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Comparative analysis of microbial fuel cell based biosensors developed with a mixed culture and *Shewanella loihica* PV-4 and underlying biological mechanism

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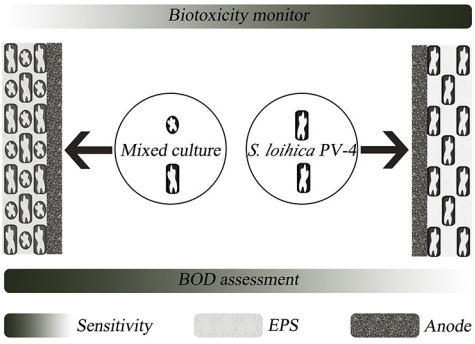
GRAPHICAL ABSTRACT



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ABSTRACT

Microbial fuel cell based biosensors (MFC-biosensors) utilize anode biofilms as biological recognition elements to monitor biochemical oxygen demand (BOD) and biotoxicity. However, the relatively poor sensitivity constrains the application of MFC-biosensors. To address this limitation, this study provided a systematic comparison of sensitivity between the MFC-biosensors constructed with two inocula. Higher biomass density and viability were both observed in the anode biofilm of the mixed culture MFC, which resulted in better sensitivity for BOD assessment. Compared with using mixed culture as inoculum, the anode biofilm developed with *Shewanella loihica* PV-4 presented lower content of extracellular polymeric substances and poorer ability to secrete protein under toxic shocks. Moreover, the looser structure in the *S. loihica* PV-4 biofilm further facilitated its susceptibilities to toxic agents. Therefore, the MFC-biosensor with a pure culture of *S. loihica* PV-4 delivered higher sensitivity for biotoxicity monitoring. This study proposed a new perspective to enhance sensor performance.

1. Introduction

Water quality monitoring is paramount for the guarantee of ecological safety and public health. Available monitoring items mostly focus on physical and chemical indicators such as pH and chemical oxygen demand. Only a few biochemical indexes represented by biological oxygen demand (BOD) are included (SEPA, 2002). Among these parameters, physical and chemical indicators enable the accurate

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determination of the concentration of specific substance in aquatic environment, but the detection process requires skilled and time-consuming operation. Moreover, these indicators fail to provide a comprehensive assessment of biotoxicity and cumulative toxicity, which makes these methods unsuitable for in situ and online monitoring. BOD index, which relates to the biodegradability of water, is evaluated by the consumption of dissolved oxygen after incubation for a fixed period (usually 5 d) (APHA, 1998). However, tediously long detection period of BOD measurement disagrees with the demand of prompt alert and leads to the deficiency of fast-response detection method. Therefore, research on novel technology and equipment for timely and efficient water quality monitoring is of crucial importance.

Microbial fuel cells (MFCs) are devices which convert chemical energy from organic matters into electricity with the catalysis of electroactive microorganisms (Logan, 2009). Previous studies have validated the feasibility to use MFC based biosensor (MFC-biosensor) to monitor BOD concentration and biotoxicity in water environment (Kim et al., 2003; Kim et al., 2007; Modin and Wilén, 2012). Kim et al. (2003) developed a dual-chamber MFC for BOD quantification and observed a good linear relationship between Coulombic yield and BOD concentration. Thereafter extensive efforts have been made to improve sensor performance from the aspects of configuration design and operation parameter. For instance, scaling down the anode volume from 25 mL to 5 mL and the optimization of fuel-feeding rate could reduce the response time of BOD test to 36 min (Moon et al., 2004), while another study managed to extend the linear range to 1280 mg/L by applying an external voltage to MFC-biosensor (Modin and Wilén, 2012). Research on the assessment of biotoxicity via MFC-biosensor started later than BOD sensor. The first MFC based toxicity sensor was reported by Kim et al. (2007). They installed it in a wastewater treatment plant and successfully alerted the shocks of several heavy metals and polychlorinated biphenyl. Since then, significant progress has been made to enhance the sensitivity and stability of detection process (Shen et al., 2013; Lorenzo et al., 2014; Jiang et al., 2015; Xu et al., 2015).

Anode biofilm is considered as the main sensing element for both BOD concentration and biotoxicity monitoring. Fluctuations in water quality would affect the metabolic activities and electron transfer processes of electroactive microorganisms and further lead to visible changes of output current or voltage (Jiang et al., 2018). Hence, electroactive biofilm colonized on the anode surface is a key factor to sensor performance. MFC-biosensors in most of researches were constructed with mixed cultures (Liu et al., 2014; Lorenzo et al., 2014; Wu et al., 2014; Xu et al., 2015; Yu et al., 2017). Only a few publications concerned about the application of pure strain as sensing element. In a recent study, Atci et al. (2016) developed a MFC-biosensor with a pure culture of *Geobacter sulfurreducens* and observed a good linear correlation between current signal and acetate concentration. As for biotoxicity sensor, a pure culture of *Shewanella oneidensis* MR-1 was employed for the first time to detect formaldehyde (Wang et al., 2013). However, the influence of the two types of inocula on the performance of MFC-biosensors has not been revealed yet.

When studying the temporal-spatial changes of biofilms in microbial electrolysis cells, Sun et al. (2015) reported that various inocula (mixed culture and *Geobacter anodireducens*) would result in discrepancies in biofilm structure, viability and biomass density and further affected electricity generation directly. Since electricity generation was an important factor for BOD quantification via MFC-biosensor (Modin and Wilén, 2012), different inocula might impact the sensor performance for BOD determination. On the other hand, the contents of extracellular polymeric substances (EPS) in biofilms might vary with different inocula as well. Due to the protective effect of EPS against toxic agent invasion (Miao et al., 2017), various inocula might also influence the sensitivity of MFC-biosensor for biotoxicity monitoring.

Therefore, this study aimed to investigate the effect of inoculum on the sensitivity of MFC-biosensor, which has not been revealed yet and might propose a new perspective to enhance the sensor performance. *Shewanella loihica* PV-4, which was a widely applicable strain of electroactive microorganisms with the superior capacity of extracellular electron transport (Kim et al., 1999; Newton et al., 2009), was selected to represent the pure culture inoculum and to develop pure culture MFC-biosensors. Meanwhile, the effluent taken from a stably operating MFC based on mixed culture was utilized to construct traditional mixed culture MFC-biosensors. Herein, a systematic analysis of the effect of inoculum on sensor sensitivity was provided by employing the two types of MFC-biosensors for both BOD and biotoxicity determination. Furthermore, anodic biofilm properties and the production of EPS were both assessed to illustrate the underlying biological mechanism.

2. Materials and methods

2.1. Chemicals and reagents

The inoculum of the mixed culture was collected from the effluent of an acetate-fed MFC which had been steadily operated for over one year. *S. loihica* PV-4 was purchased from American type culture collection and preserved in a −80 °C refrigerator. Before inoculation, *S. loihica* PV-4 was twice activated in a shaking flask (23.0 °C, 200 rpm) containing Luria-Bertani medium overnight. During all the experiments, the same anolyte and catholyte were used in these two types of MFCs. The anolyte used in this study consisted of 2.50 g $NaHCO_3$, 0.08 g $CaCl_2{\cdot}2H_2O$, 1.00 g NH_4Cl, 0.20 g $MgCl_2{\cdot}6H_2O$, 10.00 g NaCl and 7.20 g HEPES in 1.00 L deionized water. Specific volume of substrate stock solution, which contained 112.00 g/L sodium lactate and 50.00 g/L yeast extract, was added to fresh anolyte as carbon sources. Anolyte was sterilized prior to use and preserved in a clean bench (SW-CJ-1F, Airtech, China). The catholyte consisted of 10.00 g NaCl and 7.20 g HEPES in 1.00 L deionized water. For biotoxicity determination, $CdSO_4$ and chlortetracycline hydrochloride (CTC), which respectively represented the pollution of heavy metal and antibiotic, were individually dissolved in deionized water to prepare 1.0 g/L toxic stock solutions and kept at 4.0 °C after filtration. Toxic and non-toxic anolyte were then prepared by adding a specific volume of toxic stock solution and the same volume of solvent in fresh anolyte, respectively.

2.2. MFC construction and start up

Eight MFCs (MFC1-8) with dual-chamber configuration were constructed and divided into two groups. MFC1-4 were designed to investigate the effect of inoculum on sensor sensitivity for BOD and biotoxicity determination while the other group (MFC5-8) was set for the analysis of biofilm. The total volumes of anode and cathode chambers were 14 mL and 42 mL, respectively, and the two chambers were separated by a proton exchange membrane (Nafion 117, Hesen Electric Corporation, China). A 3.0 cm × 2.5 cm piece of carbon cloth (HCP330, Hesen Electric Corporation, China) with ammonia pretreatment was used as anode while cathode consisted of two pieces of 0.5 mg/cm^2 Pt loaded carbon paper with an area of 2.0 cm × 2.0 cm. Anode and cathode were connected with an external resistor of 330 Ω via titanium wire. After fabrication, all the MFCs were sterilized and inoculated in the clean bench. Then, both mixed microbial consortium and *S. loihica* PV-4 were diluted to the same viable cell concentration, and were immediately used to inoculate MFC1-8 with the same inoculum size of 30% (v/v). MFC1-2 and MFC5-6 were inoculated with the mixed consortium while the others with *S. loihica* PV-4. All the anode chambers were finally filled with sterile anolyte containing 1.12 g/L sodium lactate and 0.50 g/L yeast extract as fuel, and all the operation parameters were consistent in each MFC except for inoculum. Self-circulation at a flow rate of 2 mL/min was operated inside the anode chamber via a peristaltic pump (BT100-1L, LongerPump, China) to obtain a hydraulic retention time of 7 min. Anolyte was forced to work in the flow-by-anode mode to improve sensor performance (Jiang et al., 2015). In addition, all MFCs were refreshed simultaneously in