2016). The effectiveness of the proposed device was evaluated in terms of sensitivity, precision, and accuracy in accordance with BOD₅ value, detection range, and stability.

Method

Material

All chemicals used in this study were of analytical grade and purchased from HiMedia and Merck. Preparation and dilutions of solutions were done with drinking water which was used instead of phosphate-buffered saline (PBS) and deionized water for ensuring appropriate osmotic pressure needed for the survival of the cells (Liu et al. 2013), unless otherwise stated.

Preparation of stock solutions

Both glucose-glutamic acid (GGA) (Karube et al. 1977) and synthetic wastewater formulated by the Organisation for Economic Cooperation and Development (OECD) (OECD 2010) solutions were used as test solutions in this study. GGA stock solution, equivalent to a BOD₅ of 200 ± 10 mg/l, was simply prepared from 150 mg/l of glucose and 150 mg/l of glutamic acid, whereas OECD stock solution, equivalent to a BOD of 170 ± 10 mg/l, was more complexly handled from 15.0 mg/l of peptone, 11.0 mg/l of beef extract, 3.0 mg/l of urea, 0.7 mg/l of NaCl, 0.4 mg/l of CaCl₂·H₂O, 2.8 mg/l of K₂HPO₄, and 0.2 mg/l of MgSO₄·7H₂O (Liu et al. 2000).

Preparation of simple BOD sensing system

Packed-bed tube

Porous ceramic rings which are commercially available for aquarium filters were purchased from a local store, crushed into smaller particles of approximate size between 0.1 and 0.2 mm, disinfected with chlorine, and then rinsed thoroughly with clean water before being used as a cheap porous carrier for microbial immobilization. Three grams of the prepared ceramic particles was transferred into a U-shaped polyurethane (PU) tube (200 mm length and 8 mm inner diameter) prior to being incubated with natural bacteria consortium presented in the wastewater which was collected in the aerobic biological tanks of the Binh Hung municipal wastewater treatment plant (MWWTP) located at Ho Chi Minh City, Vietnam.

Cultivation

The collected wastewater was saturated with air and circulated through the packed-bed tube at room temperature and a flow rate of 50 ml/min by means of a submersible pump. Its bioactivity was evaluated by measuring the changes in DO responses of a GGA solution, equivalent to a BOD₅ of 10 mg/l, every 2 h. The cultivation process was done when no statistical significance was observed between the two consecutive measurements, resulting in a ready-to-use packed-bed bioreactor (PBBR). When not in use, the air-saturated tap water was circulated through it at the same conditions for preservation.

Measurement system and procedure

Figure 1 shows a schematic representation of the BOD sensing system based on a simple PBBR. DO concentrations were measured by a commercial DO probe DO-802 (Apel Instruments, Vietnam) with a measurement range of 0–20 mg O₂/l and resolution of 0.1 mg/l. The output voltage signals of the DO probe were recorded by a self-made data acquisition and processing system. All data were recorded at 1 s intervals.

As can be seen in here, the measurement system was operated based on a reversing flow mode which allows using only one probe for determination of DO contents of both the influent and effluent streams. The air-saturated sample was firstly introduced into the packed-bed tube by means of a peristaltic pump. After reaching the steady state DO response (DO_{out}), the sample flow was reversed to reach the DO probe without passing through the packed-bed tube to obtain the initial DO value of sample (DO_{in}). At the same time, the sample was isolated inside the tube and its organic compounds were degraded by the immobilized bacteria for a desired period prior to being released into the DO probe by reversing the flow of

sample, causing a decrease of DO response. Due to continuous feeding sample, the DO response increased until the steady state value (DO_{out}) was obtained again, resulting in a peak on the DO profiles. Figure 2 shows the resulting DO profiles of different isolation periods and the obtained parameters which would be used for BOD₅ estimation. For continuous mode, the change in DO response (ΔDO_0) was calculated as the difference between the steady state values of the influent and effluent (DO_{in} – DO_{out}), whereas the peak height was employed to determine the change in DO response for batch mode (ΔDO_t).

Since the method exhibits baseline noise, the limit of detection was determined by using the signal-to-noise method in which the lowest concentration of the analyte that would produce a signal equal to a certain value of noise-to-signal ratio is estimated (Shrivastava and Gupta 2011). Accordingly, a signal-to-noise ratio (SNR) of 3 is generally accepted for estimating the limit of detection. It is calculated as follows:

$$SNR = \frac{2H}{h}$$

where H is the height of the peak and h is the peak-to-peak background noise.

Determination of operating parameters

Being a tropical region, there is no significant change in water room temperature in Ho Chi Minh City, especially during the study period (from May to October 2018). Moreover, it was reported that the temperature of most discharged wastewater sources here varied from 29.7 °C to less than 32 °C (Project and Unit 2003). Thus, the effect of temperature was neglected in this study to simplify the measurement system and procedure towards actual applications.

Other operating parameters that could affect the performance of the BOD sensing system such as pH (from 5 to 9), flow rate (from 39 to 47 ml/min), and isolation time (from 1 to 8 min) were optimized using a GGA solution, equivalent to a BOD₅ of 10 mg/l. The initial pH, flow rate, and isolation time were fixed at 7 and 39 ml/min and 2 min. All experiments, including the ones described later, were conducted in triplicate, unless otherwise stated. Optimization was conducted by varying one variable while keeping the other unchanged. In general, the optimization target is to maximize the change in DO response as it reflects the performance of the system. However, the effect of isolation time was completely studied by assessing the corresponding sensitivity, range, and linearity of the calibration curve which was done by plotting the change in DO responses versus corresponding BOD₅

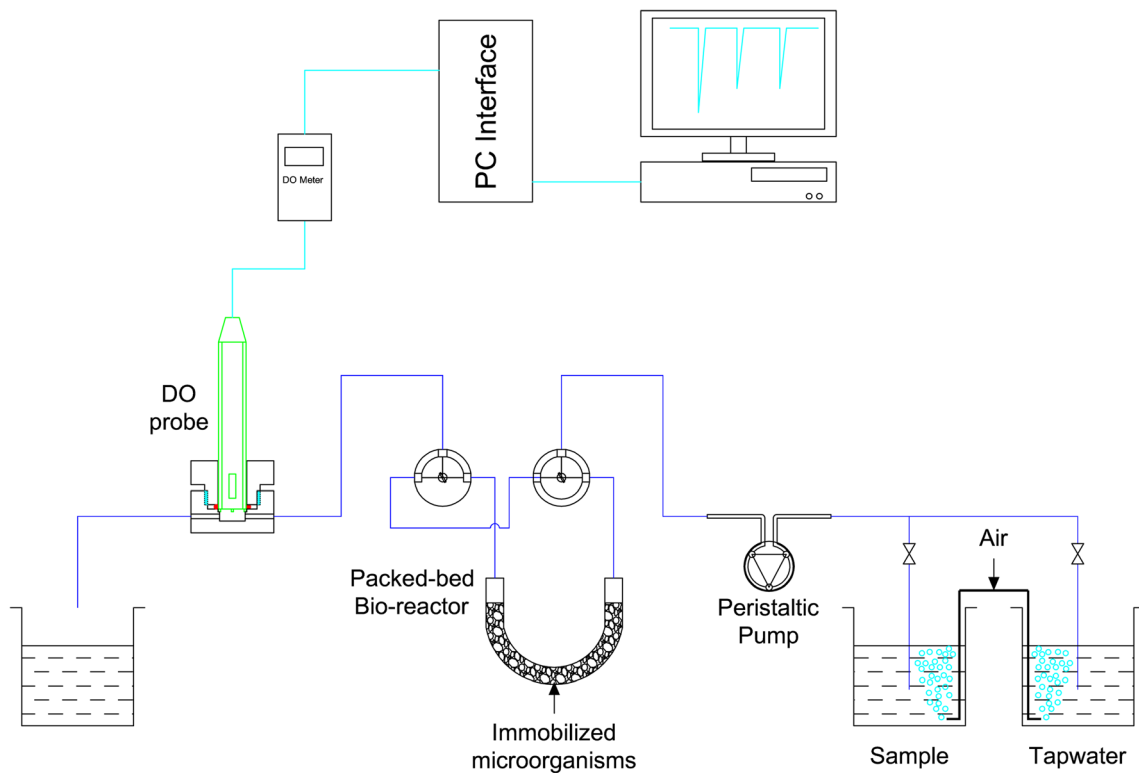


Fig. 1 Schematic diagram of the measurement system based on packed-bed bioreactor

values determined by the standard BOD₅ method (Rice et al. 2012) of a series of GGA solutions.

Repeatability, reproducibility, and stability

Generally, the coefficient of variance (CoV) or the relative standard deviation (RSD), which is indicative of consistency of the received data, must be used to assess the repeatability and reproducibility of the PBBR (Guideline 2005). It is defined as the ratio of the standard deviation of all obtained

values and to their mean and usually expressed in percentage terms. A GGA solution, equivalent to a BOD₅ of 10 mg/l, was employed again for such assessments.

After being cultivated, the as-prepared PBBR was continuously operated at optimum conditions. The time interval between two consecutive signals was fixed at 5 and 15 min. The stable change in DO responses was used to calculate the repeatability of the measurement system. The life time of the PBBR was examined both in terms of continuous operating and storage conditions.

The reproducibility of different as-prepared PBBR was assessed on 12 ones of which six ones were cultivated at different time during 3 months. Both output information such as the change in DO response and corresponding BOD₅ which was estimated from the calibration curve of the specific PBBR were used to calculate the CoV of reproducibility.

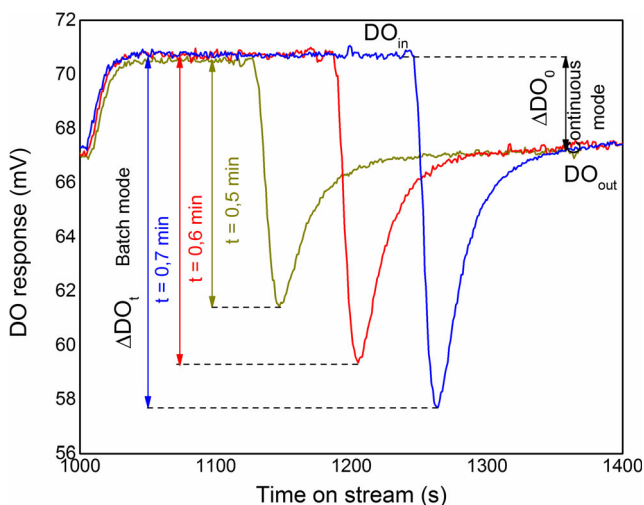


Fig. 2 DO profiles at different isolation periods

Authentic wastewater analysis

The BOD of the influent and effluent wastewater, which was collected every Monday morning from Binh Hung MWWTP during July, August, and October 2018, was estimated using two different PBBRs under optimum conditions. Calibration of the specific PBBR was done with both GGA and OECD solutions prior to measurements.

Results and discussion

Cultivation

The curves presented in Fig. 3 show a lag phase up to 12 h followed by a sharp rise to reach a stable response at 30 h. Therefore, all the packed-bed tubes in this study were cultivated for at least 36 h prior to use as PBBRs.

The surface morphologies of the porous ceramic carrier before and after cultivation were examined using a scanning electron microscope (JEOL 6400) and shown in Fig. 4. The typical size of bacteria was reported to be between 1 and 3 μm (Willey et al. 2008); thus, the pores of ceramic carrier are too big to retain them simply by size exclusion. However, its irregular surface provided the area available for bacterial adhesion and protected them from shear stress, resulting in bio-film formation after a simple cultivation process.

Effect of operating parameters

pH of samples

It is widely accepted that the pH of surrounding environment has a considerable influence on the activity of any microorganism. Therefore, the optimum pH range for specific bacterial consortium to break down organic matter present in a sample should be determined. It has been observed that the desirable pH range for a specific microorganism is narrow (Seo et al. 2009; Zhao et al. 2017), whereas that for consortia coming from authentic activated sludge is much wider (Kumlanghan et al. 2008; Liu et al. 2012). As can be seen in Fig. 5, the PBBR exhibited maximum response at a wide pH range of 6.5–8.5, which is also the recommended range for

aerobic wastewater treatment (Gray 1989), in both operating modes. Within this range, the performance of PBBR appeared to be statistically insensitive to pH changes. This finding is consistent with other studies emphasizing the greater ease of use of natural microorganisms for BOD estimation. Practically, the pH values of the prepared standard solutions and diluted authentic wastewaters were in this range so they were used as prepared without adjusting the pH.

Flow rate

It has been reported that flow rate significantly affects the performance of both BOD biosensors based on the continuous flow through (Liu et al. 2013; Liu et al. 2012) and flow injection operating modes (Niyomdecha et al. 2017; Seo et al. 2009). A very low flow rate (typically few milliliters per minute) has been required for ensuring a detectable change in DO response although it would obviously cause longer response time. In addition, it has been concluded that complete elimination of the flow dependence of the DO response due to oxygen consumption seems to be impossible in the case of using oxygen electrodes even if using the most precise ones (Helm et al. 2018; Suzuki et al. 2001). Flow dependence appears to be worse at a low flow rate region, resulting in remarkable errors if there is any clogging in the system. Therefore, the flow rate should be as high as possible to limit errors caused by varying flow rates during measurements and to enhance the response time. However, this is not straightforward for the current flow-type devices.

In this study, the semi-continuous type system was developed to detect BOD continuously at a much higher flow rate (37 to 77 ml/min). Generally, the DO probe is more accurate at higher DO levels, resulting in a larger increase in the received values with streams of higher DO contents compared with that of lower ones when the flow rate increases. In the case of both streams having nearly equal values, flow dependence appears to be neglected. Therefore, as shown in Fig. 6, ΔDO_0 seems to slightly decrease with the flow rate only due to the decrease in residence time, but actually, it was confirmed to be statistically insensitive to the change in this high flow rate region. In batch mode, the DO of the sample after isolation period was much lower than the DO_{in} value, so ΔDO_t increased with the flow rate and reached 11.38 mV at 53 ml/min, whereas no significant change was observed at higher flow rates due to much smaller flow dependence. Moreover, a significant improvement in the SNR was obtained with increasing flow rate from 37 ml/min (Fig. 7a) to 53 ml/min (Fig. 7b) due to the limitation of the pulse issue of the peristaltic pump. Therefore, the flow rate of 55 ml/min was chosen for the following experiments.

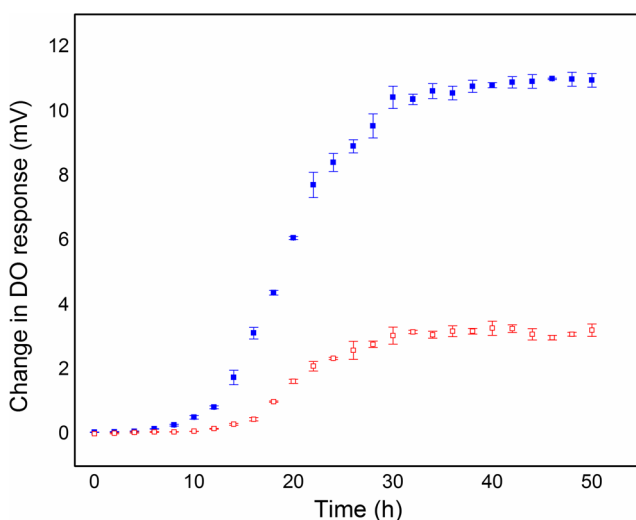


Fig. 3 Effect of cultivation time on the responses of the PBBR in batch mode (solid square) and continuous mode (open square)

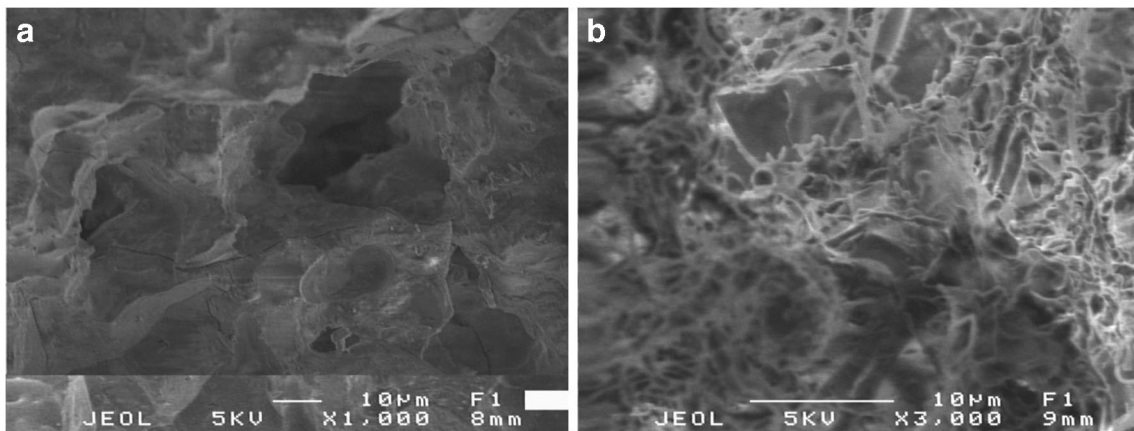


Fig. 4 SEM images of the ceramic carrier before (a) and after cultivation (b)

Isolation time

There are two methods to process the response of any biosensor based on oxygen electrodes: (i) the peak-baseline method in which the peak height, peak slope, or peak area is employed and (ii) the equilibrium method in which the difference between two steady state responses of the biosensor before and after introducing samples is used in quantitative calculations. Generally, the former method is more preferable than the later one both in terms of response time and ease of computation. Although the dynamic transition method in which the rate of change in DO response is used for BOD estimation is based on peak-type signals, it is practically infeasible due to its fluctuating signals and low SNR (Yang et al. 1997). Therefore, the peak-baseline method is only suited for the flow injection-type devices in which a small amount of sample needs to be injected by means of a complex injection system, resulting in complexity in manufacturing, operating, and maintenance. Meanwhile, the equilibrium method that is widely used for the

batch-type or flow through-type devices is time-consuming, low sensitivity, and not suited for online BOD monitoring.

In this study, peak-type signals, as shown in Fig. 2, were produced by using the semi-continuous operating type system that was proposed to take advantages of the above methods and devices. All samples, including drinking water as can be seen in Fig. 7, produced DO profiles that exhibit SNR greater than 3. Practically, the BOD₅ of drinking water was found to be zero; thus, it is reasonable to conclude that 0 mg/l is the lower limit of detection of the studied method.

Table 1 showed that all PBBRs which were cultivated at different times behaved similarly. The sensitivity increased, whereas the linear range decreased with increasing isolation time. During the isolation period, the PBBR works as a BOD bottle in the standard BOD₅ method; thus, longer isolation time caused a greater amount of oxygen consumed by microorganisms to degrade the organic matter present in the sample, resulting in a higher ΔDO_t. Consequently, the sensitivity of the biosensor can be significantly improved by simply

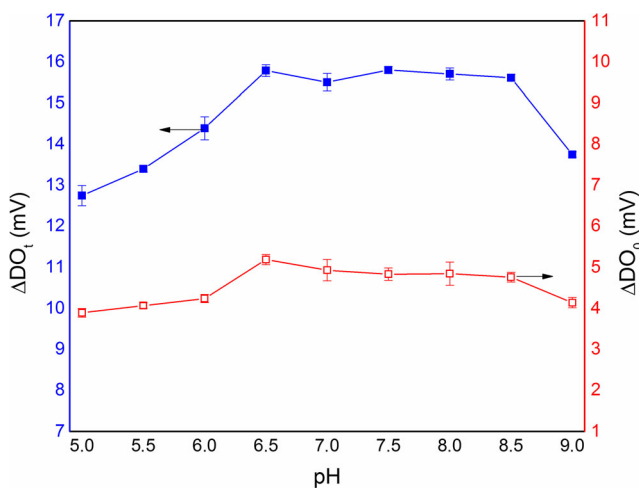


Fig. 5 Effect of pH on the responses of the PBBR in batch mode (solid square) and continuous mode (open square)

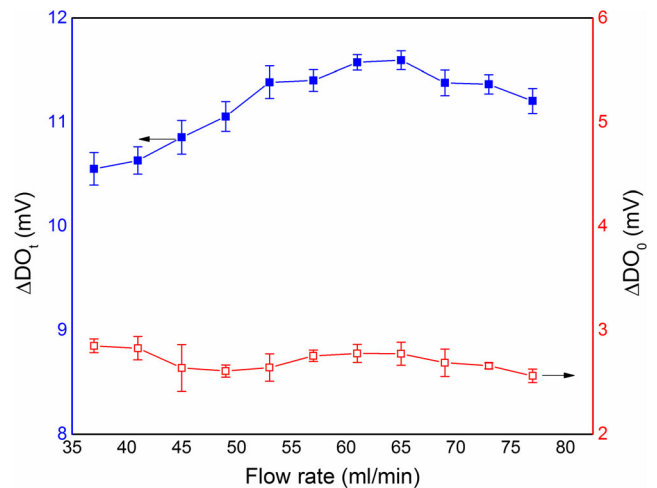
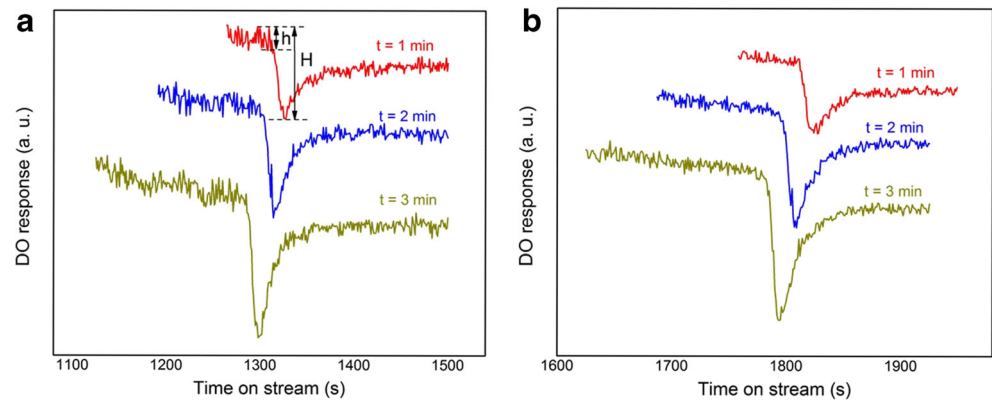


Fig. 6 Effect of flow rate on the responses of the PBBR in batch mode (solid square) and continuous mode (open square)

Fig. 7 DO profiles of drinking water at different isolation periods and flow rate of 37 ml/min (**a**) and 53 ml/min (**b**)



increasing the isolation time up to 4 min. Although the sensitivity continued to be slightly increased, longer isolation period is even worse both in terms of analysis time and precision. On the other hand, the greater the BOD or the higher biodegradable organic matter content, the more rapidly oxygen is depleted. Among the proposed models to describe mathematically the reaction occurring during BOD test, the first-order kinetic is the most widely accepted in which rate of biodegradation is proportional to the concentration of nutrient as long

as the DO content is always above 2 mg/l during the BOD test (Dimitrov et al. 2007; Leduc et al. 1988; Li et al. 2009; Nyholm 1991). However, it was observed that there might be another critical DO content at which the used microorganisms behave differently, causing a change in reaction rate constants. As a result, increasing the isolation time significantly reduced the upper limit of the linear range. In other words, the linear range of a BOD biosensor based on batch-type bioreactor is inversely proportional to its sensitivity. This finding is

Table 1 Performance of different PBBR at different isolation time

Cultivation date (BOD ₅ of collected WW, mg/l)	Isolation time, min	Sensitivity, mV/(mg/l)	Linearity (Adj- R^2)	Linear range, mg/l
2 July 2018 (77)	0	0.13619	0.9958	0–30
	2	0.4596	0.9913	0–20
	4	0.6589	0.9676	0–13
	6	0.6757	0.9240	0–13
	8	0.6903	0.9533	0–13
9 July 2018 (99)	0	0.3871	0.9940	0–17.5
	1	0.7752	0.9834	0–12.5
	2	0.8406	0.991	0–10
	3	1.0141	0.9892	0–10
16 July 2018 (93)	0	0.4388	0.998	0–15
	1	0.8643	0.9832	0–12.5
	2	0.8945	0.991	0–10
	3	1.3068	0.990	0–10
13 August 2018 (49)	0	0.1169	0.9923	0–35
	2	0.3719	0.9938	0–25
	4	0.6139	0.9863	0–13
	6	0.7334	0.9897	0–13
	8	0.7829	0.9836	0–13
27 August 2018 (62)	0	0.1373	0.9918	0–30
	2	0.3844	0.994	0–20
1 October 2018 (72)	0	0.1633	0.9936	0–30
	2	0.3731	0.9941	0–20

consistent with the results that have been shown in other previous studies even though it has not been mentioned (Kumlanghan et al. 2008; Raud et al. 2012). Further study is being conducted to complement the present data and confirm this observation.

By balancing between a high sensitivity, a short analysis time, and a wide linear range, an isolation time of 2 min was selected for further experiments.

Reproducibility, repeatability, and stability

The PBBRs that were prepared at the same time provided responses that have the mean and standard deviation of 8.07 and 0.07 mV, respectively, giving a CoV of 0.9%. Meanwhile, those prepared at different times exhibited a much higher CoV that can reach up to 36% due to the large difference in their performances as can be seen in Table 1. Since the composition of collected wastewater which was used as the cultivation solutions changes with time, the population and activity of the microbial community may be affected, resulting in the different performance of the PBBRs prepared at different times. The PBBRs which were cultivated on 7 July and 16 July using the much higher BOD wastewater compared to the others exhibited extreme microbial activities, giving the largest sensitivities. As a result, they produced the much larger responses with the same GGA standard solution, attributing to the high CoV as mentioned before. However, this large variation in DO response did not seem to have a significant effect on the precision of BOD₅ estimation when the specific calibration equations for each PBBR were used as can be seen in Fig. 8. Overall, the mean and standard deviation of the estimated BOD values using GGA as standard solution that were obtained from different PBBRs were 10.11 and 0.2 mg/l,

respectively, resulting in a good CoV of 2%. The results also showed that there was no statistically significant difference observed between the prepared PBBRs.

It was observed that each sensor gave fairly constant responses with a maximum SD and CoV of 0.25 mV and 2.2%, respectively, for three consecutive measurements. However, the SD of the estimated BOD obtained from the prepared PBBRs was significantly larger, except for the ones cultivated on 7 July and 16 July due to their high sensitivity. The maximum SD and resulting CoV of the estimated BOD were 0.45 mg/l and 4.5%, respectively, in the case of using the PBBR prepared on 13 August because of its lowest sensitivity.

Continuous operational stability studies were conducted with two PBBRs that were cultivated on 2 July and 9 July. The time interval between two measurements was firstly set at 15 min for the 2 July PBBR as in all previous experiments. This time interval was chosen for ensuring the steady state of continuous mode. Figure 9 showed that it took 3 h to reach the stable response after short-term storage without nutrient added. The response remained constants for the first 8 h with a CoV less than 0.7% and then seemed to slightly increase with time. However, the repeatability was still acceptable during 22 operating hours with a CoV of only 1.8%. After 30 h, ΔDO_t increased up to 11.61 mV, equivalent to 10.9% compared to the stable value.

It was observed that pumping sample, in which its BOD is within the linear range, into the PBBR for 5 min after 2 min isolation is enough to reach the steady state of continuous mode. Therefore, 7 July PBBR was operated with a 5-min time interval between two isolation stages. The acclimation process was done after only 1 h, then the response remained constant for 7 h with a small CoV of 1.4% before sharply increasing up to 17.95 mV, equivalent to 16.3% compared to the stable value, at the end of experiment. It appears that the

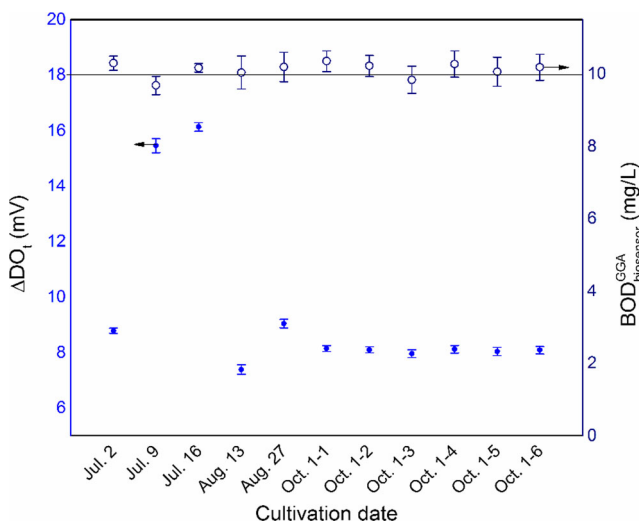


Fig. 8 Change in DO response (open circle) and estimated BOD values using GGA as standard solution (solid circle) obtained from different PBBRs

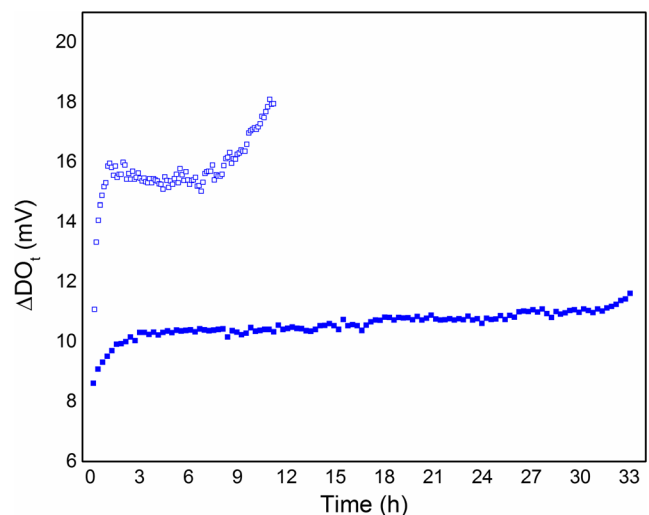


Fig. 9 Operational stability of the PBBRs cultivated on 2 July (solid square) and 9 July (open square)

difference in the time interval between two isolation stages is mainly responsible for difference in stabilities of the two PBBRs. During the isolation period, the closed static environment could enhance the growth of microorganisms. However, the subsequent flowing stage in which a high flow rate employed provides sufficient shearing force to achieve self-cleaning and maintain a stable microbial population. As a result, a shorter time interval may be insufficient for this purpose. A 17-min analysis cycle with the response time less than 2.5 min, including 2 min of isolation and 15 min of flowing, is more adequate for online BOD monitoring compared to that of other reported biosensors (Jiang et al. 2018; Jouanneau et al. 2014).

Stability of the PBBR after a short-term storage was studied from 30 August to 3 September 2018 by using a GGA standard solution, equivalent to BOD 10 mg/l. The sensor was continuously operated for about 13 h. After that, the air-saturated tap water was circulated through it at the same conditions for storage until the next day. Figure 10 showed that only the responses obtained from the first day slightly increased with time and reached a stable value after 12 h. Basically, this period is needed for the microorganisms to get acclimated to the new nutrient environment. It can be seen that the responses produced from the next 3 days were statistically similar with a mean of 9.26 mV and a maximum CoV of 1.4%. On the fourth day, ΔDO_t slightly increased to a mean of 9.34 mV with the same CoV (1.4%). This means that the PBBR can still be used later on for BOD₅ estimation as long as new calibration curves are generated before measurements. It should be noticed that no decrease in the response obtained from the tested PBBRs was observed during our study, whereas the responses of biosensors which have been studied elsewhere (Kumlanghan et al. 2008; Li et al. 2016; Li et al. 2017)

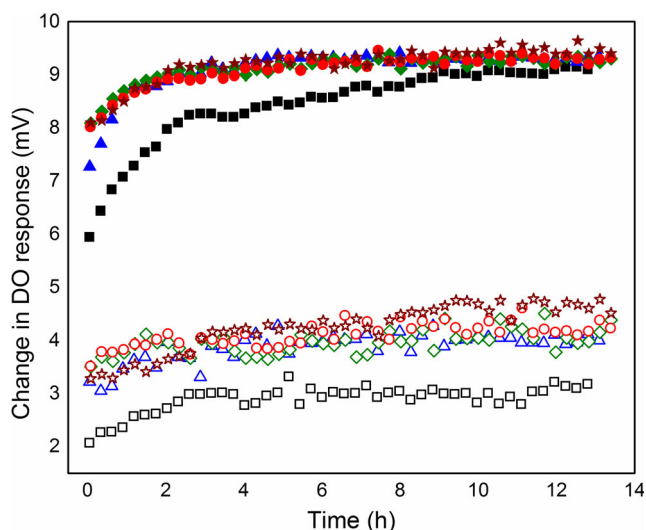


Fig. 10 Performance of the 27 August PBBR on 30 August (square), 31 August (triangle), 1 September (circle), 2 September (diamond), and 3 September (star)

decreased after a certain period of time. Stability of the studied PBBRs is obviously much lower than the other developed biosensors which were reported to be stable over a period of few days (Li et al. 2016), 15 days (Kumlanghan et al. 2008), 40 days (Li et al. 2017), or up to 2 months (Liu et al. 2013); however, it should be noticed that none of them were assessed continuously but only few measurements were performed everyday during the study period. Meanwhile, our PBBRs were observed to be stable over 80 consecutive measurements, comparable to the newly developed sensor (Niyomdech et al. 2017). Data on stability from longer-term studies are collected; however, it is not really necessary for actual application since a new PBBR can be easily made with almost no cost within 2 days to replace the used one.

Authentic wastewater analysis

Authentic wastewater samples were taken from the influent and effluent of the Binh Hung MWWTP before disinfection. Their BOD₅ values were determined by the conventional 5-day method and compared with the estimated BOD values that were obtained from the prepared PBBRs using both GGA and OECD as standard solutions. As can be seen in Fig. 11, all sensors gave underestimated results as compared to the BOD₅, especially in the case of measuring the effluent; however, the sensors calibrated with OECD solution generated much more accurate results than those calibrated with GGA. Particularly, the former and the latter underestimated BOD₅ by about 3–5% and 29–56% in the case of measuring the influent and by 27–51% and 61–83% in the case of measuring the effluent, respectively. Such underestimation may be attributed to the difference between the measurement methods and the complex composition of authentic wastewater (Kumlanghan et al.

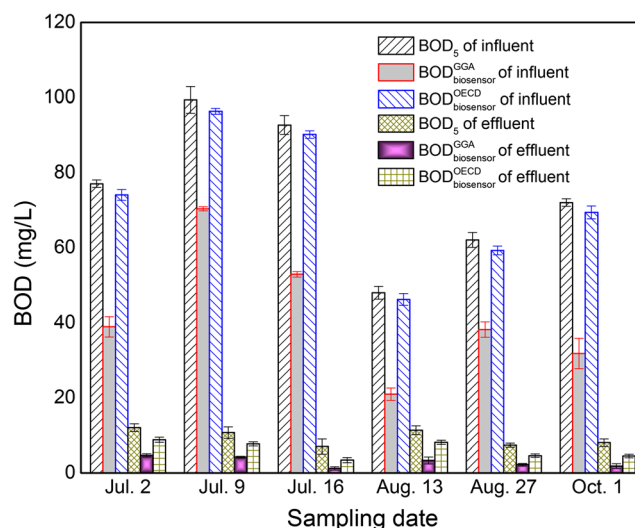


Fig. 11 BOD₅ and estimated BOD of authentic wastewater samples

2008). Short analysis time is both advantageous and disadvantageous of the developed BOD biosensors since there is not enough time to complete the degradation of most of biodegradable substances as the traditional method does (Jouanneau et al. 2014; Pasco et al. 2004). Practically, the responses obtained from biosensors depend on the relative proportions of the easily degradable components and the less degradable ones and the degradation ability of the employed microorganisms. Every microorganism is limited in its ability to utilize organic compounds; thus, using the microbial community which has been naturally acclimated to the target wastewater could enhance the estimation accuracy. However, during biological treatment process, the easily degradable compounds will be more completely removed than the less degradable ones. As a result, the relative proportion of the hardly biodegradable components will be higher in the effluent compared to the influent, giving a higher underestimation of BOD₅.

GGA contains only glutamic acid and glucose which is a preferable carbon source for most microorganisms; thus, it will be degraded rapidly, giving much higher response than those containing more complex and hardly degradable components such as OECD solution and authentic wastewater (Catterall et al. 2003; Pasco et al. 2004; Raud and Kikas 2013; Raud et al. 2012). Therefore, GGA is not appropriate for calibration of the rapid BOD biosensors although it can be used as standard solutions for the BOD₅ assay.

Conclusions

A low-cost BOD biosensor based on a packed-bed bioreactor that would be easy to self-build for on-site and online monitoring of BOD in both influent and effluent of a wastewater treatment plant was successfully developed. Immobilization of the natural microbial consortium collected from the target wastewater treatment plant on a cheap porous carrier was done simply by adhesion. As a result, the whole system costs only 3000 US dollars which is about one-tenth compared to the cost of the cheapest commercial BOD sensor (Reshetilov et al. 2013). Moreover, a new PBBR can be easily made for replacement by any one unskilled in this field with almost no cost within 2 days.

Semi-continuous operating mode was demonstrated to be suited for measuring BOD continuously at a high flow rate to limit errors caused by varying flow rates during measurements and to enhance the response time. Peak-type signals were produced with SNR greater than 3 for the blank measurements, resulting in a limit of detection of 0 mg/l. Sensitivity, detection range, precision, accuracy in accordance with BOD₅ value, and stability of the prepared PBBR were proved to be acceptable for rapid online BOD estimation of municipal wastewater.

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