A novel bioelectrochemical BOD sensor operating with voltage input

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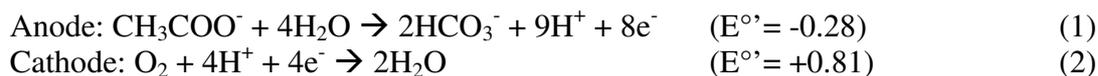
Abstract
Biochemical oxygen demand (BOD) is a measure of biodegradable compounds in water and is, for example, a common parameter to design and assess the performance of wastewater treatment plants. The conventional method to measure BOD is time consuming (5 or 7 days) and requires trained personnel. Bioelectrochemical BOD sensors designed as microbial fuel cells (MFCs), which are systems where bacteria convert organic matter into an electrical current, have emerged as an alternative to the conventional technique. In this study, a new type of bioelectrochemical BOD sensor with features that overcome some of the limitations of current MFC-type designs was developed: (1) An external voltage was applied to overcome internal resistances and allow bacteria to generate current at their full capacity, and (2) the ion exchange membrane was omitted to avoid pH shifts that would otherwise limit the applicability of the sensor for wastewaters with low alkalinity. The sensor was calibrated with an aerated nutrient medium containing acetate as the BOD source. Linear correlation (R^2=0.97) with charge was obtained for BOD concentrations ranging from 32 to 1280 mg/L in a reaction time of 20 h. Lowering the reaction time to 5 h resulted in lowering the measurable BOD concentration range to 320 mg/L (R^2=0.99). Propionate, glucose, and ethanol could also be analyzed by the sensor that was acclimated to acetate. The study demonstrates a way to design more robust and simple bioelectrochemical BOD sensors that do not suffer from the usual limitations of MFCs (high internal resistance and pH shifts).

1. Introduction
Biochemical oxygen demand (BOD) is a measure of the content of biodegradable organic matter in water. BOD is conventionally determined by measuring the dissolved oxygen (DO) concentration of a water sample before and after a 5 or 7 day incubation at 20°C (Greenberg et al. 1992). Thus, the test is time consuming and it also requires skilled personnel to obtain reproducible results. Alternative techniques have been investigated aiming for a more rapid, accurate, and simple BOD test. Several researchers have constructed biosensors based on DO probes and the use of immobilized microorganisms as the biological recognition element (Liu and Mattiasson 2002). Such biosensors generally give good correlation between signal and BOD concentration, but suffer from poor operational stability and typically have a low measurement range.

Microbial fuel cells (MFCs), which have shown operational stability of over five years, have emerged as a more robust alternative to determine BOD (Chang et al. 2004, Kim et al. 2003a). The MFC technology is based on the ability of a wide range of naturally occurring microorganisms to oxidize organics and use a solid-state electrode as electron acceptor. A MFC consists of two electrodes, an anode where organics (e.g. acetate) are oxidized and a cathode where e.g. oxygen is reduced to water. The reactions and their standard redox potentials at pH 7 can be expressed as Equations 1 and 2:

Anode: $CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$ (E°'= -0.28)   (1)

Cathode: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ (E°'= +0.81)   (2)

Electrons flow from the anode to the cathode through an external circuit while ions migrate through the liquid to maintain charge balance. Since the overall reaction, i.e. oxidation of organics and reduction of oxygen, is thermodynamically favorable, electrical energy can be recovered from the external circuit. Typically a resistor is connected between the anode and cathode. By measuring the voltage across the resistor, the current flowing in the system can be calculated using Ohm's law. The current or charge transferred in the MFC can be correlated with BOD concentration.

Two-compartment MFCs with anode and cathode separated by a Nafion cation exchange membrane have been investigated as BOD sensors in several studies. The water to be analyzed is fed to the anode chamber where a biofilm on the anode surface converts organics into an electric current. An aerated buffer solution is fed to the cathode chamber where oxygen is reduced to water on the cathode surface (Chang et al. 2004, Kim et al. 2003a, Kim et al. 2003b, Moon et al. 2004). Kumlanghan et al. (2007) used a similar MFC setup, but instead of enriching a bioelectrochemically active culture on the anode, a stable anaerobic microbial consortium was maintained in a separate reactor, which was fed to the MFC for each analysis. The idea was that a fresh inoculum for each analysis would generate a faster response than a biofilm culture growing on the anode surface (Kumlanghan et al. 2007). An operationally simpler MFC-based was developed by Di Lorenzo et al. (2009). Instead of using an aerated solution fed to a cathode chamber, a gas-diffusion cathode was pressed against a cation exchange membrane. Oxygen reached the cathode by passive diffusion from air and no catholyte pumping was required (Di Lorenzo et al. 2009a, Di Lorenzo et al. 2009b). Peixoto et al. (2011) investigated a submersible MFC (Min and Angelidaki 2008) as BOD sensor. This design avoids pumping of the anolyte; however, air must be continuously sparged to the cathode (Peixoto et al. 2011).

In a MFC-based BOD sensor, the BOD concentration can be correlated with either charge or current. Charge represents the amount of substrate converted into current and is obtained by integrating the current over time. Correlation between charge and BOD concentration can be obtained by batchwise feeding the sample to the anode, measuring the resulting current peak, and calculating the amount of charge passed through the external circuit for the duration of the current peak. The current measures the rate of oxidation of organic matter by microbes on the anode (i.e. charge per unit time). Theoretically, a high BOD concentration would lead to a higher current. However, this is only true to a certain extent. It is well known that microbial substrate utilization rate reaches a saturation level with increasing substrate concentration in accordance with Monod growth kinetics. Thus, the correlation between current (i.e. substrate utilization rate) and BOD concentration is only linear at low BOD concentration.

In a previous study, charge was correlated with BOD concentration up to 520 mg/L in a two-chambered system; however, a linear correlation could be observed only up to 206 mg/L BOD (Kim et al. 2003a). It was stated that the low coulombic yield at the high concentration was caused by the poor buffering capacity of the wastewater. Today, it is well known that the use of ion exchange membranes to separate anode and cathode in MFCs and other bioelectrochemical systems leads to a pH gradient between the two chambers (Harnisch and Schröder 2009). As shown in Equations 1 and 2 above, protons are liberated by the anode reaction and consumed by the cathode reaction. Thus, to maintain pH balance in a MFC, the flow of electrons must be accompanied by an equal flow of protons or hydroxide ions through

the ion exchange membrane. However, in wastewater and the nutrient media typically used in MFC studies, other ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+ are present in much higher concentrations than H^+. When a cation exchange membrane is used, migration between the chambers will therefore be accomplished mostly by these other ions, causing the pH to rise in the catholyte and drop in the anolyte (Rozendal et al. 2006). A low anolyte pH will inhibit the bioelectrochemical activity, which means that the alkalinity rather than the BOD concentration, may determine the current generated in a MFC.

Several studies have correlated current with BOD concentration. Chang et al. (2004) observed linear correlation between current and BOD concentration up to 100 mg/L. Higher concentration could be measured by model fitting or by lowering the flow rate through the sensor (Chang et al. 2004). A low flow rate increases the hydraulic residence time in the sensor which results in bringing the steady-state BOD concentration down to the linear range. However, an increased hydraulic retention time would also increase the response time of the BOD sensor.

In this study, we demonstrate a novel type of bioelectrochemical BOD sensor, which addresses some of the limitations of previous MFC-type designs. The sensor has the following features:

- No ion exchange membrane: By omitting the ion exchange membrane between the anode and cathode, the sensor does not suffer from decreasing anolyte pH, which in conventional designs can be detrimental to the biological activity at the anode.
- Batchwise feeding: The transferred charge is correlated with BOD concentration. Thus, microbial kinetics does not limit the range of the sensor, which would be the case if current was correlated with BOD concentration.
- Input voltage: Previous bioelectrochemical BOD sensors have operated as MFCs with a *resistor* connected between anode and cathode. In this sensor, an *external voltage* is applied to boost the current generation in the system. The current generated in MFCs is often limited by factors such as internal resistance and catalysis of oxygen reduction at the cathode. By input of an external voltage, the microorganisms at the anode are allowed to generate current at their full capacity.

As discussed in the introduction, several MFC-based BOD sensors have been developed that allow measurements of BOD concentration in significantly shorter time than the conventional technique; however, these sensors are often not capable of accurately measuring high BOD concentrations. A sensor with the special features we describe above could potentially be useful for a wider BOD concentration range. In this paper, we demonstrate the new type of bioelectrochemical BOD sensor using an acetate-containing nutrient medium as feed solution. We also investigate the response of the sensor when the BOD source is changed from acetate to other organic compounds.

2. Materials and methods

2.1 Experimental design
A bioelectrochemical reactor was constructed and the experiment was carried out in four steps: (1) The reactor was incubated with acetate as carbon source until a stable bioelectrochemical activity could be observed, (2) the reactor was characterized electrochemically and the appropriate operating conditions were determined, (3) the use of the reactor as a BOD sensor was demonstrated by correlating transferred charge with acetate

BOD concentration, and (4) the response of the sensor, which was acclimatized to acetate, was investigated for organic substrates including propionate, glucose, and ethanol.

2.2 Bioelectrochemical reactor

The bioelectrochemical reactor was 9 cm long, 0.7 cm wide, and 2 cm deep. It had two openings for influent and effluent liquid, and one opening for a reference electrode. The reactor contained a 9-cm long, 0.615-cm diameter graphite rod anode (Alfa Aesar). A gas diffusion cathode closed one of the 9×0.7 cm^2 sides of the reactor chamber. The other five sides of the rectangular reactor chamber were closed by clear acrylic plastic. The cathode was made of carbon fiber paper (Avcarb) coated with carbon nanoparticles (Cabot Black Pearls 2000) and 30% polytetrafluoroethylene (PTFE) on the liquid-facing side. The air-facing side was coated with a 40% PTFE solution containing 200 mesh graphite powder (Alfa Aesar) to prevent liquid leakage from the chamber. A Whatman filter with a pore size of 6 µm was pressed against the liquid-facing side of the cathode. The liquid volume of the reactor was 11.8 mL. A schematic of the reactor is shown in Figure 1.
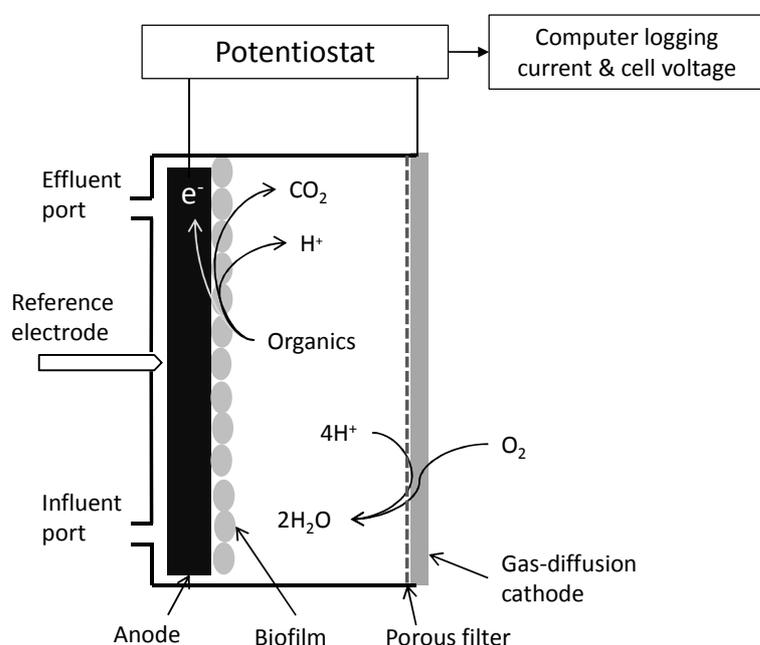

Figure 1. Schematic of bioelectrochemical reactor used as BOD sensor.

2.3 Operation

A nutrient medium was supplied to the reactor. The nutrient medium consisted of (in mg/L): 2925 NaCl, 100 MgSO$_4$•7H$_2$O, 100 CaCl$_2$•2H$_2$O, 100 NH$_4$Cl, 3879 KH$_2$PO$_4$, 12455 K$_2$HPO$_4$, 2 FeCl$_2$•4H$_2$O, 0.05 HBO$_3$, 0.05 ZnCl$_2$, 0.03 CuSO$_4$, 0.5 MnCl$_2$•4H$_2$O, 0.05 (NH$_4$)Mo$_7$O$_{24}$, 0.05 AlCl$_3$, 0.05 CoCl$_2$, 0.05 NiCl$_2$, 0.1 Na$_2$SeO$_3$, and 0.05 Na$_2$WO$_4$•2H$_2$O. Unless otherwise specified, the carbon source was 20 mM of sodium acetate.

The experiment, which was conducted for a total of 163 days, can be divided into six phases:
1. The reactor was inoculated with sludge from a wastewater treatment plant. A mixture of aerobic and anaerobic sludge was used to obtain a microbially diverse inoculum. During the first 22 days, anaerobic nutrient medium was circulated through the reactor chamber. The cell voltage was initially controlled at 0 V (day 0-14), then a voltage of 0.1 V was applied to the reactor.

2. From day 23 and onwards, nutrient medium was fed batchwise to the reactor chamber. The reaction time for each batch was initially 40 h (until day 61) but was later shortened to 20 h. For each batch run, the cell voltage was controlled at 0 V, 0.5 V, or 0.8 V (positive value means that a voltage was applied to the reactor).
3. Between day 81 and 97 the reactor was not in operation to investigate how it would respond to an inactive period. Operation resumed on day 98.
4. Between day 105 and 112, the reactor was characterized electrochemically by varying the cell voltage from open-circuit up to 0.8 V and measuring the resulting current, anode- and cathode potentials.
5. Between day 113 and 130, the response of the reactor was calibrated with acetate concentration.
6. Between day 131 and 163, the response of the reactor was investigated when the carbon source was exchanged from acetate to propionate, glucose, or ethanol.

2.4 Analytical methods
A Gamry series G750 potentiostat was used to control the cell voltage of the reactor and record voltages and currents. Electrode potentials were measured against Ag/AgCl reference electrodes (Bas Inc.); however, in the paper the potentials are given against the Standard Hydrogen Electrode (SHE) with an assumed offset of 0.20 V. Nutrient medium with specific BOD concentrations were prepared using an analytical balance with an accuracy of ±0.1 mg.

2.5 Calculation
The coulombic efficiency is the fraction of the theoretical amount of charge equivalents in the substrate fed to the reactor that is captured as an electrical current. It is calculated according to Equation 3 below:

$$Coulombic\ efficiency = (100\%) \times \frac{8 \times \int_0^t I dt}{(BOD) \times V \times F} \tag{3}$$

where I is the current (A), t is time (s), BOD is the feed concentration in g/L, V is volume (L), F is Faraday's constant (96485.3 C/mol-electrons), and 8 is a conversion factor (gBOD/mol electrons).

3. Results

3.1 Enrichment
During the initial 22 day enrichment with nutrient medium circulating through the reactor chamber, the current increased from 0 to 0.6 mA with a cell voltage of 0 V (i.e. short-circuit condition), and then further increased up to 1.0 mA with an applied voltage of 0.1 V (Figure 2).
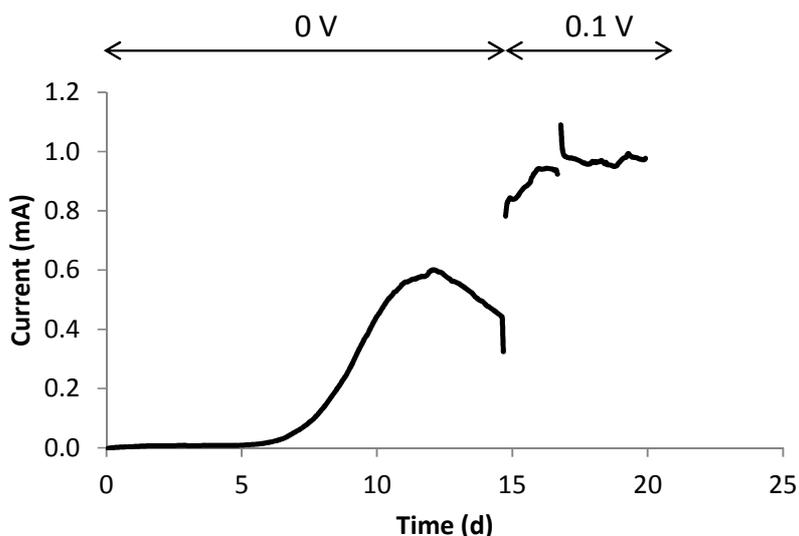
Figure 2. Enrichment of electrochemically active biofilm in the reactor. Nutrient medium was recirculated through the reactor from a 1-L bottle. Initially, the cell voltage was controlled at 0 V (short-circuit); after 14 days a 0.1 V input voltage was applied to the reactor.

The enrichment was continued with batchwise feeding of nutrient solution to the reactor. The charge passed through the external circuit for a specified reaction period of either 40 h or 20 h is shown in Figure 3. Up to day 63, a reaction period of 40 h was used. The total charge passed through the circuit in 40 h increased from about 40 C at the beginning to about 90 C at the end of the enrichment period. A cell voltage of 0.5 V and 0.8 V gave similar results; therefore, incubation at 0.8 V was stopped after 60 days. A cell voltage of 0 V resulted in about 60 C passing through the external circuit. Thus, an applied voltage increased the current in the system. From day 64, a reaction time of 20 h was used. The charge was stable around 70 C at a cell voltage of 0.5 V and 40-50 C at 0 V. Although the reactor was inactive for 17 days between day 81 and 97, the charge quickly reached previous levels once operation resumed.
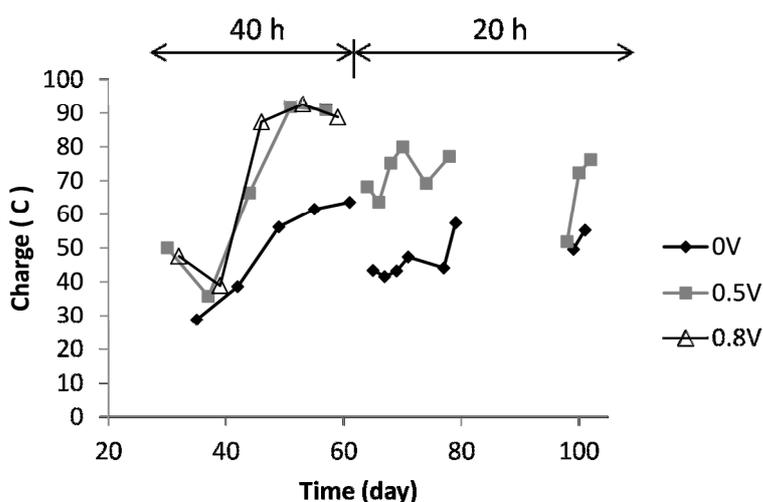

Figure 3. Charge passed through the external circuit for reaction times of 40 or 20 hours. The cell voltage of the reactor was controlled at either 0, 0.5, or 0.8 V.

7

3.2 Electrochemical characterization

During the enrichment period, an applied voltage of 0.5 V resulted in a higher current and more charge transfer than a cell voltage of 0 V (Figure 3). However, an applied voltage of 0.8 V did not improve the performance compared to 0.5 V. To further characterize the reactor and determine an appropriate operating voltage, 20 h batch runs with varying cell voltage ranging from open-circuit conditions up to 0.8 V were carried out (Figure 4). One run was carried out at each applied voltage.
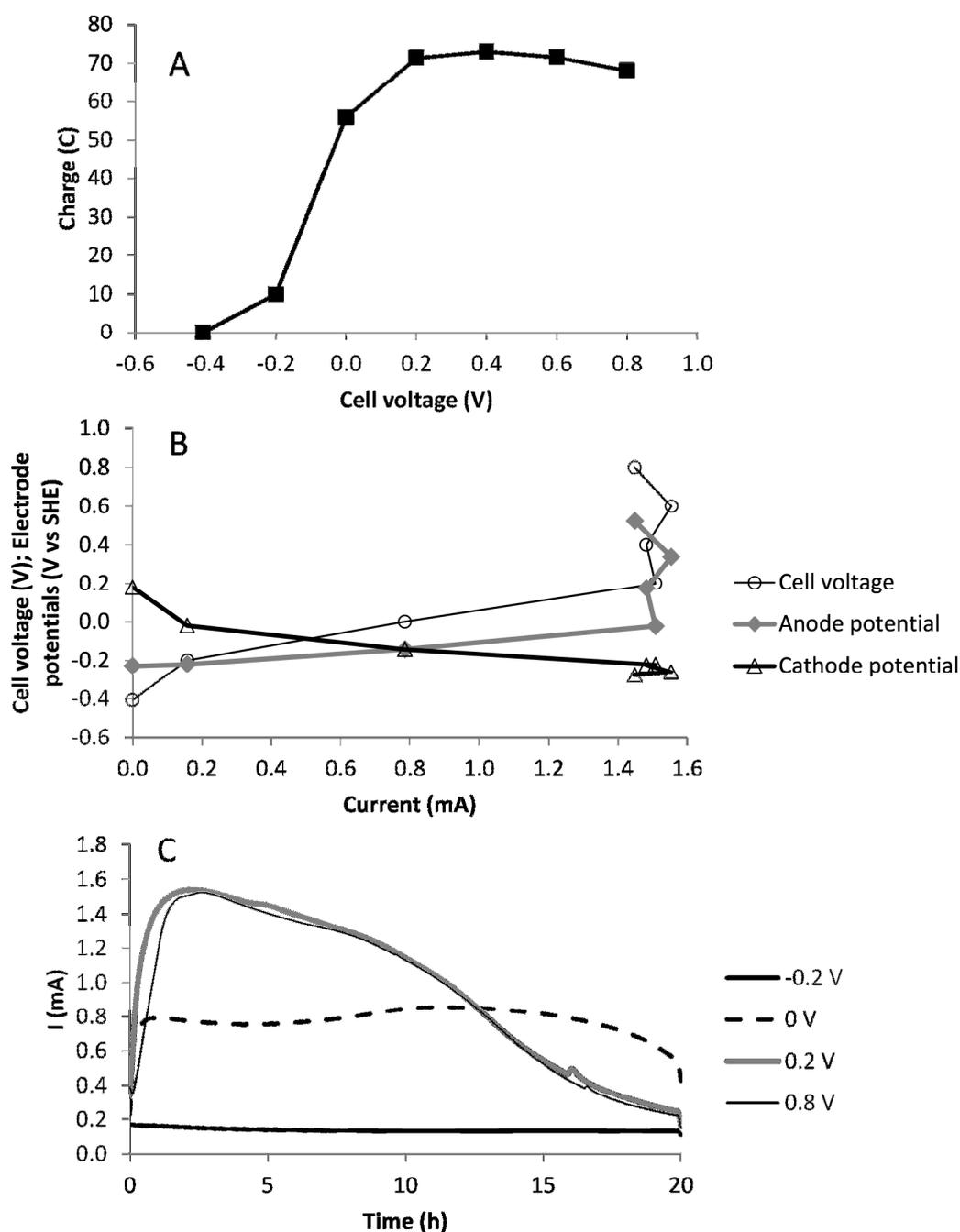

Figure 4. Electrochemical characterization of the reactor. (A) Charge passed through the circuit for 20 h reaction periods with varying cell voltages (positive values mean an external voltage is supplied to the reactor). (B) Cell voltage, anode- and cathode potentials as functions of current. (C) Current profiles with time for four selected cell voltage conditions.

As shown in Figures 4A and 4C, the open circuit voltage was -0.41 V. Increasing the cell voltage up to 0.2 V increased charge transfer in the system. Further increasing the voltage beyond 0.2 V did not increase charge transfer (Figure 4A). The current for a few selected 20 h runs are shown in Figure 4B. Cell voltages of 0.2 and 0.8 V generate similar current profiles. At 0 V, the current was 0.8 mA, which is lower than the current at 0.2 V for the first 15 hours, but higher during the last 5 hours of the run. High current means rapid consumption of organic substrate. Thus, at 0.2 V, the substrate in the reactor in reactor is rapidly consumed, which leads to low current at the end of the run when substrate is depleted. The internal resistance of the reactor was 296 Ω and was calculated from the slope of the cell voltage curve (Figure 4C) between -0.2 V and 0.2 V. Figure 4C shows that at an applied cell voltage of 0.2 V, the anode potential is about 0 V vs SHE. Further increasing the input voltage increases the anode potential, but does not result in higher current. Thus, an input voltage of 0.2 V was chosen for the rest of the experiment.

3.3 Calibration with acetate

The reactor was operated with 0.2 V input voltage for 20 h batch runs. Each batch run was started by feeding nutrient medium with a known concentration of acetate. The nutrient medium was always saturated with air before being fed to the reactor to ensure the same concentration of dissolved oxygen in each sample. The charge transferred through the external circuit could be calculated for different time points during the 20 h runs. The correlations between BOD concentration and transferred charge after 5, 10, 15, and 20 h are shown in Figure 5. Two runs were carried out for each BOD concentration except 0 mg/L (one run) and 1280 mg/L (3 runs). For a reaction time of 20 h, there was a linear correlation (coefficient of determination R^2=0.97) between charge and BOD concentration up to 1280 mg/L. For a reaction time of 15 h, a BOD concentration of up to 1280 mg/L had a linear correlation with a R^2 value of 0.94. Up to 640 mg/L, the linear correlation was better with a R^2 of 0.99. For a reaction time of 10 h, there was a good correlation up 640 mg/L (R^2=0.99). For a reaction time of 5 h, the correlation up to 640 mg/L had a R^2 of 0.97, and up to 320 mg/L the R^2 was 0.99. The results demonstrate that the reactor could be used as a sensor for BOD. They also show that for a higher BOD concentration, a longer reaction time is needed to obtain good correlation between charge and BOD concentration.
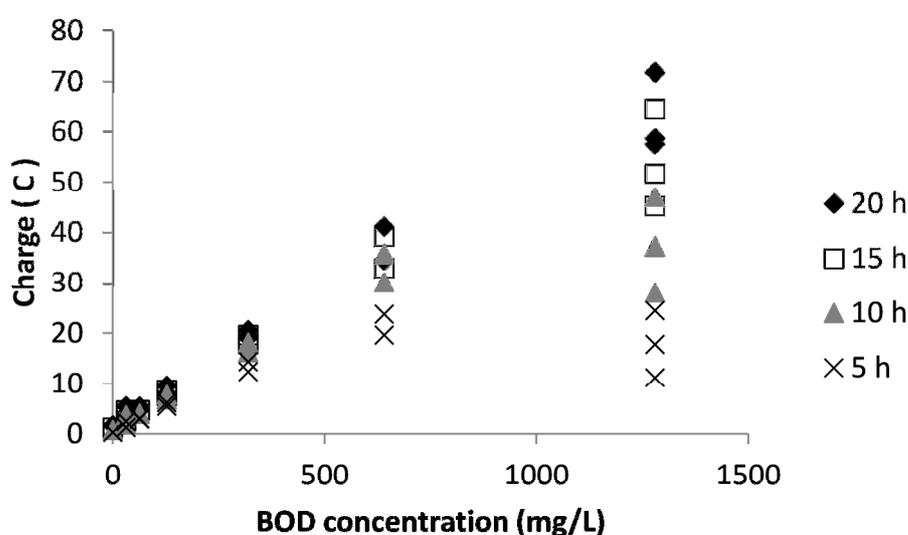

Figure 5. Correlation between charge and acetate BOD concentration for different reaction times.

3.4 Testing other carbon sources

Although the reactor had been acclimated to acetate, the responses for three other organic compounds were tested, viz. propionate, glucose, and ethanol. During this experimental period, runs with acetate and runs with the other three organic compounds were carried out alternatively (i.e. a run with e.g. ethanol was followed by a run with acetate before the next run with a different concentration of ethanol, etc.). Three BOD concentrations were tested for each compound. For propionate, glucose, and ethanol, one run was carried out at each concentration. For acetate, three (at 64 and 320 mg/L) or four (at 640 mg/L) runs were carried out at each concentration. The current generation with propionate, glucose, or ethanol compared to that obtained with acetate is shown in Figure 6. The current is higher with acetate than with any of the other compounds. This is not surprising since the microbial culture was acclimated to acetate. Propionate and ethanol give similar current profiles. Glucose gives a current profile that initially increases slowly, and then reaches a peak after about 5 to 10 hours incubation. The reason may be that glucose is first fermented to simpler compounds, such as hydrogen and acetate (Freguia et al. 2008), which are utilized by the electrochemically active microbes on the anode. For acetate, a high current peak in the beginning of the incubation period can be seen. The current rapidly drops as acetate is consumed in the reactor. For propionate, glucose, and ethanol, the current peak is lower but more extended in time. For these compounds the rate of substrate oxidation is lower, so the organic compounds remain in the reactor at higher concentration for longer time. This means that although the maximum current is lower, the transferred charge at the end of the incubation is not necessarily that much lower for the other compounds compared to acetate. The repeated runs with acetate show the stability of the reactor over time. The coefficient of variation for transferred charge after 20 h ranges from 2.7% at 64 mg/L BOD to 8.4% at 640 mg/L.
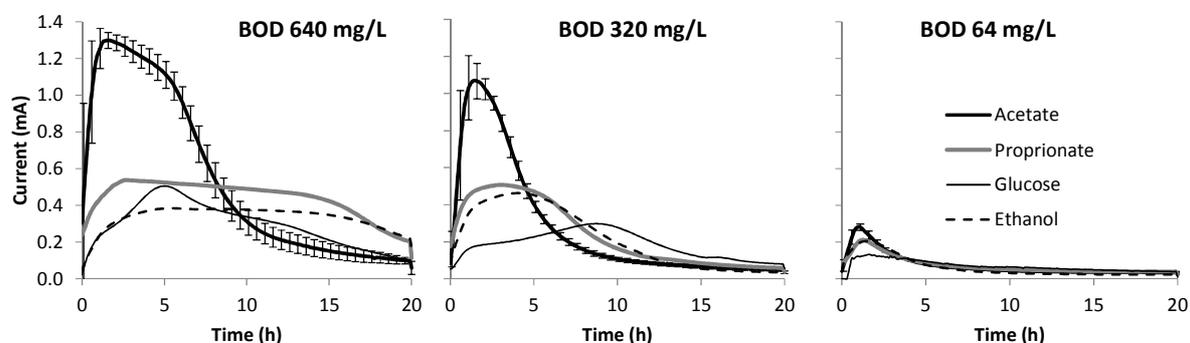

Figure 6. Current as a function of time for various organic compounds and BOD concentrations. Error bars (standard deviations of 3 to 4 repeated runs) are shown for acetate.

Transferred charge as a function of BOD concentration is shown in Figure 7 for reaction times of 5, 10, 15, and 20 h. The linear correlations and fits associated with Figure 7 are shown in Table 1. Longer reaction time gives a better linear correlation between charge and BOD concentration, which can be seen from R^2 values (Table 1). This is because a long reaction time allows more complete conversion of the substrate into current. At shorter reaction times, only a portion of the substrate fed to the reactor may have been converted into current. The slope of the linear correlation increases with increasing reaction time and varies depending on the compound that is the source of BOD. If the calibration line for acetate was used to measure propionate samples, the real BOD concentration would be underestimated. However, with longer reaction time, the underestimation decreases. For example, for a 10 h reaction

time, the acetate calibration will underestimate the BOD concentration by 53% if propionate is the source of BOD (calculated from the slope of the calibration lines). However, for a reaction time of 20 h, the underestimation is only 23%.
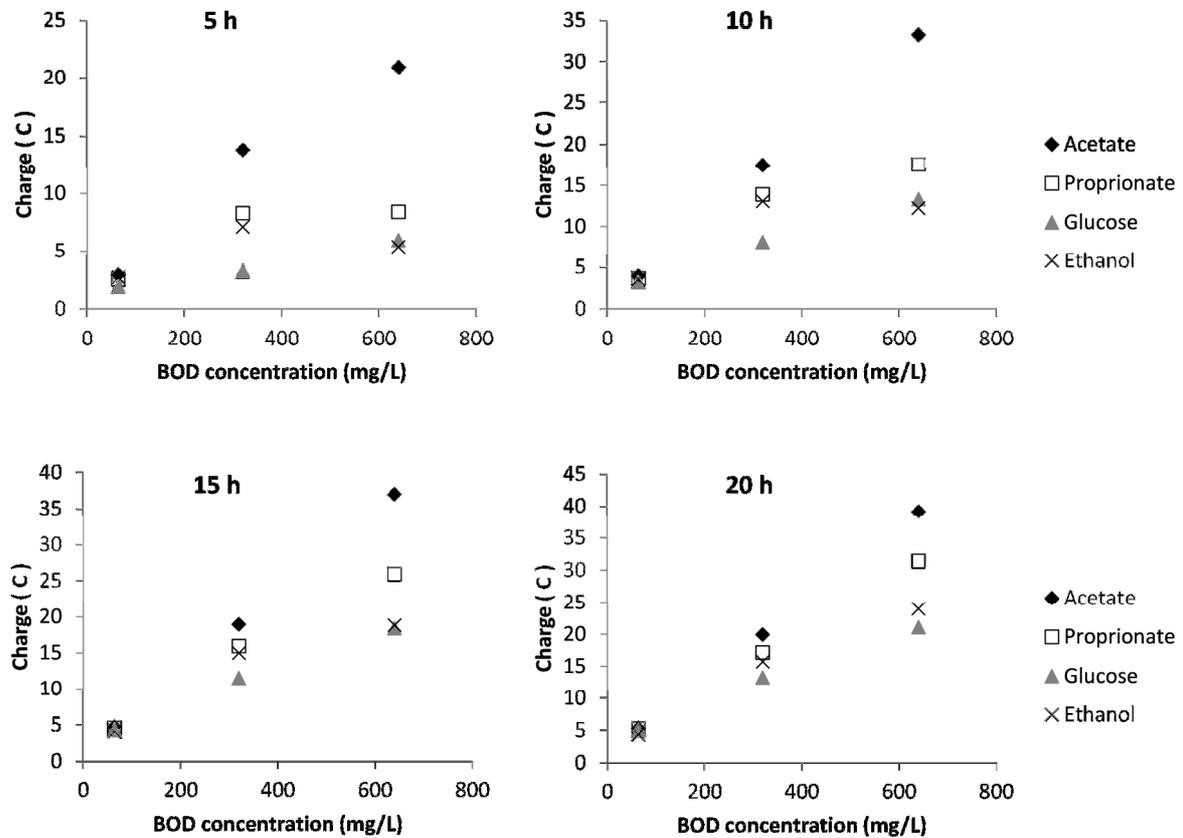

Figure 7. Transferred charge for different time points versus BOD concentration for runs with acetate, propionate, glucose, and ethanol.

The coulombic efficiency varied depending on reaction time and initial BOD concentration. With 64 mg/L BOD as acetate, the coulombic efficiency increased from 32% after 5h to 58% after 20h. For propionate, glucose, and ethanol, the coulombic efficiency after 20 h was 57%, 55%, and 59%, respectively. With 640 mg/L, it was 43%, 34%, 23%, and 26% for acetate, propionate, glucose, and ethanol, respectively. A high coulombic efficiency is desirable for accurate determination of BOD. Although, the reactor did not contain an ion exchange membrane and was fed with air-saturated water, coulombic efficiencies over 50% could be obtained. Oxygen entering the reactor with the feed solution and through the gas-diffusion cathode resulting in aerobic oxidation of BOD was most likely the main cause for lowering the coulombic efficiencies.

Table 1. Equations for the linear correlation between charge (C) and BOD concentration, and R^2 values for the fits.

BOD source & reaction time	Linear correlation	R^2
Acetate		
5h	C=0.031*(BOD)+2.00	0.966
10h	C=0.051*(BOD)+0.89	1.000
15h	C=0.056*(BOD)+1.05	1.000
20h	C=0.059*(BOD)+1.40	1.000
Propionate		
5h	C=0.010*(BOD)+3.07	0.711
10h	C=0.024*(BOD)+3.67	0.896
15h	C=0.037*(BOD)+2.86	0.990
20h	C=0.045*(BOD)+2.43	1.000
Glucose		
5h	C=0.007*(BOD)+1.27	0.981
10h	C=0.017*(BOD)+2.25	0.998
15h	C=0.024*(BOD)+3.03	0.994
20h	C=0.028*(BOD)+3.65	0.994
Ethanol		
5h	C=0.004*(BOD)+3.59	0.305
10h	C=0.014*(BOD)+4.68	0.618
15h	C=0.025*(BOD)+4.02	0.891
20h	C=0.034*(BOD)+3.23	0.977

4. Discussion

Applying an external voltage to a bioelectrochemical BOD sensor, as opposed to operating it as a MFC, can increase the rate of biological oxidation of organics at the anode and simplify the design of the sensor. The cell voltage generated in a bioelectrochemical system depends on the anode and cathode potentials and current flowing in the system according to Equation 4 (a negative cell voltage is here defined as output from the system whereas a positive cell voltage means input of external energy to the system).

$$Cell\ voltage = (E_{ED}+\eta_{anode}) - (E_{EA} - \eta_{cathode}) + I \times R_{ohmic}$$

(4)

where E_{ED} and E_{EA} are the reduction potentials associated with the electron donor redox couple (e.g. CO_2/acetate) and electron acceptor redox couple (e.g. O_2/H_2O), respectively (V vs SHE). η refers to the overpotentials at the anode and cathode electrodes (V). I is the current (A) and R_{ohmic} is the resistance associated with the circuitry, connections, and ion migration through the electrolyte (Ω).

The reduction potentials associated with the elector donor and electron acceptor can be calculated using the Nernst equation and depend on the concentrations of these compounds. The overpotentials (η) depend on the catalysis of the electrode reactions. In a bioelectrochemical BOD sensor, it is desirable that only the electron donor concentration at the anode determines the cell voltage (and thereby the current). The conventional MFC-type design therefore requires close proximity between the anode and cathode to minimize R_{ohmic}, good supply of oxygen to the cathode to maximize E_{EA}, and the use of a good cathode catalyst to minimize $\eta_{cathode}$. By input of an external voltage, the design of the sensor is simplified

because the applied voltage can cancel out the voltage losses associated with e.g. IxR_{ohmic} and $\eta_{cathode}$. An external voltage can also maximize the rate of organics oxidation at the anode (i.e. the current) by driving the anode to a higher potential, which allows the microorganisms utilizing the anode as electron acceptor to oxidize organics at their maximum rate. In this study, the current in the system increased with an applied voltage of 0.2 V as compared to a cell voltage of 0 V or -0.2 V.

A potential drawback of applying a voltage to the system is that other reactions, such as water oxidation at the anode, could contribute to the observed current. Thus, the applied voltage should not be too high. In this study, the observed current when the reactor was fed with water containing 0 mg/L BOD was very low (0.02 mA) and could be attributed to endogenous decay. This confirms that it was biological oxidation of organic compounds at the anode that resulted in current generation in the reactor.

The response time is an important aspect of BOD sensors. Although the reactor used in this study had a response time between 5 and 20 hours, which is longer than some previous MFC-based BOD sensors (e.g. Moon et al. 2004), its design allows measurement of a wide BOD concentration range. When the reactor was calibrated with acetate, longer reaction time resulted in better linear correlation with high BOD. This is because BOD concentration was calibrated with transferred charge. All of the substrate fed to the reactor must be converted into charge to obtain a good correlation. At higher BOD concentration, the reactor is fed a larger amount of substrate, which takes longer time for the microorganisms to oxidize. To minimize the response time, the reactor should be designed with a high current-to-volume ratio. This could likely be achieved by increasing the anode surface area-to-volume ratio (in this study the reactor had 1.47 cm^2/mL). Since the reactor is operated with an applied voltage, the cathode surface area does not necessarily need a corresponding increase, which facilitates design.

When the BOD source was changed from acetate to propionate, glucose, and ethanol, the response of the reactor changed. Although all three compounds could be converted to current in the reactor, the magnitude of the current was lower. Lower current led to more drawn out current peaks, which in turn caused longer response time. It is interesting to notice though, that the difference in the linear correlation for acetate compared to the other three compounds decreased with increasing reaction time. This implies that a reactor calibrated for a certain compound or mix of compounds could also be used to measure the BOD concentration for another mix of compounds if the reaction time is long enough. This is an important aspect for the practical application of the BOD sensor in a wastewater treatment plant where the composition of the organic compounds fed to the reactor may change rapidly.

The addition of sodium acetate and propionic acid, which ionize in solution, could affect the internal resistance of the reactor. However, the addition of 1280 mg/L BOD as sodium acetate to the nutrient medium used in this study only caused an increased ionic strength of 6.7%. Thus, the effect of changing ionic strength on the current profiles and charge transfer observed for various BOD concentrations were likely negligible.

In this study, air-saturated nutrient medium was fed to the reactor. The regular supply of oxygen did not seem to have a negative effect on the long term performance of the reactor. However, it would likely increase the detection limit of the BOD sensor. One can assume that the oxygen fed to the reactor is used as electron acceptor before the anode. This means that if water with 8 mg/L of dissolved oxygen is fed to the reactor, about 8 mg/L of BOD will be

consumed without an accompanying current generation. In this study, BOD concentrations ranging from 32 to 1280 mg/L were used, which is well beyond the range of the theoretical impact of DO in the influent water. For measurement of lower BOD concentrations, addition of respiratory inhibitors (Chang et al. 2005) or sparging with nitrogen gas could potentially be used to reduce the effect of dissolved oxygen in the influent water.

Salinity, alkalinity, and DO concentration could affect the output of the sensor. Low salinity (i.e. low ionic conductivity) leads to higher internal resistance of the reactor. In theory, the current generated in this type of sensor should be not be significantly affected by salinity changes because the input voltage forces the anode potential to a higher value and overcome internal voltage losses that limit current generation. Low alkalinity could potentially lead to lower current generation because of localized pH drops at the anode electrode. However, even if the magnitude of the current is affected, the transferred charge should be similar if the reaction time is increased. The DO concentration of the influent water will primarily have an impact on the ability to accurately measure low BOD concentrations. The effects of salinity, alkalinity, DO concentration, and changes in carbon substrates as well as possible interaction effects should be further investigated in future studies. The response and stability of the sensor should also be tested with real wastewater instead of a nutrient solution.

5. Conclusions
A new type of bioelectrochemical BOD sensor was developed. The sensor operates as a microbial electrolysis cell with an input voltage to overcome internal resistances which would otherwise limit current generation by microorganisms on the anode. An input voltage of 0.2 V was needed to obtain maximum current generation.

The BOD sensor was demonstrated with an aerated nutrient medium containing acetate as the BOD source. A linear correlation between BOD concentration and transferred charge was obtained in 20 h for BOD concentrations ranging from 32 mg/L to 1280 mg/L. The range of linear correlation decreased with lower reaction time. For example, in 5 h a good linear correlation (R^2=0.99) was obtained up to a BOD concentration of 320 mg/L.

The reactor was acclimated to acetate but could still generate current when fed with propionate, glucose, or ethanol. The current peaks with these compounds were lower and more drawn out. Using the calibration with acetate to measure BOD concentrations of propionate, glucose, and ethanol gave an underestimation of the true BOD concentration. However, the underestimation decreased with increasing reaction time.

By applying an input voltage and not using an ion exchange membrane, the reactor demonstrates a way to design bioelectrochemical BOD sensors that are not limited in performance by high internal resistance of pH shifts across an ion exchange membrane.

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