ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Submersible Microbial Fuel Cell Sensor for Monitoring Microbial Activity and BOD in Groundwater: Focusing on Impact of Anodic Biofilm on Sensor Applicability

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ABSTRACT: A sensor, based on a submersible microbial fuel cell (SUMFC), was developed for in situ monitoring of microbial activity and biochemical oxygen demand (BOD) in groundwater. Presence or absence of a biofilm on the anode was a decisive factor for the applicability of the sensor. Fresh anode was required for application of the sensor for microbial activity measurement, while biofilm-colonized anode was needed for utilizing the sensor for BOD content measurement. The current density of SUMFC sensor equipped with a biofilm-colonized anode showed linear relationship with BOD content, to up to 250 mg/L ($\sim 233 \pm 1$ mA/m^2), with a response time of <0.67 h. This sensor could, however, not measure microbial activity, as indicated by the indifferent current produced at varying active microorganisms concentration, which was expressed as microbial adenosine-triphosphate (ATP) concentration. On the contrary, the current density (0.6 ± 0.1 to 12.4 ± 0.1 mA/m^2) of the SUMFC sensor equipped with a fresh anode showed linear relationship, with active microorganism concentrations from 0 to 6.52 nmol-ATP/L, while no correlation between the current and BOD was observed. It was found that temperature, pH, conductivity, and inorganic solid content were significantly affecting the sensitivity of the sensor. Lastly, the sensor was tested with real contaminated groundwater, where the microbial activity and BOD content could be detected in <3.1 h. The microbial activity and BOD concentration measured by SUMFC sensor fitted well with the one measured by the standard methods, with deviations ranging from 15% to 22% and 6% to 16%, respectively. The SUMFC sensor provides a new way for in situ and quantitative monitoring contaminants content and biological activity during bioremediation process in variety of anoxic aquifers.

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KEYWORDS: submersible microbial fuel cell; biosensor; microbial activity; biodegradable organic matter; groundwater; bioremediation

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Introduction

Contamination of groundwater with various organic pollutants is one of the most severe environmental and public health problems (Salanitro et al., 2000; Semprini and McCarty, 1991). There is a pressing need to monitor microbial activity and pollutants in groundwater, because it could give direct insight on natural attenuation of contaminants (Stelzer et al., 2006; Van der Meer et al., 1998). However, most of monitoring approaches are often costly, time consuming, and not applicable to the subsurface environment (Chapelle and Lovley, 1990; Fossing and Jorgensen, 1990). Therefore, efforts should be made to pursue an innovative monitoring technology, which is reliable and rapid, yet simple, noninvasive, and cost-effective. Recent advances in microbial fuel cells (MFC) suggest that this technology has most promising potential for in situ assessment of biodegradation and related microbial activity in groundwater.

MFCs are bioelectrochemical reactors in which microorganisms mediate direct conversion of chemical energy stored in organic matter into electrical energy (Bond et al., 2002; Katuri and Scott, 2010; Logan and Regan, 2006; Parameswaran et al., 2010; Rabaey et al., 2005; Rosenbaum et al., 2010). Various organic compounds and sources of wastewater have been successfully utilized for power generation in MFCs (Liu et al., 2005; Logan et al., 2005; Mathis et al., 2008; Wang et al., 2009). Additionally, variety of anaerobic bacteria (e.g., *Geobacter* spp.), involved in contaminants reduction, are capable of transferring electrons to a solid electrode and generating electricity in MFCs (Anderson et al., 2003; Bencheikh-Latmani et al., 2005; Tront et al., 2008a; Watson and Logan, 2010). In general, with a mature biofilm-colonized anode, current generation of MFC is correlated with substrate concentration, and thus, the current can be used as a measure of contaminants concentration. Several types of MFC-based biosensor have

been developed for monitoring of biochemical oxygen demand (BOD) in wastewater and showed good stability, accuracy, and wide detection range when compared with other types of biosensors (Chang et al., 2004, 2005; Di Lorenzo et al., 2009; Kim et al., 2009; Kumlanghan et al., 2007). As another possible application of MFC sensor, microbial activity assessment has been recently proposed (Tront et al., 2008a,b; Williams et al., 2010). The idea behind is the response of current generation to the different substrate (BOD) concentrations, somehow reflects the activity of the biofilm colonized on the anode (Tront et al., 2008a). However, these assessments are indirect, and no quantitative measurement of microbial activity is available. Moreover, the detected activity mainly originates from the anodic biofilm, which might overshadow the real microbial activity of the monitored site (e.g., groundwater). Thus, more accurate, direct, and quantitative approaches should be pursued for monitoring of microbial activity in groundwater. It was found that inoculum type and concentration can affect the bacterial adhesion to the fresh anode, which can subsequently influence the electricity generation of the MFCs (Jiang et al., 2009). Therefore, we assumed that the current production from fresh anode electrode could serve as a proxy for monitoring of microbial activity. In this way, the microbial activity could be monitored independent of BOD concentration, and the influence of biofilm activity could also be avoided.

Besides, most of the existing MFC sensors are surface applicable systems (Di Lorenzo et al., 2009; Kumlanghan et al., 2007; Liu and Mattiasson, 2002), no information about their application in groundwater is available. Pumping and water distribution systems could be applied, but it will obviously increase the cost for continuous monitoring (Istok et al., 1997; Vandenbohede et al., 2008). Such considerations indicate that novel sensor configurations that are applicable in subsurface are now given high priority.

In this study, we report an innovative submersible MFC (SUMFC) sensor. In this novel SUMFC design, specially designed anode chamber was not needed, which makes it easy to apply in groundwater and other natural anaerobic environments, thereby avoiding the cost for extensive sampling, pumping, and distribution. Therefore, the objective of this study was to (i) test the sensor with either biofilm-colonized or fresh anode for BOD and microbial activity monitoring in artificial groundwater; (ii) evaluate the effect of different operation parameters on the performance of the sensor; and (iii) verify the applicability of the sensor in real contaminated groundwater. These results have practical implications for development of a sensor, as part of in situ bioremediation activities, capable of providing real time, quantitative information on the rate and nature of biodegradation processes.

Materials and Methods

Groundwater

Artificial groundwater was prepared by adding acetate (or glucose) (10–300 mg-BOD/L) to tap water, without addition of nutrients, buffer, vitamins, or minerals. For the microbial activity monitoring experiments, the fresh domestic wastewater, collected from primary clarifier (Lundtofte Wastewater Treatment Plant, Lyngby, Denmark, receives both domestic and industrial wastewater and stormwater, and it has a capacity of 114,000 person equivalents), was added into the artificial groundwater to reach different active microorganism concentrations expressed as microbial adenosine-triphosphate (ATP) concentrations (0–13.04 nmol/L). Wastewater collected at the same site, was used for the BOD experiment. The wastewater was first filtrated with 0.45-μm membrane filter, and then diluted with tap water to reach different BOD concentration (10–300 mg-BOD/L). For testing the effect of inorganic solid content on the sensor performance, soil was collected from site ZEITZ (Leipzig, Germany). Typical soils of this area are Haplic Chernozems, Haplic and Luvic Phaeozemz, Calcaric Regosols and Haplic Luvisols (soil classification according to ISSS/ISRIC/FAO, 1994) (Hildmann and Wunsche, 1996). The soil was first dried at 105°C for 24 h. The dried soil was heated in a muffle furnace (550°C, 16 h) to remove the organic content as previous described (Konen et al., 2002), and then added to the artificial groundwater. Four natural contaminated groundwater samples (Table I) were collected from site ZEITZ, where was contaminated with more than 770,000 tons of benzene and groundwater contamination up to 1,000 mg/L were found (Schirmer et al., 2006).

Table I. Characteristics of real groundwater taken from wells at ZEITZ.

Samples	BOD[a] (mg/L)	BOD[b] (mg/L)	ATP[c] (nmol/L)	ATP[b] (nmol/L)	pH	Conductivity (μs/cm)	Nitrate (mg/L)	Sulfate (mg/L)
1	251 ± 10	231 ± 3	0.081 ± 0.005	0.064 ± 0.006	8	980 ± 10	4.2 ± 0.2	25.6 ± 0.3
2	269 ± 10	250 ± 5	0.101 ± 0.005	0.082 ± 0.004	7.8	658 ± 10	—[d]	20.8 ± 0.1
3	147 ± 10	120 ± 6	0.234 ± 0.005	0.198 ± 0.004	7.3	855 ± 6	4.3 ± 0.3	49. ± 2.0
4	92 ± 10	75 ± 2	0.199 ± 0.005	0.155 ± 0.005	7.5	956 ± 8	—	63.4 ± 0.6

[a]Measured by APHA method.
[b]Measured by SUMFC sensor.
[c]Measured using Lumin (ATE)/Lumin(EX) reagents and a luminometer.
[d]Not detected.

SUMFC-Based Sensor and Its Operation

The SUMFC-based sensor was constructed as described by Min and Angelidaki (2008), and used as sensor in this study. As shown in Figure 1, the sensor was composed of an anode electrode and a nonconductive polycarbonate plate made rectangular cathode chamber (3 cm × 3 cm × 1 cm, 9 cm^3), which were submersed in a glass reactor (total volume of 1,000 mL, liquid volume of 500 mL). The glass reactor was closed with a rubber stopper having several openings. Through these openings, tubes were inserted for retrieving liquid and gas samples, and for wires connections. Five hundred milliliters of groundwater was filled into the glass reactor, and the SUMFC was submersed 3 cm below the water surface. At the bottom of glass reactor, a magnetic bar was ensuring mixing. The anode and cathode were connected to a 1,000 Ω resistor outside the glass reactor with copper wires (Fig. 1). The fresh anode electrode was a piece of not wet-proofed carbon paper (3 cm × 3 cm, 9 cm^2, Toray carbon paper, E-TEK division, USA). The biofilm-colonized anode electrode, the same size as the fresh electrode, was taken from a submersible MFC reactor. The reactor was inoculated and operated with the wastewater taken from the primary clarifier as above for electricity generation in the last 2 months. During these 2 months, the submersible MFC was operated in batch mode in the laboratory (25°C, pH 7.0) and refilled with wastewater every 7 days. After 2 months operation, a thick biofilm attached on the surface of anode can be seen. The cathode electrode was a 5% wet proofed carbon paper (3 cm × 3 cm, 9 cm^2, Toray carbon paper, E-TEK division, USA), and one side of the electrode contained Pt catalysts (0.5 mg/cm^2 with 20% pt, E-TEK). The Pt coated side was hot pressed with a proton exchange membrane (Nafion 117, DuPont Co., Fayetteville, NC) prepared as previously described (Min and Logan, 2004). The distance of the anode and cathode electrode was approximately 0.5 cm in the reactor. Electrical connections and pretreatment of electrodes were done as previously described (Zhang et al., 2009).

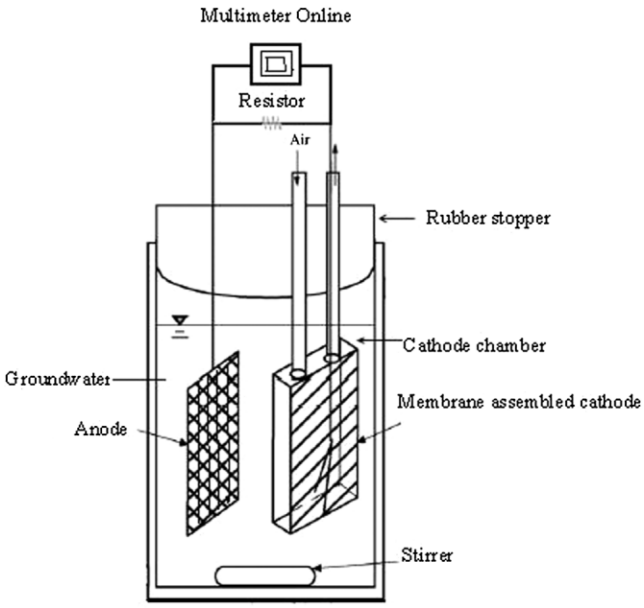

Figure 1. Schematic diagram of the SUMFC sensor.

Initially, the artificial groundwater was used to evaluate the sensor with different anode electrodes, and afterwards the sensor was validated with real contaminated groundwater. Two different anodes, biofilm-colonized anode and fresh anode, were tested in the sensor. These two electrodes were used alternately according to the purpose wished. Unless otherwise stated, the glass reactor was installed in a temperature-controlled chamber (25°C) and stirred (250 rpm) using a magnetic bar to ensure effective mixing. The initial pH of all solutions was adjusted with 1 M HCl or NaOH solutions to 7.0, and the cathode chamber was connected with a plastic tube, which was open to the air. The conductivity was adjusted to 2.5 ms/cm with a KCl (0.1 M) solution. In order to find the effect of operational parameters (e.g., temperature, pH, airflow rate, stirring rate, conductivity, nitrate, and sulfate concentration) on the performance of SUMFC, the conditions of these parameters were adjusted according to the Plackett–Burman (PB) experimental design (Table II). Before starting each batch, the groundwater was filled into the reactor and purged with nitrogen gas for approximately 30 min to remove oxygen. All electrode transfers, inoculation procedures, and groundwater replacement were conducted in an anaerobic glove box (Coy Laboratory Products, Inc, Grass Lake, MI). During operation, no precautions were taken to maintain anaerobic conditions in the glass reactor. All experiments were carried out in duplicate.

PB Experimental Design and Statistical Analysis

Plackett–Burman (PB) design was employed to screen out the important operation parameters with respect to their main effects (Zhao et al., 2009). For this purpose, seven operation parameters were chosen as follows: (X_1) temperature, (X_2) pH, (X_3) airflow rate, (X_4) stirring rate, (X_5) nitrate, (X_6) sulfate, and (X_7) conductivity. Twelve trials were generated by the software Design Expert 7.0 (Table II). Additionally, another batch without substrate addition was used as control. All the trials were conducted in triplicate. The average measurement from the triplicates was used as responded value. The experimental results analysis and related model selection were performed as previously reported (Zhao et al., 2009). According to Student's *t*-test for ANOVA, *P*-values <0.0500 were considered to have significant effect on the response, while values >0.1000 indicate the variables are not significant. If the effect was negative, it indicates that the required value for increase of current density was equal or less than the low value in the PB design. If the effect was positive, it indicates that the required value for increase of current density was equal or greater than the high value in the PB design.

Table II. Plackett–Burman design matrix for seven variables of current generation conditions and related response.

Trial	X_1 (temperature, °C)	X_2 (pH)	X_3 (airflow rate, mL/min)	X_4 (stirring rate, rpm)	X_5 (nitrate, mg/L)	X_6 (sulfate, mg/L)	X_7 (conductivity, ms/cm)	Current density[a] ± SD[b], mA/m^2	Current density[c] ± SD, mA/m^2
1	30	5.5	10	500	50	10	2.5	178 ± 2	7.8 ± 0.5
2	15	7	5	500	50	10	5	172 ± 5	7.5 ± 0.3
3	30	7	5	250	10	50	2.5	242 ± 3	12.0 ± 0.5
4	15	5.5	10	250	50	50	2.5	116 ± 2	5.8 ± 0.3
5	15	5.5	5	500	10	50	5	127 ± 3	6.3 ± 0.1
6	15	7	10	500	10	10	2.5	159 ± 2	7.2 ± 0.3
7	30	7	10	250	10	10	5	265 ± 1	13.4 ± 0.2
8	15	5.5	5	250	10	10	2.5	119 ± 3	6.0 ± 0.1
9	15	7	10	250	50	50	5	213 ± 4	10.5 ± 0.3
10	30	5.5	10	500	10	50	5	222 ± 3	11.0 ± 0.4
11	30	7	5	500	50	50	2.5	230 ± 2	11.5 ± 0.5
12	30	5.5	5	250	50	10	5	196 ± 3	9.7 ± 0.3

[a]Response value from the observed average current density of SUMFC operated with biofilm-colonized anode, 300 mg-BOD/L was used for the test.
[cb]SD represents standard deviations from a triplicate experiment.
[c]Response value from the observed average current density of SUMFC operated with fresh electrode, active microorganisms concentration used for the test was 6.52 nmol-ATP/L.

Analytical Methods

Standard method APHA (American Public Health Association, 1998) was employed to analyze chemical oxygen demand (COD) and BOD. Conductivity was determined using a CDM 83 conductivity meter (Radiometer), and pH was measured with a PHM 210 pH meter (Radiometer). Nitrate and sulfate were quantified by ion chromatography (Dionex DX-300 fitted with an AS-9-HC column and conductivity detector, Dionex Co., Sunnyvale, CA). ATP was measured using Lumin (ATE)/Lumin (EX) reagents (Celsis No. 92687) and a luminometer (advance coupe, Celsis, Landgraaf, the Netherlands). ATP content was calculated from an ATP standard curve prepared from ATP standard salt (Celsis No. 92521) dissolved in Lumin (PM) buffer (Celsis No. 92588) and diluted in sterile tap water (Ignar et al., 1998).

The voltage (V) across an external resistor (1,000 Ω) in the MFC circuit was monitored with 0.5 h intervals using a multimeter (Model 2700, Keithley Instruments, Inc., Cleveland, OH) linked to a differential multiplexer (Model 7701, Keithley Instruments, Inc.). The current intensity (I) was calculated according to the Ohm's law, $I = V/R$, where V is the voltage and R (1,000 Ω) is the resistance. Current density (J) was calculated as $J = I/A$, where A is the projected surface area of the anode electrode.

Results and Discussion

The Response of the SUMFC Sensor Equipped With Biofilm-Colonized Anode to Different BOD Concentrations in Artificial Groundwater

The SUMFC sensor equipped with biofilm-colonized anode was tested with artificial groundwater containing different BOD content (10–250 mg-BOD/L). As shown in Figure 2A, the current density increased stepwise as the BOD concentration increased from 10 to 250 mg/L in the acetate (or glucose) modified artificial groundwater. A linear relationship, between current density and BOD was observed (Fig. 2B). The current density increased linearly with BOD concentration up to 250 mg/L in the acetate (to ~233 ± 1 mA/m^2) and glucose (to ~221 ± 1 mA/m^2) amended artificial groundwater, respectively. However, the increase was not significant when the BOD concentration of acetate (or glucose) higher than 250 mg/L, which suggests that the sensor was nearly saturated at this concentration of the fuel. Current density similar to background was observed for tap water without external BOD addition (≤0.1 mA/m^2, data not shown). In addition, the SUMFC sensor was further tested with real wastewater that was filtrated by 0.45-μm membrane filter and diluted with tap water at BOD concentration of 10–300 mg/L. Similar result was observed as in the acetate or glucose modified groundwater (Fig. 2), which further confirmed the applicability of SUMFC sensor equipped with biofilm anode for BOD monitoring. The similar current generation of SUMFC sensor, with artificial wastewater spiked with acetate (or glucose) and the real wastewater, was due to the ubiquitous of easily biodegradable substances, such as acetate and other decomposition intermediates in anaerobic subsurface environments (Lovley and Chapelle, 1995; Williams et al., 2010). The current generation and BOD detection range (~250 mg/L) obtained here were higher than that in most of previous studies (Chang et al., 2004; Kim et al., 2009; Kumlanghan et al., 2007; Williams et al., 2010), which might be due to the reduction of ohmic loss in current compact configuration. Additionally, it was found that the response time of the sensor was increasing with BOD. The response time for BOD values below 100 mg/L was <0.17 h, while it was nearly 0.67 h for BOD concentrations higher than 150 mg/L (Fig. 2A). This observation was consistent with previous studies (Kim et al., 2003; Moon

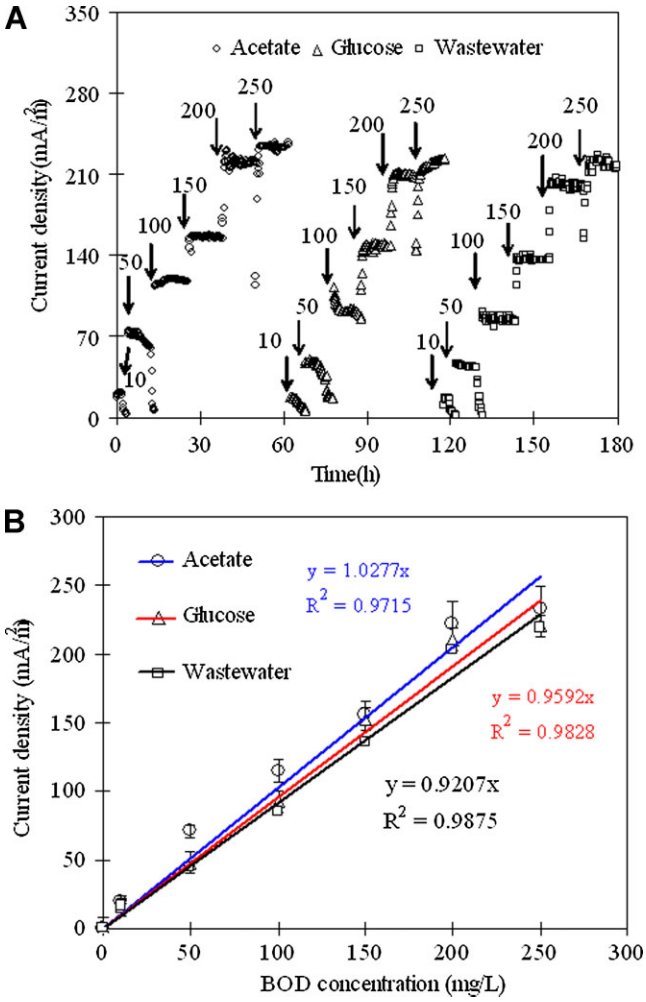

Figure 2. Current generation of the SUMFC sensor equipped with biofilm-colonized anode in the artificial groundwater containing different BOD concentrations. **A**: Time course of current generation. **B**: Correlation between current density and BOD concentration. Arrows indicate the points of reactor batch feeding, and the numbers indicate the BOD concentration (mg/L) fed into reactor. (Operation temperature, 25°C; stirring rate, 250 rpm; pH, 7.0; conductivity, 2.5 ms/cm; microbial activity in groundwater, 0 nmol-ATP/L.). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

et al., 2006). The increase of response time with BOD concentration might be due to substrate diffusion limitation to the biofilm or temporary carbon storage inside the bacterial cells during initial high substrate concentrations (Freguia et al., 2007; Tront et al., 2008b). The above results confirm the importance of biofilm colonized on the surface of anode for BOD monitoring. Several repetitions of the BOD experiments over 5 months in the laboratory showed a good reproducibility. The unique submersible configuration makes SUMFC sensor more applicable than other type of BOD sensors in subsurface environments. Abrevaya et al. (2010) reported a cylindrical reactor, without anode chamber, for extraterrestrial microbial life detection. Ferricyanide was used as electron acceptor in their device. Meanwhile, ferricyanide is a toxic compound, which could limit the device's practical application as a subsurface sensor. Further optimization of system design parameters, such as reactor size, electrode material and surface area, microbial efficiency, mass transfer, or cathode design could further enhance SUMFC sensor performance (Tront et al., 2008a). A mini-MFC design with reticulated vitreous carbon electrodes and flow tubing was reported by Biffinger et al. (2007). Although it is a two-chamber system, its miniature architecture provides a new perspective for future sensor design.

The Response of the SUMFC Sensor Equipped With Biofilm-Colonized Anode to Different Microbial Activity in Artificial Groundwater

ATP is an energy carrying molecule occurring in all living cells, and is generally used as a proxy for total content of living microorganisms (Delahaye et al., 2003; Magic-Knezev and van der Kooij, 2004). Thus, ATP is an excellent indicator for the presence of microbial activity. To evaluate the impact of the anodic biofilm on microbial activity monitoring, the biofilm-colonized sensor was submersed in the artificial groundwater (300 mg-BOD/L), which was inculcated with wastewater bacteria to reach different active miroorganism concentrations (0–13.04 nmol-ATP/L). The corresponding current generation was monitored and shown in Figure 3. However, no correlation between current and microbial activity was observed; current density of 242 ± 5 mA/m^2 was produced regardless of amount of active microorganisms in the groundwater (e.g., 0, 0.02, 13.04 nmol-ATP/L) (Fig. 3). The control (without organic matter addition) did not generate any electricity. The indifferent response of biofilm-colonized anode to the different microbial activity in the

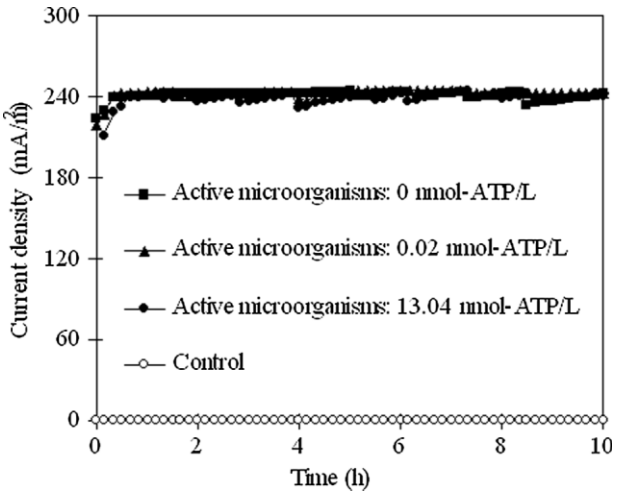

Figure 3. Current generation of the sensor equipped with biofilm-colonized anode in the artificial groundwater containing different active microorganism concentrations. Control: no substrate addition. (Operation temperature, 25°C; stirring rate, 250 rpm; pH, 7.0; conductivity, 2.5 ms/cm; BOD, 300 mg/L.)

groundwater, observed above, indicated that the microbial communities in the biofilm were adequate for electricity generation, thereby overshadowing the possible exoelectrogenic activity of the microorganisms in the groundwater. The results suggest that using of biofilm-colonized anode in the sensor could somehow detect the activity of the biofilm, but it was not suitable for monitoring the microbial activity in the groundwater.

The Response of the SUMFC Sensor Equipped With Fresh Anode to Different Microbial Activity in Artificial Groundwater

To evaluate the role of the fresh electrode for microbial activity monitoring, the biofilm-colonized anode was changed to the fresh electrode in the sensor. The sensor was submersed in the artificial groundwater, which was also inoculated with wastewater bacteria to form different active microorganism concentrations (0–13.04 nmol-ATP/L). As shown in Figure 4A, the current was produced immediately and increased stepwise as the active microorganisms concentration increased from 0.02 to 13.04 nmol-ATP/L (300 mg-BOD/L was applied). The autoclaved abiotic and substrate controls (0 mg-BOD/L) did not generate any electricity (Fig. 4A). A corresponding linear relationship between current density and active microorganisms concentration was established as shown in Figure 4B. There were two linearity ranges, the current density first increased linearly with active microorganisms concentration from 0 to 0.13 nmol-ATP/L (0.6 ± 0.1 to 1.86 ± 0.1 mA/m^2, $R^2 = 0.984$), and then from 0.13 to 6.52 nmol-ATP/L (1.86 ± 0.1 to 12.4 ± 0.1 mA/m^2, $R^2 = 0.97$). However, the relationship ceased to be linear when the active microorganisms concentration was higher than 6.52 nmol-ATP/L, which suggested the system was nearly saturated. The results above indicated that the current generated by fresh anode was depending on the amount of living cells in the groundwater. The microbial ATP concentration, rather than the biomass concentration, was used here as an expression of active microorganisms concentration. ATP is considered to be suitable parameter for estimation of microbial activity, due to its linear relationship with the microbial cell mass (Horiuchi et al., 2003; Lee et al., 2006; Oades and Jenkinson, 1979). Additionally, measuring ATP is much less time and labor intensive, compared to dry cell biomass weight determination (Lee et al., 2006). Moreover, ATP content is correlating only with living cells, while biomass weight determination does not distinguish between living and dead cells. When 100 mg/L of 2.4-dinitrophenol (DNP), which is a microbial activity inhibitor was added into the artificial groundwater containing active microorganisms concentration of 13.04 nmol-ATP/L as a control, no current density was generated (Fig. 4A). The result further confirmed the correlation between the current production from the fresh anode and microbial activity in the artificial groundwater.

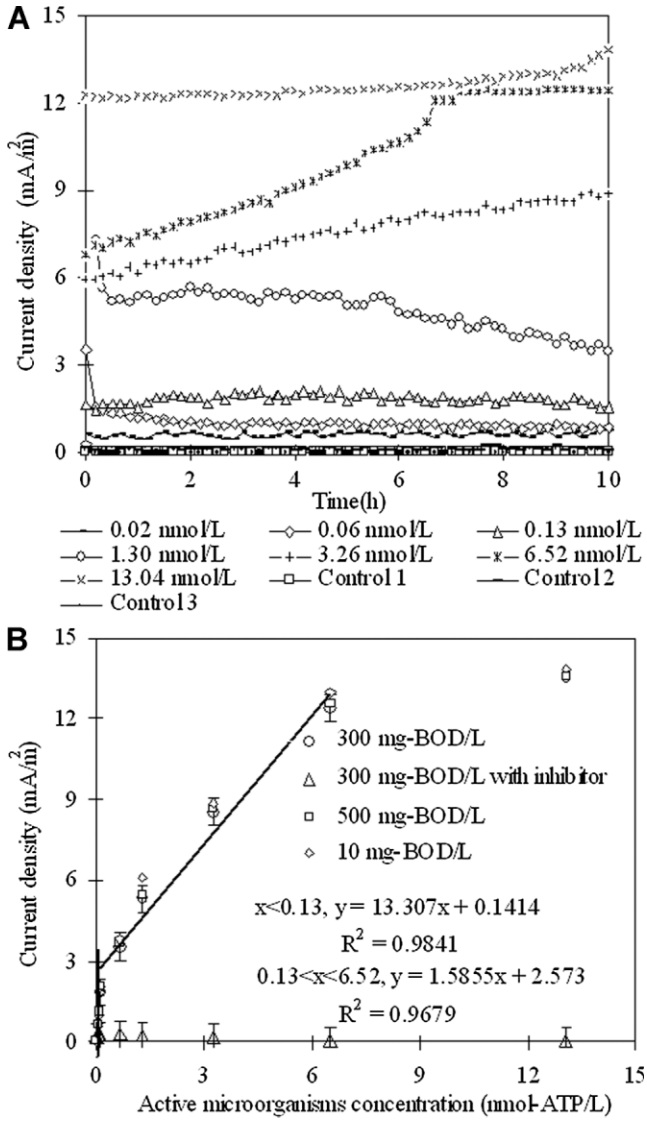

Figure 4. Current generation of the sensor equipped with fresh anode in the artificial groundwater. **A**: Time course of current generation with different active microorganism concentrations. **B**: Correlation between current density and active microorganism concentrations at different BOD levels. Control 1: an autoclaved abiotic control; Control 2: inhibitor addition; Control 3: without substrate addition. (Operation temperature, 25°C; stirring rate, 250 rpm; pH, 7.0; conductivity, 2.5 ms/cm.)

The relationship between the current density generated by the fresh anode and active microorganisms concentration (expressed in microbial ATP concentration) was also investigated at different substrate concentrations (10, 300, or 500 mg-BOD/L). No significant differences were observed (Fig. 4B). This was because, when the fresh electrode was used as anode, the microbial activity was the limiting factor for current generation, thus not disclosing the differences of BOD content. The result suggests that using of the fresh anode in the sensor could diminish the influence of substrate concentration to the microbial activity monitoring, which was occurring in previously studies (Kumlanghan et al.,

2007; Tront et al., 2008a). Importantly, the microbial activity detection range (~6.52 nmol/L of microbial ATP) obtained in this study covered the microbial ATP concentration contained in natural groundwater (Hammes et al., 2010), suggesting the sensor applicability. Moreover, as the biofilm formation is a long-term process, which normally takes 1–2 months (Zhang et al., 2009), the fresh anode could keep long-term accuracy without cleaning. The long-term stability of fresh anode is the subject of further study.

Effect of Operation Parameters on the Performance of SUMFC Sensor

To further study the effect of operation parameters on the performance of the sensor, PB design was employed and the results are summarized in Tables II and III. Increasing of stirring rate and nitrate concentration more than 250 rpm and 10 mg/L, respectively, negatively affected current density, regardless type of anode, while oppositely high temperature (up to 30°C), initial pH (up to 7), and conductivity (up to 5 ms/cm) showed significantly positive effect (Table III). It is worth noting that the stirring rate showed significantly negative effect on the current density generated by fresh anode, while the current density generated by biofilm-colonized anode was not significantly affected. The above opposite behavior could be explained by the negative effect of stirring on the attachment of electrochemically active bacteria on the fresh electrode. Stirring was probably disturbing the approach of exoelectrogenic microorganisms to the anode for transferring their electrons to the anode, when a fresh electrode was applied, while when a biofilm electrode was applied, microorganisms were already strongly attached on the electrode and could tolerate high shear forces (Pham et al., 2008). Nitrate is a prevalent electron acceptor in aquifer environments (Chang et al., 2005). Presence of nitrate could have caused inhibition of the current generation, due to consumption of electrons for reduction of nitrate, and thereby showed negative effect in this study. Thus, the combination of high level of temperature (≥30°C), pH (≥7) and conductivity (≥5 ms/cm) and low level of nitrate (≤10 mg/L) and stirring rate (≤250 rpm), were favorable for higher current generation, which might therefore improve the sensitivity of SUMFC sensor.

The effect of inorganic solid content on the performance of the SUMFC sensor was further studied (Fig. 5). The current density generated by the biofilm-colonized electrode decreased gradually with inorganic solid content from 0 (242 ± 5 mA/m^2) to 0.75 kg/L (142 ± 2 mA/m^2) in artificial groundwater (acetate, 300 mg-BOD/L). Similarly, the current density produced by the fresh electrode decreased sharply with inorganic solid content from 0 (12.4 ± 0.1 mA/m^2) to 0.5 kg/L (2.3 ± 0.2 mA/m^2). The decrease of current with increasing inorganic solid content could be explained by the inefficient transportation of protons due to physical hindrance, when the inorganic solid content was increased (Kumlanghan et al., 2007). These results underline that the SUMFC sensor needs calibration by taking environmental conditions into account before applying in real samples.

Performance of the SUMFC Sensor in Real Contaminated Groundwater

To further verify the applicability of the SUMFC sensor, four real groundwater samples (well 1–4) taken from contaminated ZEITZ site were used. The fresh anode was initially used in SUMFC sensor for microbial activity detection, after that, it was replaced by the biofilm-colonized anode for BOD detection. An example of current generation with the real sample (well 1) is shown in Figure 6. Current density (approximately 0.44 mA/m^2) was generated immediately after the sensor was submerged in the contaminated groundwater (Fig. 6). The current density increased gradually with operation time and reached its highest level

Table III. Estimated effect and confidence level in PB design for current generation in SUMFC sensor.

	BOD monitoring (biofilm-colonized anode)			Microbial activity monitoring (fresh anode)		
Variables	Effect	*P*-value	Significance	Effect	*P*-value	Significance
Temperature	71,07	0.0003	+[a]	3,68	0.0005	+
pH	53,80	0.0008	+	2,58	0.0020	+
Airflow rate	11.00	0.1363	N[b]	0,45	0.2788	N
Stirring rate	−10,77	0.1427	N	−1,02	0.0474	+
Nitrate	−4,73	0.4682	N	−0,52	0.2239	N
Sulfate	9,90	0.1693	N	0,92	0.0633	N
Conductivity	25,20	0.0130	+	1,35	0.0198	+

[a]Positively significant.
[b]Not significant.

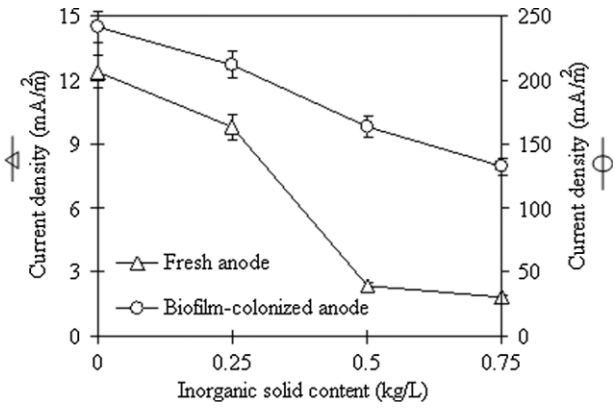

Figure 5. Effect of inorganic solid content on the current density of SUMFC sensor in the artificial groundwater. (Operation temperature, 25°C; stirring rate, 250 rpm; pH, 7.0; conductivity, 2.5 ms/cm; acetate, 300 mg-BOD/L; microbial activity in groundwater, 6.52 nmol-ATP/L.)

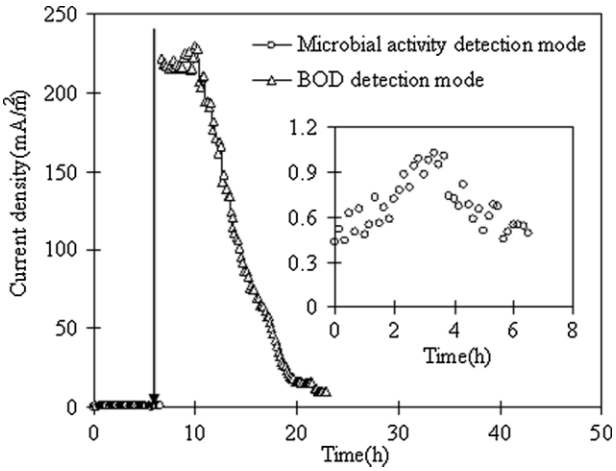

Figure 6. Current generation of the SUMFC sensor in the real contaminated groundwater. Arrows indicated the points of changing the fresh anode to the biofilm-colonized electrode. (Operation temperature, 25°C; stirring rate, 250 rpm; pH, 8.0; conductivity, 980 ± 10 μs/cm.)

(0.99 ± 0.01 mA/m^2) after 3 h. According to the linear relationship established in Figure 4B, the microbial activity in the real groundwater was estimated to a microbial ATP level of 0.064 ± 0.006 nmol/L, which was close to 0.081 ± 0.005 nmol/L of ATP measured by standard method (Ignar et al., 1998). After switching to the biofilm-colonized anode, a stable current density (222 ± 1 mA/m^2) was produced immediately ($\leq$0.08 h), resulting in a total response time <3.1 h for both microbial activity and BOD monitoring (Fig. 6). The BOD calculated according to the acetate and glucose curve (Fig. 2B) was equal to 216 ± 5 and 231 ± 3 mg/L, respectively, which was closed to 251 ± 10 mg/L tested by the standard method (APHA, 1998). The current generations with other samples were showed the same trends, and the results were summarized in Table I. The deviations between the ATP concentration measured by the sensor and standard method (Ignar et al., 1998) were ranging from 15% to 22%, while the deviations between the BOD measured by the sensor and APHA method were ranging from 6% to 16% for these four real samples. Repeated BOD measurements by the sensor showed standard deviations from ±2% to ±6%, which are lower than the deviation from conventional BOD 5-day tests, where deviation of ±15% is allowed (Kumlanghan et al., 2007). The deviations of sensor estimations of BOD and microbial activity, compared to the standard method, might have been due to the influence of some characteristics of the real groundwater (e.g., pH, conductivity, electron acceptors, and microbial community composition) (Chang et al., 2005; Kumlanghan et al., 2007). However, the deviations were within the acceptable levels of such analyses. The current sensor configuration permitted to switch the anode electrode easily, which could fulfill the requirement for in situ application.

Conclusion

This study demonstrates that choice of fresh or biofilm-colonized anode was a key point for the application of SUMFC sensor. The sensor equipped with fresh anode was excellent for monitoring microbial activity, whereas, the sensor equipped with biofilm-colonized anode was appropriate for BOD measurement. By changing the anode in the same sensor, BOD of up to 250 mg/L and active microorganisms concentration of up to 6.52 nmol-ATP/L could be measured based on a linear relation with current density, respectively. Operation conditions such as temperature, initial pH, and conductivity affected the sensitivity of the sensor. The applicability of SUMFC sensor was verified with real groundwater samples. Microbial activity (expressed as microbial ATP concentration) and BOD were determined accurately in <3.1 h, with deviations ranging from 15% to 22% and 6% to 16% when compared with standard methods, respectively. The simple, compact SUMFC sensor showed promising potential for direct, inexpensive, and rapid monitoring of biodegradable organic matter and biological respiration activity in groundwater.

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