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SHORT RESEARCH AND DISCUSSION ARTICLE

Simple approach for the rapid estimation of BOD_5 in food processing wastewater

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Abstract

A simple approach was developed for the rapid and accurate estimation of 5-day biochemical oxygen demand (BOD_5) in food processing wastewater. Immobilization of the natural microbial consortium that was collected from an aerobic compartment of a food processing wastewater treatment plant was simply performed by adhesion using a low-cost porous carrier. *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Streptomyces*, whose salt-tolerance and ability to break down organic compounds have been widely reported, were found to be predominant. These microorganisms may cause an enhancement of the bioreactor response in the presence of sodium chloride. Consequently, a modified glucose-glutamic acid (GGA) calibration standard was proposed in which an appropriate amount of NaCl was added; this solution was found to be more effective in terms of accuracy and practicality than both conventional GGA and the synthetic wastewater recipe from the Organisation for Economic Cooperation and Development (OECD). The calibrated self-built packed-bed bioreactor exhibited good precision of 3% or less in predicting BOD_5 in influent, which is similar to the performance of the most common commercial biochemical oxygen demand (BOD) bioreactors. There was a statistical agreement between the results obtained from this rapid BOD biosensor and the conventional methods, even when testing treated wastewater samples.

Keywords Bioreactor · BOD biosensor · GGA · Calibration standard · Dilution factor · Salt-tolerant bacteria

Introduction

In Vietnam, the food processing industry is a key part of the national development strategy for the period to 2025. This sector has been expanding rapidly, with an annual growth rate of 9%, and it has significantly contributed to Vietnam's gross domestic product over the past 5 years (Huong et al. 2017).

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However, it is apparent that the rapid growth in the food processing industry is a leading cause of environmental deterioration. Wastewater from seafood processing and canned food factories poses pollution problems due to its high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), which are 500–8000 mg O_2/L and 400–6500 mg O_2/L, respectively (Dieu 2003; Hoa et al. 2017); thus, the timely measurement of COD and BOD plays a crucial role in the prevention of environmental pollution. However, while COD can be measured immediately, the standard technique for determining BOD is problematic, in that it requires 5 days and involves complex procedures, as well as measurement devices (Jouanneau et al. 2014). Although BOD biosensors have recently been shown to have potential as effective devices for fast determination and online monitoring of pollution levels in wastewater, they cannot produce real BOD_5 values (Ejeian et al. 2018); only predicted BOD_5 values are obtained, and their accuracy depends on calibration and using the correct standard solution.

Many kinds of BOD biosensor have been studied and have shown high correlation with the standard method when using

simple simulated wastewaters; however, only a few biosensors have been tested with authentic wastewater to assess their accuracy and precision (Kibena et al. 2013; Liu et al. 2016; Raud et al. 2012b). These biosensors include bioluminescent bacteria (Costa et al. 2018; Sakaguchi et al. 2007), microbial fuel cell biosensors (Kharkwal et al. 2017; Pasternak et al. 2017; Wu et al. 2020), biosensors with entrapped microorganisms (Kibena et al. 2013; Raud et al. 2012b), and bioreactors (Liu et al. 2013; Wang et al. 2010). Glucose-glutamic acid (GGA) solution, which is used as the standard solution for the determination of BOD_5 in the traditional method, has been widely used for the calibration of BOD sensors (Reshetilov et al. 2013). GGA only contains two substrates, which are suitable nutrient sources for most microorganisms; thus, GGA is consumed more rapidly than material containing more complex and less readily biodegradable compounds, such as actual wastewater (Liu and Mattiasson 2002). In practice, this difference does not affect the results obtained from the traditional method, which requires 5 days for almost complete degradation of biodegradable components; however, GGA may give unreliable results in the case of fast prediction of BOD_5 using a biosensor. Theoretically, the less readily biodegradable components in wastewater samples may not be assimilated due to the short duration of the analysis, resulting in an underestimation of BOD_5 (Arlyapov et al. 2012; Pasco et al. 2004; Pham et al. 2019; Raud et al. 2012a). On the other hand, GGA calibration has also been reported to give an overestimation of BOD_5, although no explanation has been provided (Liu et al. 2000; Oota et al. 2010; Zhao et al. 2017). Thus, in order to improve the agreement between biosensors and traditional methods of BOD_5 measurement, more complex synthetic wastewaters have been developed and tested in place of GGA. Some of the specific recipes that have been reported as better standard solutions are confidential (Jia et al. 2003), while others have been described in detail (Chee et al. 2000; Tanaka et al. 1994). However, the most widely used is the synthetic wastewater recipe from the Organisation for Economic Cooperation and Development (OECD) (Kibena et al. 2013; Raud et al. 2012b). Preparation of these complex solutions is labor-intensive and time-consuming, and they can be degraded more rapidly than GGA due to microbiological contamination. Moreover, although they contain some representative components of wastewater, such as meat extract, peptone, tannic acid, lignin sulfonic acid, sodium lauryl sulfate, gum arabic, urea, and inorganic salts, they cannot be considered as representative of all kinds of wastewater, or consequently, as universal standards. Thus, designing a simple but effective standard for calibrating BOD biosensors focused on each specific case should be particularly valuable. In this work, modifying the GGA calibration solution by adding a metabolic promoter or inhibitor of the most abundant pollutant-degrading bacteria in the target wastewater was suggested to resolve over- or underestimation of BOD_5. This approach is obviously much simpler than alternatives that increase the complexity of the composition of the calibration solution.

Due to their wide detection range, activated sludge and natural microbial populations are employed as bio-receptors in all marketed BOD biosensors (Endress+Hauser; LAR). Unfortunately, neither the commercial BOD biosensors nor others with promise are cheap enough to be widely deployed or simple enough to be self-built in developing countries (Jouanneau et al. 2014; Reshetilov et al. 2013). Therefore, in our previous work, a disposable BOD bioreactor for on-site prediction of BOD, which is easy to self-build by simple immobilization of the existing microbial consortium on porous ceramic carriers, was developed and successfully used for the prediction of BOD_5 in municipal wastewater influent (Pham et al. 2019). However, even though it has been reported that different dilutions of the same wastewater sample could give different results (Rastogi et al. 2003b), the detailed analytical procedure which can help define the appropriate dilution factor to estimate BOD_5 in an unknown wastewater sample has not been provided. This paper aims to set out a simple approach, including finding the dilution factor and calibration standard for the rapid estimation of BOD_5 in food processing wastewater. The proposed procedure is expected to be able to simplify the analytical process as well as to shorten the analysis time compared to conventional methods, while avoiding under- or overestimation of BOD_5. Thus, the procedure's effectiveness was evaluated in terms of repeatability, reproducibility, stability, and accuracy against BOD_5 values that were determined using the traditional 5-day method.

Method

Material

All chemicals used in this work were of analytical grade and were procured from Merck (Germany) and HiMedia (India). Unless otherwise stated, the standard solution and samples were prepared and diluted using drinking water, which can be used instead of phosphate buffer saline and distilled water to ensure appropriate osmotic pressure for proper cell functioning (Liu et al. 2013).

Preparation of stock solutions

GGA (Karube et al. 1977), a mixture of OECD and GGA (OECD-GGA) (Kumlanghan et al. 2008) and modified GGA, which is discussed later in this paper, were used as standard solutions for calibration of BOD_5. GGA stock solution was simply made by dissolving 150 mg of D-glucose and 150 mg of glutamic acid in 1 L of distilled water, whereas OECD-GGA stock solution was a more complex preparation

comprising 7.5 mg/l of peptone, 5.5 mg/l of beef extract, 3.0 mg/l of urea, 0.7 mg/l of NaCl, 0.4 mg/l of $CaCl_2.H_2O$, 2.8 mg/l of K_2HPO_4, 0.2 mg/l of $MgSO_4.7H_2O$, 7.5 mg/l D-glucose, and 7.5 mg/l glutamic acid (Liu et al. 2000). BOD_5 values were determined to be 192.76 ± 7.97 mg/l and 115.36 ± 9.37 mg/l ($n = 15$), respectively.

Preparation of a simple BOD sensing system and measurement procedure

The packed-bed bioreactor (PBBR) was prepared according to the process described in our previous work, excepting that incubation was carried out with the natural bacteria consortium present in samples collected from the aerobic compartment of the wastewater treatment plant located at Saigon Food JSC, Ho Chi Minh City, Vietnam (Pham et al. 2019).

Figure 1 shows a schematic diagram of the BOD sensing system based on a PBBR. The semi-continuous mode was employed to allow determination of the dissolved oxygen (DO) values of both the influent and effluent by only one DO probe (DO-802, Apel Instruments, Vietnam). The air-saturated sample was first introduced into the PBBR using a peristaltic pump until a steady-state DO response (DO_{out}) was reached. Next, the sample flow was reversed until the initial DO value of the sample (DO_{in}) was attained; the sample was isolated inside the PBBR, and its organic compounds were degraded for 2 min. The sample flow was then reversed for the isolated sample to be released into the DO probe resulting in a decrease in DO response. Since the fresh sample was fed constantly, the DO response increased until the steady-state value (DO_{out}) was obtained, again causing a peak in the DO profile (H_{peak}). The output voltage signals of the DO probe were continuously recorded at 1-s intervals using a self-made data acquisition and processing system.

Microbiological analyses

A qualitative analysis of the type of microorganisms immobilized on each biocarrier was performed after the chemical loading studies.

All biocarriers were removed from the PBBR and mixed vigorously with 30 ml of NaCl solution 0.85% in a 50-ml glass vial. The supernatant liquid was then serially diluted (10-fold dilution), and 100 μl of each dilution was spread-plated onto NA, PDA, Gause I media with the addition of 1% casein (Sharma et al. 2015), 1% soluble starch (Khokhar et al. 2011; Vrints et al. 2007), 1% Tween 20 (Bala et al. 2014), and 3% NaCl (depending on wastewater sample) for each group of microorganisms that could hydrolyse proteins, starches or lipids, or show salt tolerance, respectively. The NA, PDA, and Gause I plates were incubated at 30 °C in the dark for 48 h, 5 days, and 10 days. Microbial isolates of similar colony morphologies were examined for cellular morphology, gram stain reaction, and ability to degrade the same compounds. The strains of microorganism were identified by using a biochemical test (traditional methods) based on

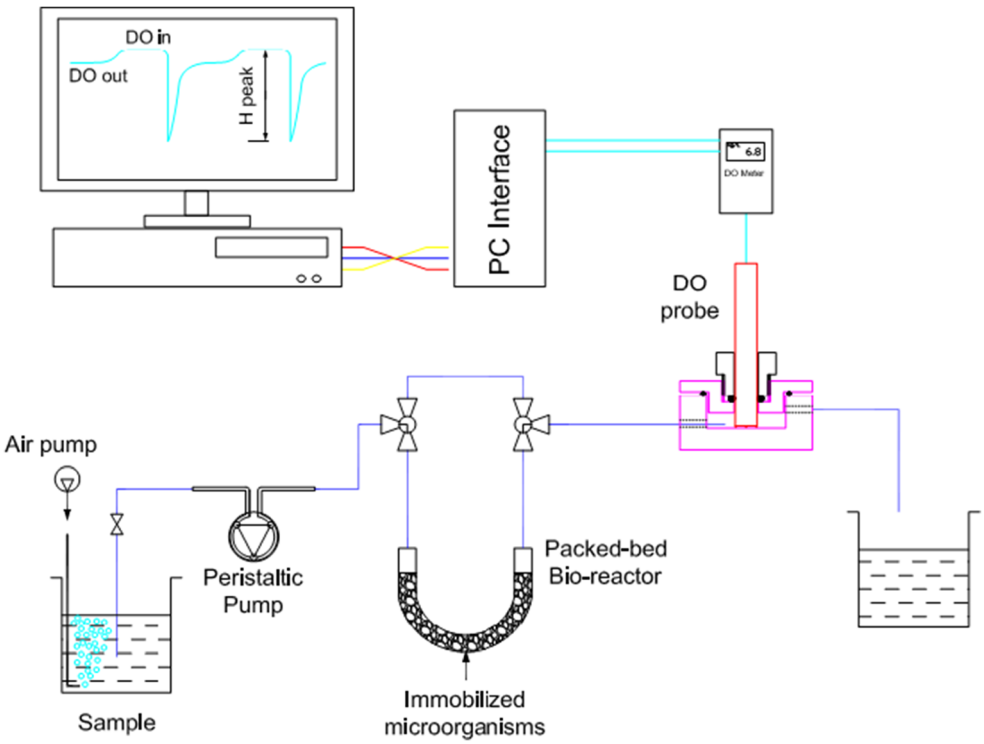

Fig. 1 Schematic diagram of the BOD biosensing system

Cowan and Steel classification (Barrow and Feltham 1993) and Bergey classification (Buchanan and Gibbons 1974).

Effect of salt concentration

Different GGA-NaCl solutions were prepared by dissolving the desired amounts of sodium chloride in a known volume of GGA solution, equivalent to a BOD_5 of 10 mg/l. By comparing the H_{peak} value of the GGA-NaCl and blank GGA solutions, which were obtained using the same PBBR, the effect of salt concentration was determined. Accordingly, inhibition and promotion levels were calculated as follows:

$$\text{Inhibition level}\ (\%) = \frac{H_{\text{peak}}^{GGA} - H_{\text{peak}}^{GGA.NaCl}}{H_{\text{peak}}^{GGA}} \times 100\% \quad (1)$$

$$\text{Promotion level}\ (\%) = \frac{H_{\text{peak}}^{GGA.NaCl} - H_{\text{peak}}^{GGA}}{H_{\text{peak}}^{GGA}} \times 100\% \quad (2)$$

All measurements were conducted in triplicate to evaluate the standard deviation.

Actual wastewater analysis

Calibration of a specific PBBR prior to measurement was carried out by examining the relationship with the BOD_5 values determined by the standard BOD_5 method (Rice et al. 2012) and the H_{peak} values of standard solutions at different concentrations. For each standard solution of known concentration, BOD_5 tests were performed in triplicate, while BOD biosensor tests were repeated until three consecutive H_{peak} values were within ± 1% of the average. Subsequently, the BOD_{bio} value of a BOD_5-unknown sample was estimated using the average H_{peak} value of three consecutive measurements and the corresponding calibration curves.

The influent and effluent wastewater samples were randomly collected from the wastewater treatment plant of Saigon Food JSC from August to October 2019. BOD_{bio} values of these samples were obtained using four different PBBRs that were cultivated at different time points over a 3-month period. The accuracy of the PBBRs was determined by comparing the resulting BOD_{bio} values with the BOD_5 values obtained using the standard method. Repeatability and reproducibility of a specific PBBR were assessed by the coefficient of variance (CoV) and standard deviation (SD), which are indicative of the consistency of the data (Guideline 2005).

In order to ensure sufficient time in the flow stage to achieve self-cleaning and maintain a stable microbial population, the time interval between two consecutive measurements was fixed at 15 min (Pham et al. 2019).

Results and discussion

Isolation and identification of immobilized bacteria

Biochemical analysis of the isolated bacterial colonies helped to identify them as *Pseudomonas aeruginosa*, *Nitrosomonas*, *Nitrobacter*, *Bacillus cereus*, and *Streptomyces*. Their bioactivities were determined and are shown in Table 1. The five isolates grew well in the presence of 3% NaCl. *Streptomyces*, *Pseudomonas aeruginosa*, and *Bacillus cereus* differ from the other microorganisms in their use of protein, which is likely to be a major component of food processing wastewater. In addition, the two former ones are able to degrade starch and cellulose, which are also present in this kind of wastewater. It can be concluded that they are responsible for the biodegradation of organic matter that causes changes in the DO response.

The occurrence of *Pseudomonas aeruginosa* in marine sources and food processing wastewater, as well as its salt-tolerant property, has already been reported (Artiga et al. 2008; Castillo-Carvajal et al. 2014; Kimata et al. 2004; Sivaprakasam et al. 2008). There have also been many studies on the salt tolerance and ability to break down proteins, starch, and cellulose of *Streptomyces* (Buntić et al. 2016; Chau et al. 2016) and *Bacillus cereus* (Kadam et al. 2013; Mlaik et al. 2015).

Effect of salt concentration

To determine the preliminary effects of salinity, experiments were performed with salt concentrations varying from 0 to 35 g/L. Figure 2a shows that adding salt up to 10 g/L can promote the response of the PBBR, whereas higher sodium chloride content causes inhibition of microbial activity. In general, wastewater from the canned food industries is characterized by high salinity (Artiga et al. 2008; Pollution Prevention in Food Canning Processes 2001). In practice, salt stress can constrain many enzymes in common microbial species and reduce cellular activity; thus, salt is considered as an inhibition factor in biological processes, especially in saline wastewater treatment (Ching and Redzwan 2017; Li et al. 2019). However, degradation of COD and BOD in saline wastewater has been reported as being enhanced by the utilization of a salt-tolerant microorganism in a biological treatment plant (Castillo-Carvajal et al. 2014). Therefore, the promotion effect observed here was probably caused by the presence of *Pseudomonas aeruginosa* in the PBBR, as shown earlier. Sivaprakasam et al. studied the effect of salinity on COD removal by different salt-tolerant bacteria and found that *Pseudomonas aeruginosa* was most effective at the lowest salt concentrations (2% w/v); its biological activity decreased with the increasing salinity of wastewater, which is consistent with our results (Sivaprakasam et al. 2008). However,

Table 1 Results of isolation and identification of biologically active microorganisms

No.	Isolation	Bioactivity					Identification
		Protein	Starch	Cellulose	Lipid	3% NaCl	
1	S1	+	+	+	–	+	*Streptomyces*
2	B1	+	+	+	–	+	*Pseudomonas aeruginosa*
3	B2	–	–	–	–	+	*Nitrosomonas*
4	B4	–	–	–	–	+	*Nitrobacter*
5	B5	+	–	–	–	+	*Bacillus cereus*

Sivaprakasam et al. did not perform control experiments with salt-free wastewater to identify whether at 2% w/v, salt is an inhibitor or promoter of *Pseudomonas aeruginosa*.

Further experiments were conducted at salt concentrations of less than 5 g/L. The smallest concentration was chosen to be similar to the relative concentration of sodium chloride in the OECD solution. As can be seen in Fig. 2b, the promotion level is proportional to the salt content with a good R-square value of 0.9974. The adjusted R-square is very close to the R-square, implying a strong linear fit. It can be seen that the presence of even a small amount of salt can greatly enhance the microbial activity compared to the blank GGA solution. These results seem to agree with those reported by Abou-Elela et al. (2010) who studied the biological activity of *Staphylococcus xylosus* at salt concentrations ranging from 5 to 30 mg/L. COD removal increased linearly as salinity increased up to 20 g/L, which is near the optimum value for *Staphylococcus xylosus,* but did not increase further for higher salt concentrations.

Effect of the standard solution

GGA and OECD-GGA, which are the most popular standard solutions, were initially employed to obtain the estimated BOD_{bio} of different wastewater samples collected from the wastewater treatment plant of the Saigon Food JSC. Since GGA contains only glucose and glutamic acid, which are quickly biodegraded by most bacteria, it produces a much higher response than the OECD solution and actual wastewater that contain more complex and less readily biodegraded components. Consequently, GGA has been reported not to be appropriate for the calibration of biosensors due to its underestimation of BOD_5 (Arlyapov et al. 2012; Pasco et al. 2004; Pham et al. 2019; Raud et al. 2012a). However, the results here were contrary to this expectation; in particular, using GGA as the standard solution resulted in an overestimation of BOD_5, as can be seen in Fig. 3. Meanwhile, no statistical difference was observed between the estimated and determined BOD_5 values in the case of OECD-GGA. It was found that GGA produced a much lower response compared to OECD-GGA at the same assigned BOD_5 value. This effect may be caused by the presence of NaCl, which was able to enhance the biological activity of the immobilized bacteria in the OECD-GGA solution, as previously confirmed. Therefore, the same amount of sodium chloride was added to GGA to produce GGA-NaCl standard solution. The best agreement between BOD_{bio} and BOD_5 was obtained using this modified calibration solution compared to the two conventional solutions, as shown in the inset in Fig. 3.

Calibration is performed automatically in most commercial BOD biosensors (Endress+Hauser 2007; Kelma 2018; LAR 2019). However, after preparation, deterioration of standard solutions progresses over time and, consequently, their assigned BOD_5 values continuously decrease, resulting in an

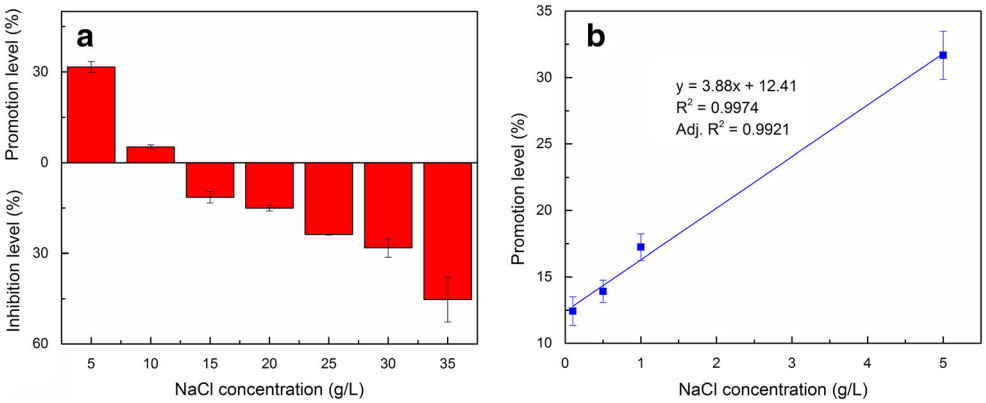

Fig. 2 Effect of sodium chloride concentration on the biological activity of the PBBR (**a**) and linear relationship of the promotion level and salt content (**b**)

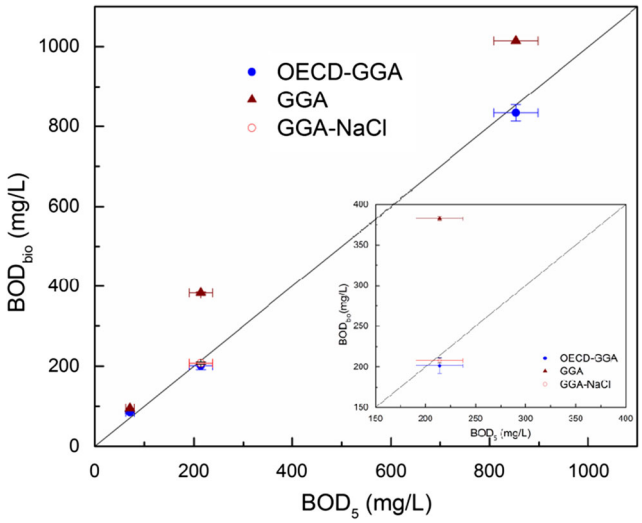

Fig. 3 Agreement between BOD_{bio} obtained from using different standard solutions and BOD_5. Inset: Zoom of one region for comparison of OECD-GGA, GGA, and GGA-NaCl standards

overestimation of BOD in the actual sample. This problem will be worse when operating under tropical conditions. As can be seen in Fig. 4, the more complex and expensive OECD-GGA standard, which is more labor-intensive and time-consuming to prepare, works well for only 6 h at 30 °C; no significant difference was found between the as-prepared and 6 h-aged solutions (P value = 0.99). Meanwhile, the GGA-NaCl standard exhibited a longer lifetime in the same conditions. Its BOD_5 remained unchanged for the first 18 h (P value > 0.86) then slightly decreased for the next 12 h; however, there was no significant difference between the as-prepared and 30-h-aged solutions (P value = 0.15). It was found that the PBBRs could operate stably and continuously for 30 h with a CoV of 10.9% (Pham et al. 2019); thus, the modified GGA-NaCl standard is suitable for continuous calibration over this period of time without the need to replace it frequently with a freshly prepared solution. This attribute not only reduces time and effort to operate the BOD biosensing system but also avoids errors caused by the preparation of the calibration solution and ensures accuracy of measurement.

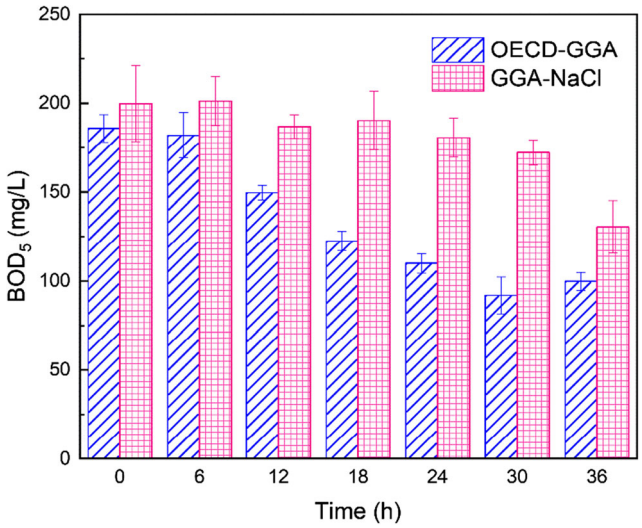

Fig. 4 Degradation of OECD-GGA and GGA-NaCl standards at 30 °C

Authentic wastewater analysis

Finding an appropriate dilution factor

With the traditional dilution method, it is recommended that several dilution factors should be tried so that at least two bottles give an acceptable minimum residual DO of 1 mg/L and DO uptake of 2 mg/L after a 5-day incubation. In addition, if the requirements for minimum residual DO and DO uptake are all met, the large variation between the obtained BOD_5 for different dilutions may be due to the toxicity of the wastewater sample (Delzer and McKenzie 2003). Although COD analysis, which is more reproducible and less time-consuming, could be performed in advance as a guide for dilution, this approach needs additional labor and hazardous chemicals and sometimes does not work due to the unexpected correlation between BOD and COD in a particular wastewater. Therefore, determining the proper dilutions for an unknown sample prior to BOD_5 measurement is not an easy task.

For any BOD biosensor that is based on aerobic respiration of microorganisms, samples must be diluted, and dilutions can be carried out automatically in commercial devices (Endress+Hauser 2007) or even in the lab-scale devices (Kumlanghan et al. 2008; Liu et al. 2012, 2013; Seo et al. 2009). However, no guide for choosing a proper dilution for an unknown sample has been provided. Even though trying different dilutions for rapid estimation of BOD in an unknown sample could be performed more quickly and more easily than with manual operating biosensors, it is impossible to choose the most appropriate dilution without defined criteria, since each will produce different estimated BOD values.

In this work, five dilutions of actual influent and effluent wastewater samples, which have BOD_5 values varying from dozens to hundreds of mg/L, were prepared by diluting different volumes of samples to a fixed volume of 2 L. As can be seen in Fig. 5, each dilution gave different DO responses, but there seems to be a point where the dilutions become effective so that the DO response increases linearly with the sample concentration and, consequently, the BOD_{bio} values obtained from such dilutions are not statistically different from BOD_5. At the lower dilution levels marked by red solid line circles, the biological activity of the immobilized microorganisms seems to be affected by toxic substances (Delzer and McKenzie 2003) or dissolved oxygen content, which has been reported to have an influence on the respiration rate (Kalinske 1971). As a result, the obtained BOD_{bio} decreased with the reducing dilution factor. Other biased results, which are

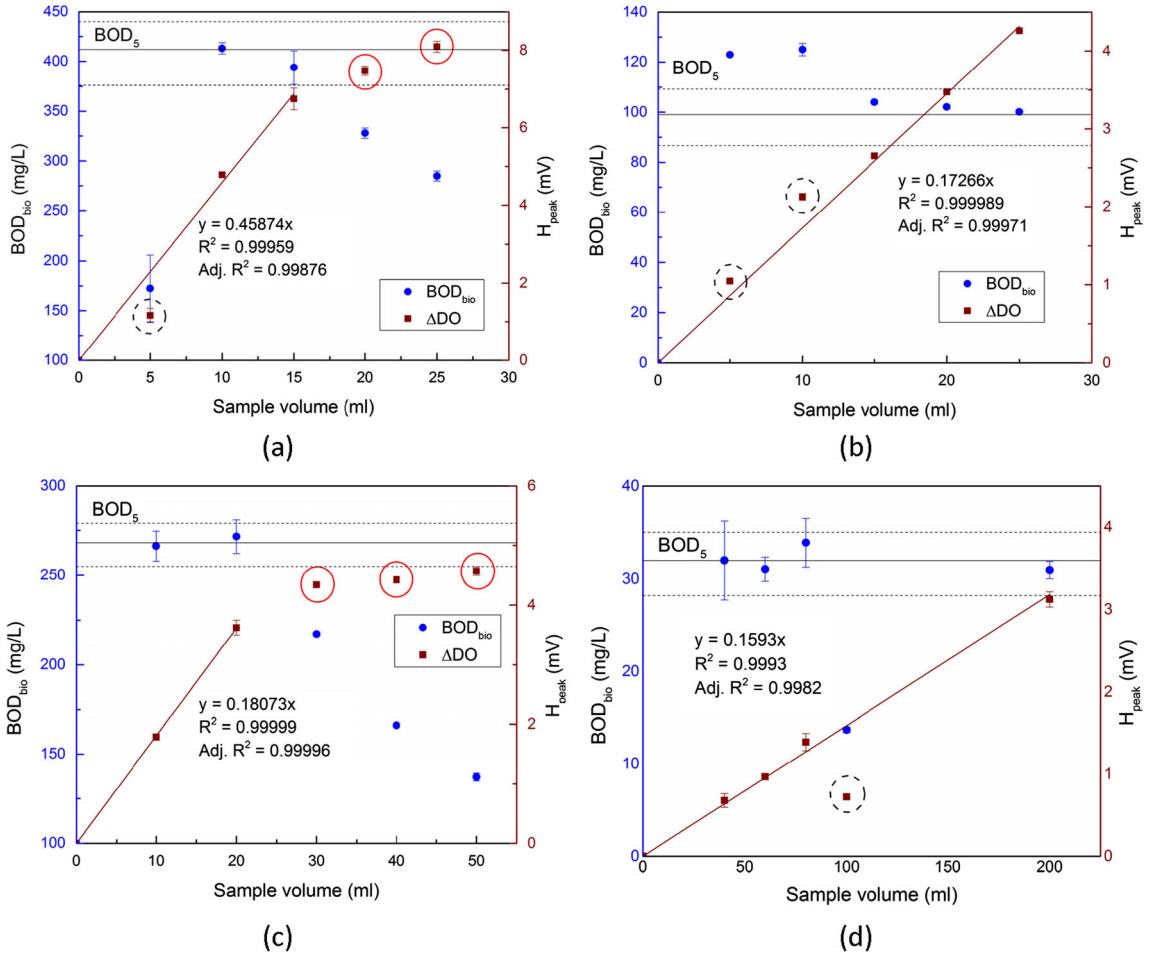

Fig. 5 Effect of dilution factor on the DO response and resulting BOD_{bio} of the influent (**a**, **c**) and effluent (**b**, **d**) wastewater samples taken on Sep. 11 (**a**, **b**) and on Sep. 24 (**c**, **d**)

marked by black dashed line circles, may be due to manual dilution errors that could be eliminated by using an automatic system in the actual application. Therefore, a proper dilution can be easily and quickly be achieved by increasing the dilution factor until at least two consecutive dilutions give proportional results.

BOD estimation of actual wastewater

The wastewater treatment plant at Saigon Food JSC is mainly based on biological treatment with activated sludge. The characteristics of its raw and treated wastewaters are presented in Table 2. It can be seen that the treatment plant works efficiently; removal of TSS, BOD, and COD were 83%, 94%, and 91%, respectively.

Since treated wastewater probably has high toxicity and low biodegradability, its predicted BOD value has rarely been reported. A few studies, including our previous one, have looked at industrial effluents but their BOD values were underestimated using the developed BOD biosensors (Arlyapov et al. 2012; Kumlanghan et al. 2008; Pasco et al. 2004; Pham et al. 2019; Rastogi et al.

Table 2 Characteristics of raw and treated wastewater from Saigon Food JSC

Parameter	TSS	COD	BOD	Total N	Total P	Oil and grease	N-NH_4^+	Coliforms
Unit	mg/l	mgO_2/l	mgO_2/l	mg/l	mg/l	mg/l	mg/l	MPN/ 100 ml
Raw wastewater	154	919	542	67.3	8.84	2.3	31	1.1×10^6
Treated wastewater	26.4	80.7	32.3	18.2	1.85	n.d	17.6	1.1×10^5

2003a; Raud et al. 2012a; Tanaka et al. 1994). In order to improve the accuracy of predicting BOD in treated wastewater, artificial solutions which consist of known amounts of arbitrarily selected refractory organic compounds from actual effluent must be used (Chee et al. 2000; Tanaka et al. 1994); however, previous results have not shown whether the observed agreement is statistically significant. Therefore, in order to confirm the actual applicability of any BOD biosensor, the differences between estimated and BOD_5 values in industrial effluents need to be examined statistically.

Raw and treated wastewater samples were taken from reservoirs after biological treatment in the wastewater treatment plant of the Saigon Food JSC in August, September, and October 2019. Their BOD_5 values were determined by the conventional 5-day method and compared with the estimated BOD_{bio} values obtained from six different PBBRs. With activated sludge as the source, it was not possible to determine the quantity and composition of microorganisms that were immobilized in the bioreactors, even though they were prepared in the same conditions (Kumlanghan et al. 2008; Pham et al. 2019). Thus, calibration using the most suitable standard solution had to be performed before sample analysis. As shown in Table 3, each PBBR, which was calibrated with the GGA-NaCl standard, had specific calibration equations. The sensitivity of a biosensor is measured by the slope of the calibration curve and was found to be related to the type and density of the immobilized bacteria (Raud et al. 2012a). In this low-cost PBBR, the type and density cannot be controlled since the immobilization was carried out by the simple adhesion of bacteria to ceramic carriers, resulting in fluctuations in sensitivity. In general, a smaller sensitivity results in a larger SD, which may indicate lower confidence and hence larger potential prediction error. However, even the least sensitive PBBR exhibited higher sensitivity than has been reported previously (Hu et al. 2017; Niyomdecha et al. 2017; Raud et al. 2012a; Zhao et al. 2017); thus, the estimated BOD values that were obtained from all prepared PBBRs are consistent with BOD_5 values in both raw and treated wastewater samples. In particular, the comparisons of all the means of the two methods gave statistically insignificant results (P value > 0.3). A good precision of 3% or less, which is similar to that of the most common commercial BOD bioreactors (Ahmed et al. 2019), was obtained in the case of predicting BOD in influent. After being treated by the biological process, the non-degradable and toxic components still remain in the effluent, whereas the almost biodegradable components are completely removed, giving a lower precision due to the sample complexity. However, the overall precision of the prepared PBBRs is acceptable as shown by the largest CoV of 12.6%, which is comparable with that (13.4%) of the conventional 5-day method.

It can be concluded that GGA-NaCl can be used as a simple and effective calibration solution in terms of preparation time, cost, and accuracy for estimation of BOD_5 in food processing wastewater. A specific calibration solution for other wastewaters can be designed and created using a similar procedure, with the following steps:

(i). Finding the most abundant pollutant-degrading bacteria in the target wastewater and any specific compound that could be present and identified as a metabolic promoter or inhibitor.
(ii). Using GGA as the standard calibration solution for estimation of BOD_5 in the target wastewater and assessing the under- or overestimation level empirically.
(iii). Modifying the GGA calibration solution by adding the promoter or inhibitor in the case of over- or underestimation of BOD_5 in the target wastewater, as required.
(iv). Repeating step (ii) with the modified GGA and step (iii) until there is no statistical difference between the estimated BOD_{bio} and BOD_5 value determined by the traditional 5-day method.

Table 3 Determination and estimation of BOD in actual wastewater

PBBR	Calibration equation	Sampling date	BOD_{bio} (mg/L)		BOD_5 (mg/L)		P value	
			Influent	Effluent	Influent	Effluent	Influent	Effluent
1	$y = 0.3657x + 0.061$	Aug. 20	886 ± 25	77.2 ± 7.1	892 ± 28	76.7 ± 4.2	0.81	0.92
2	$y = 2.1995x + 0.2156$	Sep. 5	208 ± 3	–	214 ± 24	–	0.67	
3	$y = 2.2139x + 0.2119$	Sep. 11	413 ± 6	–	412 ± 33	–	0.88	
4	$y = 0.966x + 0.0053$	Sep. 19	511 ± 7	34.8 ± 4.4	495 ± 23	31.8 ± 3.4	0.31	0.40
5	$y = 1.3256x + 0.0242$	Sep. 24	266 ± 8	–	267 ± 12	–	0.94	
6	$y = 1.9413x + 0.0281$	Oct. 11	191 ± 4	102.1 ± 6.4	193 ± 3	94.7 ± 12.7	0.62	0.41

Conclusions

A simple approach for the rapid and accurate estimation of BOD_5 in food processing wastewater based on the low-cost PBBR that can easily be self-built on-site was successfully demonstrated. The enhancement of the bioreactor response with an increase in salt concentration of up to 10 mg/L could be attributed to the presence in the PBBR of *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Streptomyces*, which are the well-known salt-tolerant and organic material degrading bacteria. Therefore, a modified GGA calibration standard, in which an appropriate amount of NaCl is added, was proposed and proven to be more effective both in terms of accuracy and practicality than the conventional GGA and OECD standards. Sensitivity, precision and accuracy in accordance with the BOD_5 values of the prepared PBBR, which was calibrated using the GGA-NaCl standard, was shown to be good enough for the rapid online estimation of BOD_5 in both the influent and effluent of food processing wastewater. The results confirmed that using the prepared specific biosensor coupled with a specific standard solution can enable accurate estimation of BOD in relevant industrial wastewater.

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