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Short communication

Biochemical oxygen demand measurement by mediator method in flow system

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ABSTRACT

Using mediator as electron acceptor for biochemical oxygen demand (BOD) measurement was developed in the last decade (BOD_{Med}). However, until now, no BOD_{Med} in a flow system has been reported. This work for the first time describes a flow system of BOD_{Med} method (BOD_{Med}-FS) by using potassium ferricyanide as mediator and carbon fiber felt as substrate material for microbial immobilization. The system can determine the BOD value within 30 min and possesses a wider analytical linear range for measuring glucose–glutamic acid (GGA) standard solution from 2 up to 200 mg L^{-1} without the need of dilution. The analytical performance of the BOD_{Med}-FS is comparable or better than that of the previously reported BOD_{Med} method, especially its superior long-term stability up to 2 months under continuous operation. Moreover, the BOD_{Med}-FS has same determination accuracy with the conventional BOD_5 method by measuring real samples from a local wastewater treatment plant (WWTP).

1. Introduction

Biochemical oxygen demand (BOD) is an international regulatory environmental index for monitoring wastewater pollutants [1]. The legislated standard test for BOD monitoring is the widely accepted 5 days method (BOD_5). However, it requires complicated procedures, skilled analysts and time-consuming. Over the last decade, several mediated methods have been developed for rapidly measuring BOD (BOD_{Med}) and showed great potential as an alternative to the standard BOD_5 assay [2–12]. Unlike the oxygen-type BOD biosensors which are limited by the electron acceptor concentration (~8.7 mg L^{-1} at 25 °C of O_2), the mediated-type BOD methods using ferricyanide as electron acceptor, is ~10,000 fold more soluble in water than O_2 [13]. Combing a large population of microbes, the BOD_{Med} method made the dilution process of sample before testing unnecessary, thus increasing the accuracy and reliability of BOD results. Furthermore, the BOD_{Med} strategy offered a simple way to resolve the problem of fluctuations of response signals resulted from the disturbance by aerated operation of oxygen-type BOD biosensors. So, the BOD_{Med} method showed remarkable improved abilities for BOD measurement [14–16].

Until now, almost all reported BOD_{Med} methods proceed in a quiescent system and no works related to arrange BOD_{Med} assay in a flow system (BOD_{Med}-FS) have been studied yet. To accelerate the application and commercialization of the BOD_{Med} approach, developing a facile flow system combined with immobilized microbes and long-term stabilities is highly demanded. Therefore, to build a high performance BOD_{Med}-FS, it is very important to find an appropriate means to immobilize the microbes, because the activity of microbes would be affected significantly when exogenous mediator is added in the flow system. More recently, our group reported a phenomenon that the negative effect of mediator toward microbes could be recovered [13] and established a BOD_{Med} bioreactor by using carbon fiber felt as the substrate for immobilizing microbes to form native biofilm [17]. Inspired by these achievements, in this work, we try to further develop a BOD_{Med}-FS with extraordinarily high biodegradation ability, superior accuracy and reliability. The present approach reported in this work would pave a way for future development of practical BOD determination systems for a wide range of applications.

2. Experimental

2.1. Materials

Broth medium was purchased from Fluka (CASO, Fluka Chemie GmbH CH-9471 Buchs) which contains casein peptone, soybean flour and peptone broth. Phosphate buffer solution (PBS, 0.12 M Na_2HPO_4/0.08 M K_2HPO_4/0.1 M KCl, pH 7.0) was used for sample dilution and flow system rinsing unless otherwise stated. To prepare standard BOD solutions with different concentrations,

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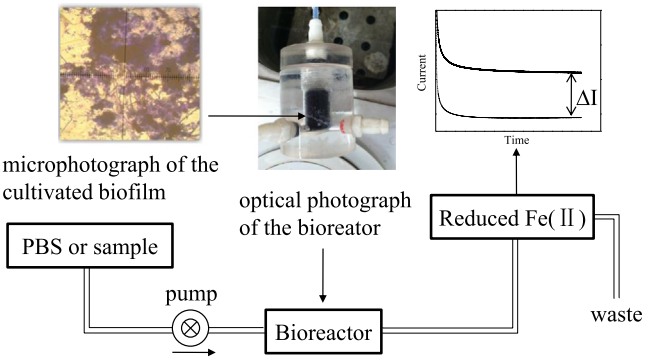

Scheme 1. Schematic diagram of the BOD_{Med}-FS. .

appropriate dilution of the glucose–glutamic acid (GGA) solution according to the American Public Health Association (APHA) method that contained 1.5 g L^{-1} glucose and 1.5 g L^{-1} glutamic acid (BOD value of ~1980 mg O L^{-1}, signed as GGA^{1980}) was used [1,18]. The BOD_5 values of the real samples were determined by the standard dilution method [1]. All chemicals used in this study were of analytical reagent grade and all solutions were prepared with sterile deionized water.

2.2. Flow system

The BOD_{Med}-FS, according to Scheme 1, was composed of a biofilm reactor (BFR) with microbes deposited on a carbon fiber felt and an electrochemical analyzer (CHI 832, CHI Co., Shanghai, China) as the signal collector. The flow system was driven by a pump and a dispensing controller (FK-1C, Baoding Longer, China). Activated sludge collected from a local municipal wastewater treatment plant (WWTP) was used as the microbial seeds for biofilm formation. 3 g of commercial CASO broth medium was pulled into a culture flask with 100 mL supernatant liquid of activated sludge. In order to optimize the measuring conditions, several parameters including incubation periods (under 35 °C and pH 7), incubation temperatures (under 24 h and pH 7) and pH values of the incubation solution (under 24 h and 35 °C) were studied. After culture, the flow system was sufficiently rinsed by PBS.

2.3. Measurement procedures

The mediator solutions were prepared by appropriate dilution of the 0.33 M potassium ferricyanide. The PBS and sample solutions were both placed in the thermostatic bath to keep consistent temperature while deaerated by nitrogen (N_2). Endogenous control solutions were prepared by adding PBS to replace samples. A fixed flow rate of 3.8 mL min^{-1} was set for the flow system. Before and after wastewater measurements, a calibration procedure (standard GGA solution) must be checked, so that the BOD_{Med}-FS can be adjusted and any influence of the sample on the sensor can be detected. After incubation, the samples were pumped out, centrifuged at 10, 000 rpm for 3 min to obtain a supernatant solution and analyzed microbially produced ferrocyanide. The response signals were collected using an electrochemical analyzer and conducted with amperometric mode. The electrode system and detailed operating instructions can be found in our previously reported study [17].

3. Results and discussion

3.1. BOD_{Med}-FS

Scheme 1 shows the established BOD_{Med}-FS used in this study and different parts of the flowing system were connected by airtight tubes. The top-left image in Scheme 1 shows an optical microscopy of the immobilized microbes on a carbon fiber felt. As seen from it, the microbes are uniformly and tightly deposited on the carbon fiber felt support which suggests the feasibility of the present flow system for BOD measurement. The mediator reduced by BFR is detected by the signal changed on the electrode. As shown in the top-right image of Scheme 1, a net current ΔI between blank and sample is observed, indicating the analytical result of sample can be quantified by the relationship of ΔI and concentration.

3.2. Balance and optimization of BOD_{Med}-FS

The long-term stability is an important factor for the practical application of BOD biosensor, which was affected by the performance of microbial activity. In general, an accordant microbial consortium to the microbes contained in the tested water was obtained by a native cultured process. However, the type of species and the amount of microbial consortium could be changed during the measuring and storing processes, especially influenced by the storage conditions. Moreover, the microbes in the flow system could be divided continuously, leading to the reproduction of new bacteria and sacrifice of old bacteria. Therefore, keeping high biodegradability of the BFR which could be affected by different storage conditions is extraordinary important when the BOD_{Med}-FS was left in an idle state. Different storage conditions were studied in this work. As shown in Fig. 1, three different storage conditions including pH 7 PBS with GGA^{200} solution (box a), tap water (box b) and tap water with GGA^{20} solution (box c) were studied in this work using our established BOD_{Med}-FS. Higher but unstable electrochemical signals were obtained in box a whereas stable signals were observed in box b and c with box b shows the lowest signal behavior. It should be mentioned here that some strange signals are appeared at the first measurement cycle in box a and box b each day which may be attributed to the measured high blank signals caused by endogenous respiration. We supposed that the bioactivity was high in the initial stage of biofilm formation and the biodegradation was un-balanced. This phenomenon could be prevented by changing the storage conditions which is the case of box c where tap water with GGA^{20} solution was used to maintain the stability of BOD_{Med}-FS. The net currents of the electrochemical signals are shown in Fig. S1.

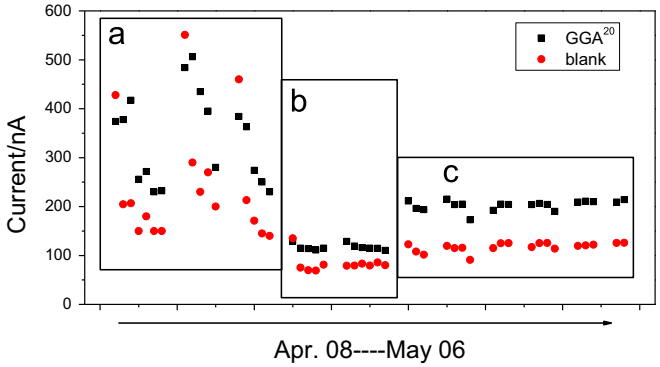

Fig. 1. Electrochemical signals for measuring GGA^{20} and blank by using BOD_{Med}-FS at storing conditions were pH 7 PBS with GGA^{200} solution (box a), tap water (box b) and tap water with GGA^{20} solution (box c).