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Novel Environmental Analytical System based on Combined Biodegradation and Photoelectrocatalytic Detection Principles for Rapid Determination of Organic Pollutants in Wastewaters

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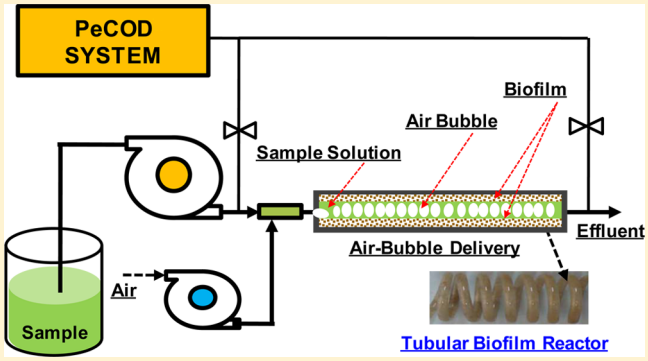
Supporting Information

ABSTRACT: This work describes the development of a novel biofilm reactor-photoelectrocatalytic chemical oxygen demand (BFR-PeCOD) analytical system for rapid online determination of biodegradable organic matters (BOMs). A novel air bubble sample delivery approach was developed to dramatically enhance the BFR's biodegradation efficiency and extend analytical linear range. Because the air bubble sample delivery invalidates the BOD quantification via the determination of oxygen consumption using dissolved oxygen probe, the PeCOD technique was innovatively utilized to resolve the BOD quantification issue under air bubble sample delivery conditions. The BFR was employed to effectively and efficiently biodegrade organic pollutants under oxygen-rich environment provided by the air bubbles. The BOD quantification was achieved by measuring the COD change (Δ[COD]) of the original sample and the effluent from BFR using PeCOD technique. The measured Δ[COD] was found to be directly proportional to the BOD_5 values of the original sample with a slope independent of types and concentrations of organics. The slope was used to convert Δ[COD] to BOD_5. The demonstrated analytical performance by BFR-PeCOD system surpasses all reported systems in many aspects. It has demonstrated ability to near real-time, online determining the organic pollution levels of wide range wastewaters without the need for dilution and ongoing calibration. The system possesses the widest analytical liner range (up to 800 mg O_2 L^{-1}) for BOD analysis, superior long-term stability, high accuracy, reliability, and simplicity. It is an environmentally friendly analytical system that consumes little reagent and requires minimal operational maintenance.



INTRODUCTION

Organic pollutions in wastewaters, especially the biodegradable organic matters (BOMs), can cause serious environmental problems.[1] These BOMs demand oxygen when degraded by natural microbes that could lead to detrimental oxygen depletion of aquatic environment.[2] The rapid determination of BOMs is therefore essential for aquatic environmental impact assessment.[3] Analytically, the concentrations of BOMs can be collectively presented by biochemical oxygen demand (BOD). Despite a variety of analytical methods developed for determination of BOD,[4−8] to date, the BOD_5 assay has been the most widely used method because of its wide applicability to different type of samples.[1] It is noteworthy that in many parts of the world, BOD_7 is employed to avoid multifarious tests over weekends. However, BOD_5 measurements must be carried out via a serial dilution over a 5-day period to validate the assay result due to its narrow analytical linear range. The required 5-day assay time is obviously unsuitable for applications where rapid response is needed. In order to meet the end-users' demand, a large body of works has been devoted to develop rapid BOD assays.

The vast majority of published rapid BOD studies have been based on the measurement of microbial respiration rate.[6−12] In these approaches, the biodegradations are achieved using microorganisms embedded/entrapped in a membrane and the

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extent of the degradation is quantified exclusively via the detection of oxygen depletion using a dissolved oxygen (DO) probe located behind the membrane. Although the rapidity of analysis could be achieved with such a sensor configuration, these approaches have a number of common drawbacks affecting their real-world performance. These approaches are only capable of selectively degrading less than 1% of total organics in a sample.[13] The low degradation efficiency can be attributed to the low oxygen solubility (9.08 mg O_2 L^{-1} at 20 °C). Also, the diffusion barrier introduced by the membrane further reduces the oxygen availability.[14] The selective degradation of easily degradable organics is because only limited types and amounts of microorganisms could be immobilized in the membrane for rapid BOD sensors.[6,7] As a result, such rapid BOD sensors could only be used for specific types of samples containing low concentrations of easily degradable organics, greatly limiting their applicability.[15−17] Similar to the case of the BOD_5 method, the rapid BOD sensors employing oxygen depletion to quantify BOD possess a narrow analytical linear range.[17−19] These drawbacks could be overcome if large portions of total BOMs presented in the sample can be rapidly and indiscriminately degraded. In effect, this could be achieved by increasing the microbial populations in presence of a sufficiently high concentration of electron acceptor.[4,13,20] In this regard, we have reported a unique approach that employs high populations of mixed microbial seeds combined with high concentrations of ferricyanide artificial electron mediator to replace oxygen as an electron acceptor to achieve rapid BOD determination.[4,13,21−25] The method has demonstrated a remarkably improved correlation to the standard BOD_5 assay, attributing to that a large portion of organics in the sample (up to 63%) can be biodegraded within 3 h.[4] Nevertheless, compared to rapid BOD assay systems using oxygen as the electron acceptor and DO probe to quantify BOD, the artificial electron acceptor approach requires a relatively long assay time and complex analytical procedures.[4,15] Also, the potential toxic effect of artificial electron acceptor such as ferricyanide could compromise the BOD detection.[26]

It is well-known that DO is the most preferred electron acceptor for the oxidative biological degradation processes because of its distinctive advantages, such as nontoxic and simple procedures.[4,6] Recently, our group developed a biofilm reactor (BFR)-based rapid BOD determination system for which the naturally occurred microorganisms were directly cultivated on the inner wall of a tubular reactor for biodegradation with a DO probe as a detector.[27] The method uses the superior biodegradation ability of the BFR to achieve rapid and indiscriminate degradation of wide spectrum of BOMs, capable of biodegrading ~20% BOMs within 99 s due to the large population of exposed microorganisms in the BFR.[28] This approach also eliminates the diffusion barrier in the embedment/entrapment methods, enabling higher biodegradation efficiency. Such a BFR system has been successfully applied to continuous online BOD determinations.[29] Similar to other oxygen sensor-based rapid BOD determination systems, the extent of the biodegradation is proportional to the oxygen consumption, which is measured by a DO probe.[27−30] However, the inherent disadvantage of low analytical linear range remains as a key limit of the BFR system, especially for heavily polluted wastewaters. The efficiency of an oxidative biodegradation system is deeply dependent on the sufficient supply of electron acceptor (commonly, dissolved oxygen).[31,32] The BFR method would be directly applicable to heavily polluted wastewaters without the need for dilutions if oxygen is continually supplied by purging the sample with air. Nevertheless, the BOD quantification by measuring the oxygen consumption prohibits the air purging because such an action will invalidate the analytical relationship between the oxygen concentration change and the extent of biodegradation. As is well-known, the inherent advantages of the BOD_5 method are its indiscriminate and near exhaustive degradation of a wide spectrum of organic pollutants, its matrix independence, and wide applicability. And the benefits of BOD sensor methods are their rapidity and ease of operation. It would be of a significant methodological advance for rapid BOD determination if an oxygen-based BOD biodegrading system embracing the advantages of both BOD_5 and BOD sensor methods, with the additional advantage of wide analytical range, could be developed.[17]

Herein, we propose and experimentally validate for the first time a novel analytical approach to rapidly determine online BOD. A novel air bubble sample delivery approach was developed to ensure sufficient oxygen supply to the BFR, enabling us to take full advantage of the BFR's high population of exposed microorganisms for rapid biodegradation of heavily contaminated wastewaters. Because the continuous oxygen supply to the BFR will invalidate the BOD quantification via the measurement of oxygen consumption by a DO probe, an innovative means is therefore investigated. The BOD values were quantified by determining the chemical oxygen demand (COD) changes (Δ[COD]) of a sample before and after biodegradation by the BFR with a photoelectrocatalytic oxidative degradation principle (PeCOD). The PeCOD method employed in this work is capable of achieving degradation and COD quantification in a single photoelectrocatalysis process at a nanostructured TiO_2 photoanode, independent of oxygen variation in the sample solution.[33] The method has the demonstrated ability to determine COD in a rapid, sensitive, accurate, and absolute manner without the need for ongoing calibration.[34−43] The proposed analytical principle and system performance were systematically validated using a range of individual organic matters, their mixtures, synthetic samples, and wastewaters.

EXPERIMENTAL SECTION

Materials and Sample Preparation. Indium tin oxide (ITO) conducting glass slides were purchased from Delta Technologies Limited. Peptone, beef extract, glucose, and glutamic acid were obtained from Sigma without further treatment prior to use. All other chemicals were of analytical grade. The synthetic sample containing mainly 150 mg L^{-1} peptone, 110 mg L^{-1} beef extract, and 30 mg L^{-1} urea (BOD_5 = 1398 ± 112 mg O_2 L^{-1}) was prepared according to the Organization for Economic Corporation and Development (OECD) Guidelines.[44] This synthetic sample is often used as a calibration standard for BOD and COD analysis systems. The glucose and glutamic acid (GGA) synthetic sample (BOD_5 = 1880 ± 150 mg O_2 L^{-1}) are usually used for BOD_5 standard, and it was prepared with 1.50 g glucose and 1.50 g glutamic acid in 1 L according to the American Public Health Association (APHA) standard methods.[1] Synthetic samples of glucose, sucrose, glycerol, isopropyl alcohol, and succinic acid were prepared with the equivalent BOD concentrations of 1500 mg O_2 L^{-1}, respectively. A mixture of the above seven synthetic samples with equal BOD concentration of each was prepared. The wastewaters used in this study were collected within the

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Changchun City in China from wastewater treatment plants (WWTPs), beverage manufacture, cake manufacture, dairy manufacture, corn manufacture, restaurants, and hospitals. All synthetic samples' pHs were adjusted between 6.0 and 8.0, and preserved according to the guideline of the standard method.[1] The wastewaters with turbidity over 200 NTU were filtered using a copper net filter system to remove large particles. High-purity deionized water (Millipore Corp., 18 MΩ) was used in the preparation of these synthetic samples. The sample for PeCOD method involved addition of $NaNO_3$ solid equivalent to 2 M as supporting electrolyte.

Preparation of Nanostrutured TiO_2 Photoanode. Aqueous TiO_2 colloid was prepared by hydrolysis of titanium butoxide according to the method described by Nazeeruddin et al.[45] The resultant colloidal solution contains 60 g dm^{-3} TiO_2 solid with particle size ranging from 8 to 10 nm. The ITO slide was used as the photoanode substrate and was pretreated to ensure its cleanness. After pretreatment, the ITO substrate was dip-coated in the TiO_2 colloidal solution. The coated substrates were then calcined in a furnace and used as the photoanodes. The details of the photoanode preparation were published in our previous work.[36−41] The thickness of TiO_2 porous film was 1.0 μm measured with a surface profilometer (Alpha-step 200, Tencor Instrument).

Tubular Biofilm Reactor Cultivation. The HF etched glass tube was used for fabricating a BFR as described in our previous work.[27−30] Activated sludge collected from a municipal WWTP was used as the cultivation solution for biofilm formation. Air-saturated cultivation solution with added nutrients was continually pumped through the functionalized tube. The cultivation process was terminated when no further decrease in COD removal was observed from the injections of the GGA solution in two consecutive intervals.

Apparatus and Methods. All PeCOD experiments were performed at room temperature in a thin layer three-electrode photoelectrochemical cell with a quartz window for illumination as used in previous studies.[41] The area, thickness, and the volume of the reaction chamber are 0.75 cm^2, 0.18 mm, and 13.5 μL, respectively. A saturated Ag/AgCl electrode and a platinum mesh were used as the reference and the auxiliary electrodes, respectively. An electrochemical workstation (CHI832, Chenhua) was used for PeCOD experiments. Illumination was carried out using a 150-W xenon arc lamp light source with focusing lenses (HF-200w-95, Beijing Optical Instruments). The detailed analytical procedures can be found from the Supporting Information, SI, and our previous published papers.[36−41]

Figure 1 shows the schematic diagram of the biofilm reactor-photoelectrocatalytic chemical oxygen demand (BFR-PeCOD) system set up and analytical detection principle. Air bubble-sample mixture was real-time generated (see the inserted picture in Figure 1) by a dispensing controller (FK-1C, Baoding Longer, China) and pumped into the BFR by a peristaltic pump at a certain flow rate during the experiment to ensure a sufficient oxygen supply for rapid biodegradation. The COD values of the original sample and its effluent from the BFR were analyzed using PeCOD method.[41] The obtained COD change (Δ[COD]) caused by the biodegradation of BFR was used to quantify the BOD value of the sample.

RESULTS AND DISCUSSION

BFR-PeCOD Analytical Principle. With normal DO probe-based BOD quantification methods, the low oxygen solubility limits the analytical linear range to ~50 mg O_2 L^{-1}.[6,7,10−12] Also, the slow recovery time of DO probe leads to a prolonged assay time. Even for a relatively low BOD concentration of 18 mg O_2 L^{-1}, a normal DO probe needs 15 min to reach the steady response and at least 35 min to completely recover from the previous test (see Figure S1 of the SI). To extend the applicability of the BFR-based method to heavily polluted wastewaters, the ability of the BFR to rapidly biodegrade high concentration organic pollutants needs to be dramatically improved. Our previous studies have suggested that the insufficient oxygen supply limits the degradation efficiency of the BFR.[27−30] In this work, we propose a novel air bubble sample delivery approach (Figure 1) that takes full advantage of the BFR's high population of exposed microorganisms to enhance the BFR's biodegradation efficiency. With such an approach, a large portion of the BFR is taken up by the moving air bubbles while only a small portion is occupied by the sample solution (Figure 1). More importantly, the sample solution inside the BFR is distributed in a thin-layer sharp around the air bubbles and in contact with the exposed microorganism film, which allows rapid diffusion of oxygen into the thin-layer liquid samples, ensuring an oxygen rich reaction environment throughout the entire degradation process. The huge population of exposed microorganisms combining with oxygen rich environment inside the BFR leads to an enhanced biodegradation efficiency even when the concentration of organic pollutants is high.

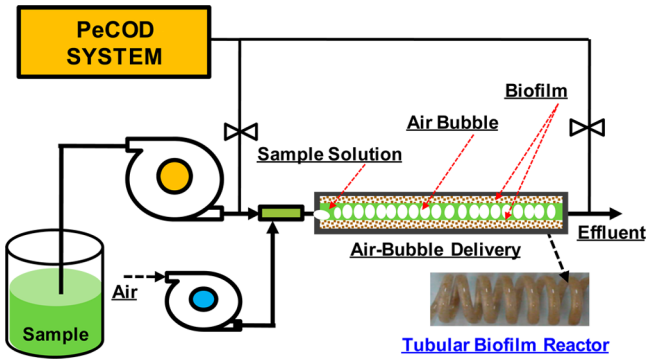

Figure 1. Schematic diagram of the BFR-PeCOD based BOD detection system and the photograph of tubular biofilm reactor.

The use of the air bubble sample delivery method will invalidate the BOD quantification via a DO probe. A new detection method is therefore needed. In this work, a rapid COD determination method, PeCOD, is innovatively utilized to quantify BOD (namely, the BFR-PeCOD method). We have previously demonstrated that PeCOD method is rapid, independent of oxygen concentration variation in sample solutions and does not require ongoing calibration,[36−41] which make it a suitable method for the proposed air bubble sample delivery BOD determination system. As illustrated in Figure S2 (SI), for a given sample, the COD values before ($[COD]_{Sample}$) and after ($[COD]_{Effluent}$) biodegradation by BFR can be rapidly determined by PeCOD. The determined COD change ($\Delta[COD] = \{[COD]_{Sample} - [COD]_{Effluent}\}$), in principle, equals to the BOD change resulting from the biodegradation by BFR. On this basis, the BOD value of the original sample can be quantified by Δ[COD] if a quantitative relationship between Δ[COD] and the original sample BOD value can be established. Figure 2 shows the relationships between the measured Δ[COD] and BOD_5 values of the

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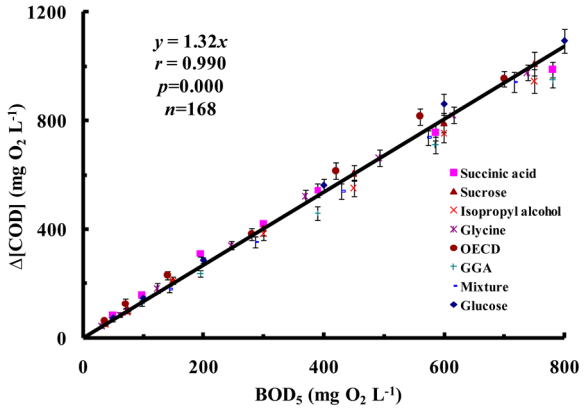

Figure 2. Correlation between Δ[COD] and standard BOD_5 values for a range of synthetic samples and their mixtures. The error bars represent deviations of three successive measurements. Temperature: 37 °C; tubular BFR length: 220 cm; and air bubble sample delivery flow rate: 1.0 mL min^{-1}.

original samples (r = 0.990, p = 0.000, n = 168). Well-defined liner relationships were obtained from all synthetic samples investigated, including different organics, their mixtures, OECD, and GGA. Compared to our previously reported oxygen probe based BFR system,[27] the analytical linear range of air bubble delivery system has been extended from 30 to 800 mg O_2 L^{-1} due to the oxygen rich environment inside the BFR. Such a linear relationship implies a linearly increased biodegradation by the BFR. It also demonstrates a dramatically enhanced degradation efficiency of BFR under oxygen rich conditions, even for substrate concentration as high as 800 mg O_2 L^{-1}. This confirms that the extent of biodegradation by the BFR is directly proportional to the substrate concentrations. The slope of the line-of-best-fit (k) obtained from the investigated synthetic samples defines a conversion factor between the measured Δ[COD] and the original sample BOD_5 value, as follows:

$$[BOD_5] = \frac{\Delta[COD]}{k} \quad (1)$$

For the BFR used in this work at a flow rate of 1.0 mL min^{-1} (the average retention time of sample solution inside the BFR is 7 min), the COD removal rate is calculated to be 84.5% for GGA as a representative under the oxygen-rich conditions created by the air bubble sample delivery system. Also, for the given BFR under the set of experimental conditions used in this work, the determined k value is fixed. It is also independent of the type and concentration of the organics in samples, implying that the biodegradation is indiscriminative and matrix-independent. Such remarkable biodegradation capabilities make the BFR system with the air bubble sample delivery approach perfectly suited to the needs of rapid BOD determination. In terms of biodegradation efficiency, this is the best performing system of all reported BOD determination systems.

The original sample and its effluent from the BFR can be separately analyzed for their COD values by PeCOD based on eq 2:[41]

$$[COD] = \frac{Q_{net}}{4FV} \times 32\,000\ (mgO_2L^{-1}) \quad (2)$$

where Q_{net} is the net charge originated from the photoelectrocatalytic mineralization of organics; F is the Faraday constant, and V is the volume of the thin-layer photoelectrochemical cell. The Δ[COD] value can therefore be obtained by the following:

$$\Delta[COD] = [COD]_{Sample} - [COD]_{Effluent} = \frac{Q_{net}^{Sample} - Q_{net}^{Effluent}}{4FV} \times 32\,000\ (mgO_2L^{-1}) \quad (3)$$

where Q_{net}^{Sample} and $Q_{net}^{Effluent}$ are the experimentally measured net charges resulted from the degradation of organics in the original sample and its effluent, respectively. According to eqs 1−3, the BOD_5 value of the original sample can be readily represented as follows:

$$[BOD_5] = BOD_{BFR-PeCOD} = \frac{\Delta[COD]}{k} = \frac{Q_{net}^{Sample} - Q_{net}^{Effluent}}{4kFV} \times 32\,000\ (mgO_2L^{-1}) \quad (4)$$

The data presented in Figure 2 have demonstrated that for a given BFR at a given flow rate, k is an experimentally determined constant that independent of the type of organics. This means that $[BOD_5]$ of the original sample can be quantified by eq 4 without the need for ongoing calibration.

Operational Conditions. The biodegradation sustainability of the BFR was first tested by continually air bubble delivering a glucose sample solution with a concentration equivalent to 400 mg O_2 L^{-1} to the BFR at 1.0 mL min^{-1}. After the injected sample reaching the end of the BFR (7 min after the injection), the effluents were collected each 10 min and their Δ[COD] values were determined (Table S1 of the SI). The determined Δ[COD] values kept almost steady within 1 h continuous sample injection via air bubble delivery, suggesting a superior sustainable biodegradation capability of BFR. This capability is like a small wastewater treatment device with a constant degradation efficiency. The results shown in Table S1 of the SI also indicate that the sample collection time for COD analysis with PeCOD can be flexible. For this work, all effluent samples were collected 10 min after sample injection and used for COD analysis.

For an online analytical system, it is highly desirable to operate easily and reduce the consumption of reagent. With normal DO probe-based BOD quantification methods, the procedure requires one to wash the system with buffer solution for cleaning and DO probe recovery purposes.[6,7] For the sample concentrations ranged from 3 to 18 mg O_2 L^{-1}, the washing procedure for one assay cycle will normally consume 80−140 mL of buffer solution and take 20−35 min at a flow rate of 4.0 mL min^{-1}. For the sample with concentration close to 30 mg O_2 L^{-1}, a much longer washing time is needed to completely recover the DO probe response to the original level. (Figure S1 of the SI). In order to simplify the operational procedures, and reduce the assay cycle time and reagent consumption, the effect of buffer solution washing on the analytical performance of the BFR-PeCOD system was evaluated in this work. Figure S3 of the SI shows the plots of Δ[COD] against BOD_5 for GGA samples. The data were collected from the consecutive injections of GGA samples from the high BOD concentration (800 mg O_2 L^{-1}) to the low concentration (25 mg O_2 L^{-1}) with

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or without buffer solution washing procedures between intervals. The slope values of 1.29 and 1.32 were obtained from analytical cycles with and without buffer washing procedures. These near identical slope values (the conversion factor, k) suggest that the buffer washing procedures can be avoided. Such an advantage greatly simplifies the system operation and dramatically reduces the assay time, making it possible to operate the BFR-PeCOD system without the need for chemical reagent.

For the BFR-PeCOD method, eq 4 can be used to obtain BOD_5 of the original sample. For a given BFR and PeCOD photoelectrochemical cell at a constant temperature, except the conversion factor k, all other parameters are either fixed or determinable parameters, independent of operational conditions. To make eq 4 applicable to unknown samples without the need for ongoing calibration, the dependent on k on the hydrodynamic conditions needs to be investigated. For a BFR (φ = 2.0 mm and L = 220 cm), the effect of air bubble sample delivery rate (0.5−2.0 mL min^{-1}) on k was investigated using a set of GGA solutions ranged from 20 to 1200 mg O_2 L^{-1} BOD at 37 °C. Excellent linear relationships between Δ[COD] and BOD_5 were obtained for all delivery rates investigated (Figure 3). It was found that an increase in the delivery rate from 0.5 to

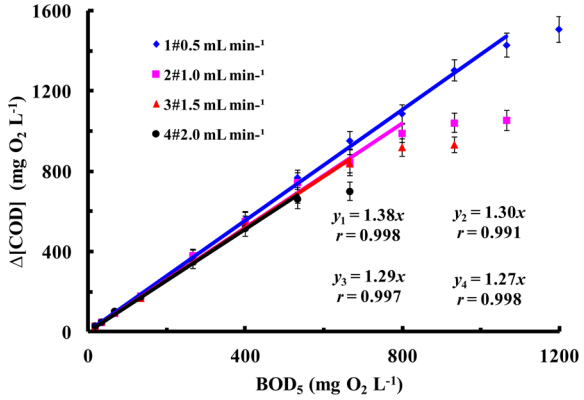

Figure 3. Effect of air bubble sample delivery flow rate on Δ[COD] values for GGA solutions. The error bars represent deviations of three successive measurements. Temperature: 37 °C, and tubular BFR length: 220 cm.

2.0 mL min^{-1} led to a slightly decreased k values from 1.38 to 1.27. Importantly, the obtained k value variations caused by the delivery rate changing were less than 8%, especially for delivery rates between 1.0 and 1.5 mL min^{-1}, only less than 1% k values changes (from 1.30 to 1.29) was observed. This implies that within the delivery rates investigated, the effect of the air bubble sample delivery rate on the biodegradation efficiency of the BFR is insignificant. This conclusion is critically important for the validity of using eq 4 for unknown sample analysis. These results confirmed that for a given BFR, the k value can be predetermined and applicable for unknown sample analysis in accordance with eq 4. The data shown in Figure 3 also revealed that the analytical linear ranges can be extended from 800 to 1100 O_2 mg L^{-1} when air bubble sample delivery rates were decreased from 1.0 to 0.5 mL min^{-1}. This can be attributed to the increased sample contact time with the microorganisms in the BFR, allowing more GGA to be biodegraded before flowing out of the BFR. Considering the effects of delivery rate on k values and analytical liner range, the used of 1.0 mL min^{-1} flow rate is deemed to be a balanced approach. This is in a strong contrast to the normal DO probe-based BOD quantification methods, where the operational parameters such as the sample injection rate and temperature must be strictly managed to minimize the endogenous respiration effect. Under such conditions, a typical analytical cycle consists of a 7 min biodegradation period and 5 min PeCOD quantification. The linear range, detection limit, reproducibility and long-term stability were evaluated under the optimized conditions using GGA as the testing samples. A linear range from 20 to 800 mg O_2 L^{-1} and a lower detection limit of 10 mg O_2 L^{-1} can be obtained. A set of relative standard deviations of 7.5%, 6.2% and 3.9% were obtained from seven successive tests of 120, 480, and 720 mg O_2 L^{-1} GGA samples, respectively. The long-term stability determines the reliability and is paramount for practical usefulness of an online analytical system. Figure S4 of the SI shows the BFR-PeCOD's long-term stability data collected over a 30-day test period for determination of glucose sample with a concentration equivalent of 400 mg O_2 L^{-1}. The results reveal no statistically significant trend change, confirming that the BFR-PeCOD system possesses superior long-term stability.

Validation by Synthetic Samples. The applicability of eq 4 for unknown sample analysis with predetermined k values needs to be validated. As shown in Figure 2, under optimal conditions, a k value of 1.32 can be obtained from a range of synthetic samples including different organics, their mixtures, OECD, and GGA. This predetermined k value was employed for the BFR-PeCOD method to quantify $BOD_{BFR\text{-}PeCOD}$ values. A set of synthetic samples with mass concentrations of 900 mg L^{-1} and experimentally determined BOD_5 values was employed for the validation experiments. The $BOD_{BFR\text{-}PeCOD}$ measurements were conducted over an 11-day period (one synthetic sample per day). Figure 4 shows the plot of $BOD_{BFR\text{-}PeCOD}$

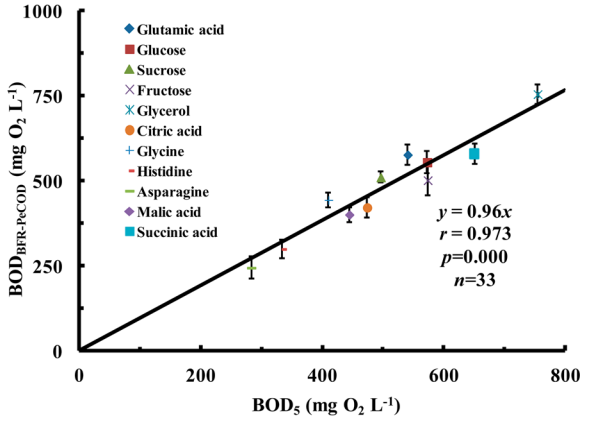

Figure 4. Correlation between $BOD_{BFR\text{-}PeCOD}$ and standard BOD_5 values for synthetic samples with a mass concentration of 900 mg L^{-1}. The error bars represent deviations of three successive measurements. Temperature: 37 °C; tubular BFR length: 220 cm; and air bubble sample delivery flow rate: 1.0 mL min^{-1}.

values determined by the BFR-PeCOD method against the BOD_5 of the synthetic samples. The slope of the plot, in theory, should be 1. The determined slope value was found to be 0.96 with good linearity (r = 0.973, p = 0.000, n = 33). Such a near unit slope value implies that the BOD value determined by the BFR-PeCOD method is essentially the same as that determined by the BOD_5 method, but with a dramatically reduced assay time, and that it can be fully automated. Considering the superior long-term stability demonstrated in Figure S4 of the SI

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and the data shown in Figure 4, it is reasonably believed that the predetermined k can indeed be used to accurately and reliably calculate BOD values of unknown samples based on eq 4 without the need for ongoing calibration.

Analysis of Wastewaters. Although the above experimental results validated the applicability of the BFR-PeCOD method for synthetic samples, the ultimate purpose of application to real samples has yet to be confirmed. The matrix effect is the most critical difference for determining synthetic and real samples. In fact, the failure of most rapid BOD methods for real sample analysis is their inability to tolerate the matrix effect of real samples, especially for wastewaters containing complex and diversified BOMs. In this work, the wastewater samples were collected from various industrial sites and WWTPs. All collected samples were analyzed by both BFR-PeCOD and conventional BOD_5 methods, respectively. For the BFR-PeCOD method, the $BOD_{BFR\text{-}PeCOD}$ values were quantified by eq 4 using a conversion factor of k = 1.32, obtained from Figure 2. The correlation between the two methods is given in Figure 5. A high significant correlation (r = 0.952, p = 0.000, n = 60) between the two methods was obtained, indicating that the two methods are in good agreement. Importantly, the slope of the principal axis of the correlation ellipse of 0.96 was obtained. This almost unitary slope value suggests that the proposed BFR-PeCOD method can be used for determination of BOD with the same accuracy as that of the conventional BOD_5 method. Given a 95% confidence interval, this slope was in the range of 0.79 and 1.13, implying there is a 95% confidence belt that the true slope lays between these two values. Considering that there are analytical errors associated with both the PeCOD and the BOD_5 measurements and that these errors contribute to scatter on both two axes, the high correlation and near unity slope value obtained confirm the applicability of the BFR-PeCOD method for wide range of wastewaters. It is noteworthy that the BFR possesses high tolerance to organic toxicants (e.g., 3,5-dichlorophenol) and heavy metal ions (e.g., Zn(II), Cr(VI), Cd(II), Cu(II), Pb(II), Mn(II), and Ni(II)) with concentrations up to 30 mg L^{-1},[28] which made this proposed method possible to be directly applied to highly polluted real samples.

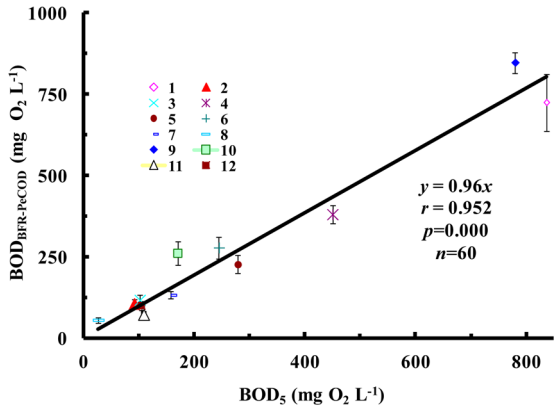

Figure 5. Correlation between $BOD_{BFR\text{-}PeCOD}$ and standard BOD_5 values for wastewater samples. The error bars represent deviations of five successive measurements. Temperature: 37 °C; tubular BFR length: 220 cm; and air bubble sample delivery flow rate: 1.0 mL min^{-1}. The samples were collected from the following: 1−3, daily wastewater treatment plants; 4−5, beverage manufactures; 6, cake manufacture; 7, dairy manufacture; 8, corn manufacture; 9−10, restaurants; and 11−12, hospitals.

In summary, we have proposed and experimentally validated a novel BFR-PeCOD analytical system which is suitable for field-based environmental monitoring applications, especially for heavily polluted wastewaters. The reported system is capable of near real-time, online determining BOD levels of a wide range of wastewaters without the need for dilution and ongoing calibration. The analytical linear range has been significantly extended to 800 mg O_2 L^{-1} with superior long-term stability, high accuracy, and reliability. It is an environmentally friendly analytical system that consumes little reagent and requires minimal operational maintenance. These advantages make the BFR-PeCOD system very promising for practical online BOMs monitoring applications. The findings revealed and the strategy used in this work pave a way for future development of practical useful aquatic organic pollution monitoring systems for a wide range of applications.

■ ASSOCIATED CONTENT

Supporting Information

The PeCOD analytical principle, response characteristic of the DO probe to a set of GGA solutions, relationship of Δ[COD] values and effluent collection time, BFR stability, and BFR linear responses comparison with and without washing. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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