# Accepted Manuscript

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Please cite this article as: Junyoung Sim, Robertson Reid, Abid Hussain, Hyung-Sool Lee , Semi-continuous measurement of oxygen demand in wastewater using biofilm-capacitance. Biteb (2018), doi:10.1016/j.biteb.2018.08.009

**Semi-continuous measurement of oxygen demand in wastewater using biofilm-capacitance**

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**Abstract**

Bio-capacitive coulombs were tested for determination of the 5-day biochemical oxygen demand ($BOD_5$) using a dual-chamber microbial electrochemical cell (MxC) operated at charging (open circuit) and discharging (close circuit) conditions. For acetate medium, the cumulative coulombs charged in a capacitive biofilm anode (open circuit) were well correlated with BOD concentrations ($R^2 \sim 0.9$). The maximum detectable $BOD_5$ concentration with the bio-capacitance MxC was close to 250 mg/L, and the cumulative coulombs were saturated for above the maximum $BOD_5$ concentration (Monod pattern). The bio-capacitance MxC sensor consistently showed high linearity between the cumulative coulombs and $BOD_5$ concentrations for domestic wastewater influent ($R^2 = 0.93$-$0.99$), despite of 1min charging. High correlation between the coulombs and $BOD_5$ concentration was also obtained for wastewater effluent at 1 min charging, which indicates that the bio-capacitance MxC sensor can semi-continuously measure $BOD_5$ concentration in wastewater at every 2 min (1min charging and 1min discharging).

## 1. Introduction

Oxygen demand (OD) is a common parameter for assessing wastewater effluent quality, which can be measured chemically (chemical OD (COD)) or biochemically (biochemical OD (BOD)). Municipalities have typically regulated organic content in wastewater effluent using the standard 5-day BOD ($BOD_5$) that represents the amount of biodegradable organic compounds present in wastewater. For instance, the federal government of Canada regulates $BOD_5$ concentration in wastewater effluent less than 25 mg/L (Canada, 2018). However, the standard $BOD_5$ measurement method is time-consuming and labor-intensive. More importantly, intermittent $BOD_5$ measurement (e.g., weekly or biweekly) does not represent a continuously-varying $BOD_5$ concentration in wastewater effluent discharged into water bodies (Jouanneau et al., 2014). In order to uninterruptedly ensure high quality wastewater effluent, a novel technology capable of frequently monitoring $BOD_5$ in wastewater is essential. Microbial electrochemical cells (MxCs) are an attractive tool that can more often measure $BOD_5$ concentration than the existing incubation-based method. In addition, the electric current generated from MxCs fundamentally reflect BOD, electron equivalent of biodegradable organic compounds expressed as $O_2$ (Lee & Rittmann, 2010), different from optical or fluorescent BOD sensors: Exoelectrogens transfer electrons from biodegradable organic matter to the anode in MxCs. Previous studies have developed $BOD_5$ monitoring tools based on this MxC's fundamental and have used electric current as a signal to determine $BOD_5$ concentrations in wastewater (Chang et al., 2004; Kim et al., 2003; Kim et al., 2009; Peixoto et al., 2011; Recio-Garrido et al., 2017). However, electric current is inherently a kinetic term that measures the oxidation rate of organic compounds by exoelectrogens (1 A = 1 C/s). Estimation of $BOD_5$ concentration with electric current can work for specific conditions, but using the kinetic term is inappropriate to estimate $BOD_5$

concentration that is the mass term in fixed volumes. Moreover, electric current (or current density) generated from MxCs does not well represent low $BOD_5$ concentration (e.g., $< BOD_5$ ~30 mg $O_2$/L) or small change of $BOD_5$ concentration (Kim et al., 2009; Modin & Wilén, 2012; Quek et al., 2015).

Cumulative coulombs in a given time are equivalent to substrate electrons oxidized by exoelectrogens and hence the cumulative coulombs can better represent $BOD_5$ concentration in a fixed volume than electric current. Several literature has reported that cumulative coulombs have higher correlation and sensitivity to $BOD_5$ concentration than electric current (Gil et al., 2003; Kim et al., 2009; Liu et al., 2018). However, the coulomb-based method still needs longer reaction times from several hours to days to measure $BOD_5$ concentration especially for real wastewater. To ensure clean wastewater effluent and protect downstream water body municipalities should improve water quality monitoring practices capable of measuring $BOD_5$ concentration more frequently without long time-delay (e.g., , minute range).

In this work, the bio-capacitance of biofilm anodes was tested for $BOD_5$ measurement. Exoelectrogens can keep oxidizing and transferring donor electrons to biofilm anodes in open circuit, electron-charging mode. Then, the electrons accumulated in biofilm anodes during the charging mode are immediately discharged in close circuit, electron-discharging mode. This study assessed that the bio-capacitance of biofilm anodes could well signal for $BOD_5$ concentration in an MxC using acetate medium. Then, charging and discharging conditions were optimized for domestic wastewater (influent and effluent). This work proved that biofilm anodes as bio-capacitors can measure $BOD_5$ concentration in every 2 minutes, enabling semi-continuous $BOD_5$ measurement.

## 2. Materials and Methods

### 2.1 Reactor configuration

A laboratory-scale MxC was designed and constructed as shown in Figure 1. It consisted of cylindrical acrylic sections and had a working volume of 4.80 mL for the anode and the cathode chamber, respectively. Carbon felt (43201, Alfa Aesar, USA) with a geometric surface area of 16 cm$^2$ was selected as the anode, while an air cathode consisting of carbon cloth with platinum/carbon black catalyst layer (0.5 mg Pt/cm$^2$; SLGDE, Fuel Cells Etc, USA) was used as the cathode. The anode and the cathode chamber were partitioned by a cation exchange membrane (CEM; CM1 7000, Membranes International, USA) with a projected surface area of 8.04 cm$^2$. An Ag/AgCl reference electrode (MF 2052, BASI, USA) was inserted ~0.5 cm away from the anode to fix the anode potential ($E_{anode}$) at -0.4 V (vs Ag/AgCl) using a potentiostat (VSP, BioLogic, France) in close-circuit operation of the MxC.

### 2.2 Inoculation and Operation

The MxC was inoculated with 5 mL of effluent from an existing MxC enriched with *Geobacter* genus (98%) (Dhar et al., 2017) that had been operated with acetate medium (25 mM acetate medium) for over 1 year. The composition of the medium was (per L of 18.2 MΩ cm MilliQ water) 2,050 mg/L $CH_3COONa$, 2,274 mg $KH_2PO_4$, 11,678 mg $Na_2HPO_4 \cdot 12H_2O$, 37 mg $NH_4Cl$, 25 mg $MgCl_2 \cdot 6H_2O$, 6 mg $MnCl_2 \cdot 4H_2O$, 0.1 mg $CuSO_4 \cdot 5H_2O$, 0.1 mg $Na_2WO_4 \cdot 2H_2O$, 0.1 mg $NaHSeO_3$, 0.01 mg $CaCl_2 \cdot 2H_2O$, 0.5 mg $ZnCl_2$, 0.1 mg $AlK(SO_4)_2$, 0.1 mg $H_3BO_3$, 0.1 mg

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$Na_2MoO_4 \cdot 2H_2O$, 0.2 mg $NiCl_2$, 5 mg EDTA, 1 mg $CO(NO_3)_2.6H_2O$, and 0.2 mg $NiCl_2 \cdot 6H_2O$. The medium was autoclaved and sparged with ultra-pure nitrogen (99.999%) for 30 min. Then, $FeCl_2 \cdot 2H_2O$ (20 mM) and $Na_2S \cdot 9H_2O$ (77 mM) were added to the medium (1 mL per L), and the medium pH was constant at $7.3 \pm 0.1$. The cathode chamber was filled with tap water in which oxygen reduction to water occurred. During acclimation of exoelectrogens, the anode and the cathode chamber were operated in batch mode until a peak current density of ~1 $A/m^2$ was replicated. Then, the MxC was switched to continuous mode by feeding acetate medium to the anode chamber using a peristaltic pump (Masterflex 7523-80, Cole-Parmer, USA) at a hydraulic residence time (HRT) of 8 h. For steady-state current density to a given acetate concentration, bio-capacitance experiments were conducted. The operation of the MxC was alternated between open circuit (electron charging) and closed circuit mode (electron discharging) using the potentiostat and the EC-Lab software for bio-capacitance tests. During open circuit mode, the biofilm oxidizes electron donor (e.g., acetate) and stores substrate electrons in the biofilm anode (bio-capacitance). During closed circuit mode, the stored electrons are discharged to the cathode and cumulative coulombs are monitored. Two charging times of 15 min and 30 min were assessed with a fixed discharging time of 1 min (closed circuit mode) using acetate medium. Current and electrode potential were monitored at every 0.01 s using the potentiostat and the EC-Lab. Operation of the MxC was repeated five cycles of open and closed circuit modes as acetate concentration was varied from 32 to 410 mg COD/L. Then, calibration curves between the cumulative coulombs and acetate concentration (as mg COD/L) were built. After completing proof-of-concept experiments with acetate medium, the bio-capacitance $BOD_5$ sensor was further validated with domestic wastewater. The wastewater discharged from primary and secondary clarifiers was regularly sampled from Waterloo wastewater treatment plant (ON,

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Canada), and then diluted with deionized water to vary $BOD_5$ concentration. Calibration curves were established as charging time was changed from 1 to 40 min.

**2.3 Chemical analysis**

Acetate concentration was quantified with a gas chromatography (GC) (Model: Hewlett Packard HP 5890 Series II) equipped with a Nukol fused-silica capillary column and flame ionization detector (FID) using helium gas as a carrier gas. The initial temperature of the column was 110°C, increasing to 195°C at the rate of 8°C/min, and then held constant at the final temperature of 195°C for 9 min. Injector and detector temperatures were 220°C and 280°C, respectively. Prior to GC-FID analyses, the liquid samples were acidified to pH ~ 2 using 1 N phosphoric acid, and then filtered using 0.2 µm membrane filter (DISMIC-25 HP, Toyo Roshi Kaisha Ltd., Japan). All liquid samples were measured in triplicates. Measured acetate concentration was converted to COD using a half reaction for acetate ($1/4 \ CO_2 + 7/8 \ H^+ + e^- = 1/8 \ C_2H_3O_2^- + 1/4 \ H_2O$), which means that 1 mM acetate is equivalent to 64 mg COD/L. The literature confirmed that calculated COD almost equals to measured COD concentration using a HACH method (Lee et al., 2008b). $BOD_5$ concentration was measured with the Standard Method (APHA, 2012), and all samples were measured in triplicates. Average data was reported with standard deviations.

## 3. Results and discussion

### 3.1 Anode potential and current generation in charging and discharging operation

Figure 2 shows the changes of anode potential and current density in the MxC run as open and closed circuit mode (five cycles); acetate concentration was fixed at 410 mg COD/L during these experiments. Anode potential was quickly stabilized and reached at -0.55 to -0.56 V (vs. Ag/AgCl) in open circuit modes of 15 min and 30 min, which was replicated in five cycles. Peak current density in closed circuit mode (1 min discharging) was increased by 2.3-2.5 A/m$^2$ and gradually decreased to ~1.6 A/m$^2$ in 1 min. The same pattern was repeatedly observed for all five cycles. This result supports that the biofilm anode works as a bio-capacitor, as reported by the literature (Kumlanghan et al., 2007; Lv et al., 2014; Ren et al., 2015; Schrott et al., 2011).

### 3.2 Correlation between bio-capacitive coulombs and acetate concentration

Cumulative coulombs in discharging mode were plotted against acetate concentration in Figure 3. Cumulative coulombs were linearly increased to acetate concentration at ~200 mg COD/L, but the coulombs were saturated at over acetate concentration of ~200 mg/L (1mM acetate = 64 mg COD/L). This pattern is very close to current density and substrate (*j*-S) curves (Lee et al. 2009; Lee, 2018; Dhar et al., 2016).

Equation 1 describes the Monod equation that correlates substrate-utilization rate with substrate concentration.

$$(-dS_d/dt) = f_e^o q_{max,app} \frac{S_d}{S_d + K_{sd,app}} X_a \qquad (1)$$

where, $S_d$ is donor substrate concentration (g COD/L), t is reaction time (d), $f_e^o$ is the fraction of electrons used for catabolism, $q_{max}$ is the apparent maximum specific substrate utilization rate (g COD/g VS-d), $K_{sd,app}$ is the apparent half-saturation concentration of substrate (g COD/m$^3$), and $X_a$ is the concentration of active microorganisms (g VS/m$^3$).

For biofilm anodes, (-dS/dt) per anode area can be expressed as current density (1g COD/d-m$^2$ = 0.14 A/m$^2$), and current density is mathematically described as below.

$$j = 0.14 f_e^o q_{max,app} X_f L_f \frac{S_d}{S_d + K_{sd,app}} \qquad (2)$$

where, $j$ is current density per geometric surface area of the anode (A/m$^2$), 0.14 is the conversion factor (0.14 A= 1 g COD/d), $X_f$ is biofilm density (g VS/m$^3$), and $L_f$ is biofilm thickness (m).

Cumulative coulombs ($j \times$t) are equivalent to current density in a given time, so the correlation between the coulombs and acetate concentration can show a Monod-like curve as shown in Figure 3A. The Monod-like curve obtained from charging and discharging operation supports that exoelectrogens keep oxidizing acetate, transfer acetate electrons to the biofilm, and store the electrons in the biofilm: the bio-capacitor. Figure 3B and 3C shows linear correlations ($R^2 \sim 0.9$) obtained between cumulative coulombs and acetate concentration < 250 mg COD/L. The linear curves suggest that cumulative coulombs established in charging and discharging operation of the MxC can estimate acetate concentration less than 250 mg COD/L. This indicates that MxCs can be used for measuring COD or BOD concentration in semi-continuous manners at every 16 min (15 min charging and 1 min discharging). The saturated coulombs to acetate concentration

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250 mg COD/L in Figure 3A mean that high organic samples should be diluted below 250 mg COD/L. Since acetate is completely biodegradable, this result also proposes the maximum detectable $BOD_5$ concentration with the bio-capacitance MxC is 250 mg/L.

### 3.3 Linearity between bio-capacitive coulombs and wastewater $BOD_5$ concentration

Correlation tests between cumulative coulombs and $BOD_5$ concentration were further tested with wastewater influent (from primary clarifier) and effluent (from secondary clarifier) as charging time was changed from 1 min to 40 min. Figure 4 shows high linearity between $BOD_5$ concentration in wastewater influent and cumulative coulombs ($R^2$ 0.93-0.99), except for a 30 min-charging condition. Interestingly, a 1 min-charging condition still showed a high linearity of $R^2$ = 0.96, which means measuring $BOD_5$ concentration at every 2 min (1 min charging and 1 min discharging). Figure 5 presents correlations between $BOD_5$ concentration in wastewater effluent and cumulative coulombs. $R^2$ coefficient became relatively low at 0.84 (40 min and 30 min charging), but it was as high as 0.93-0.99 (1 min and 5 min charging). This result suggests $BOD_5$ monitoring at every 2 min even for low organic wastewater.

It is interesting to find that $R^2$ coefficients for domestic wastewater are similar to or even higher than those for acetate medium. Syntrophic interactions among exoelectrogens, fermenters and $H_2$ consumers are required for mixed-culture biofilm anodes to utilize complex organics and fermentable substrates (Dhar et al., 2015; Dhar et al., 2016; Gao et al., 2014; Lee et al., 2008a). Despite of complex syntrophy in the anodic biofilm fed with the wastewater, high linearity ($R^2$ >0.93) between cumulative coulombs and $BOD_5$ concentration in wastewater influent and effluent was observed even at 1 min-charging time. This result supports that electrons

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transferred to the anode via the mixed and syntrophic biofilm anode can well represent biodegradable organics in the wastewater with short charging time ($< 5$ min). Charging times longer than 30 min tended to deteriorate the linearity between the coulombs and $BOD_5$ concentration for wastewater influent and effluent (see Figures 4 and 5). Microbial reactions other than anode respiration (e.g., fermentation, methanogenesis, etc.) would become more important in the biofilm at longer charging time so that the linear relationship between the coulombs and $BOD_5$ could decrease. Especially for wastewater influent, fraction of other electron sinks including methane, biomass and soluble microbial products would increase (Lee et al., 2008b). In comparison, endogenous decay current would dominate observed current for wastewater effluent having low $BOD_5$ concentration (An & Lee, 2013). More research is required to evaluate electron divergence in short and long charging times, but this study clearly shows that cumulative coulombs using bio-capacitance well estimate $BOD_5$ concentration in municipal wastewater.

The maximum measureable $BOD_5$ concentration of the bio-capacitive method is 250 mg/L, approximately, which means that this technology can be applied to most of domestic wastewaters. This study could not define a detection limit of $BOD_5$ concentration for the bio-capacitance MxC sensor mainly due to limited sensitivity and accuracy of the conventional 5 day-incubation $BOD_5$ method used for this work, although the lowest $BOD_5$ concentration tested in this work was 2 mg/L. Low organic water needs more accurate quantification methods to build regression lines, such as dissolved organic carbon or assimilable organic carbon (Quek et al., 2015).

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The present wastewater effluent monitoring practice is intermittent (weekly, biweekly, or monthly sampling and measurement), while wastewater effluent is continuously discharged to water body. Intermittent sampling and measuring approach cannot ensure clean wastewater effluent discharged to water body. Increasing frequency of sampling and measurement (like daily basis) may be an alternative to improve $BOD_5$ monitoring, but effectiveness and economical efficiency of this option are questionable. Using the new bio-capacitance $BOD_5$ method needs long-term studies to meet legislative requirements for a standard method (e.g., detection limit, reliability, sensitivity, etc.). However, the bio-capacitance $BOD_5$ sensor can be used as an alarming system to the current wastewater effluent monitoring regulations; the bio-capacitive MxC sensor enables $BOD_5$ measurement at every 2 min ($R^2$=0.96 for wastewater influent and $R^2$=0.93 for wastewater effluent). For instance, lab technicians can intensively carry out water sampling and $BOD_5$ measurements when the bio-capacitive MxC indicates $BOD_5$ concentration over 25 mg/L: provision of an alarming signal. In contrast, the technicians can follow the present monitoring practice without alarming signals from the MxC sensor. Another merit of the MxC sensor is to give $BOD_5$ concentration with an electric signal, that is readily transmitted over long distances, and hence this technology can be applied to remote and rural areas.

## 4. Conclusions

The bio-capacitance MxC sensor, that charges substrate electrons in open circuit and discharge the electrons in closed circuit, can measure low to high $BOD_5$ concentration in domestic wastewater ($R^2$=0.93-0.99), which means that the new sensor can be widely applied for wastewater treatment facilities. The bio-capacitance MxC can measure $BOD_5$ concentration at every 2 min, and this semi-continuous determination of $BOD_5$ concentration can improve the present intermittent wastewater effluent monitoring practice (weekly or monthly monitoring), better ensuring clean wastewater effluent and protecting downstream water body from wastewater.

## Acknowledgement

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Figure 1. Schematic of a microbial electrochemical cell (MxC) for the quantification of biochemical oxygen demand in water.

Figure 2. The anode potential was recorded during the 15-min and 30-min charging intervals for five cycles. (a) anode potential, and (b) current density during 1 min discharging time.

Figure 3. Relationship between acetate concentration (in COD mg/L) and cumulative coulombs for 15-min and 30-min charging interval. (A) Monod pattern curve, (B) linear correlation below 250 mg COD/L with 15min-charging time, and (C) linear correlation below 200 mg COD/L with 30min-charging time. The discharging time was fixed at 1 min.

Figure 4. The linearity between domestic wastewater and cumulative coulombs built in the MxC. Charging time was varied between 1 and 40 min, and discharging time was fixed at 1 min for the experiments. BOD$_5$ measurements were conducted in duplicate.

Figure 5. The linearity between wastewater effluent and cumulative coulombs in the MxC. Discharging time was fixed at 1 min during the experiments.

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**Highlights**

- A bio-capacitive biofilm anode was evaluated for $BOD_5$ measurement

- The maximum detectable $BOD_5$ concentration was ~250 mg/L

- High linearity between cumulative coulombs and $BOD_5$ concentrations was obtained

- The MxC sensor can measure $BOD_5$ concentration in water at every 2 min
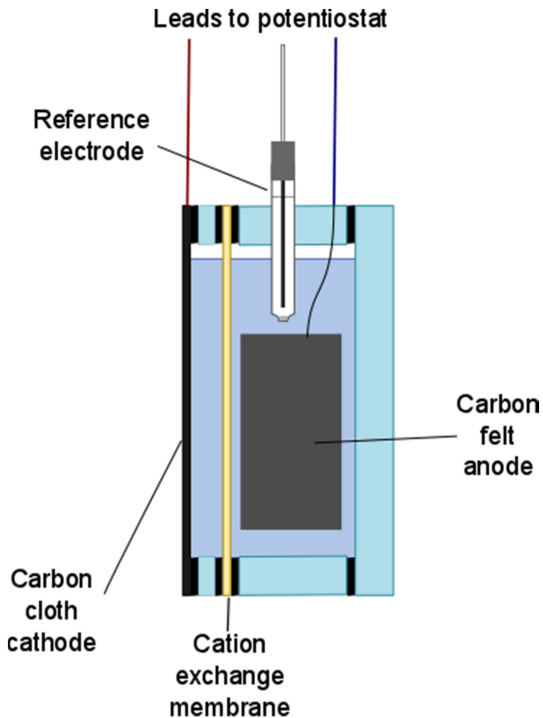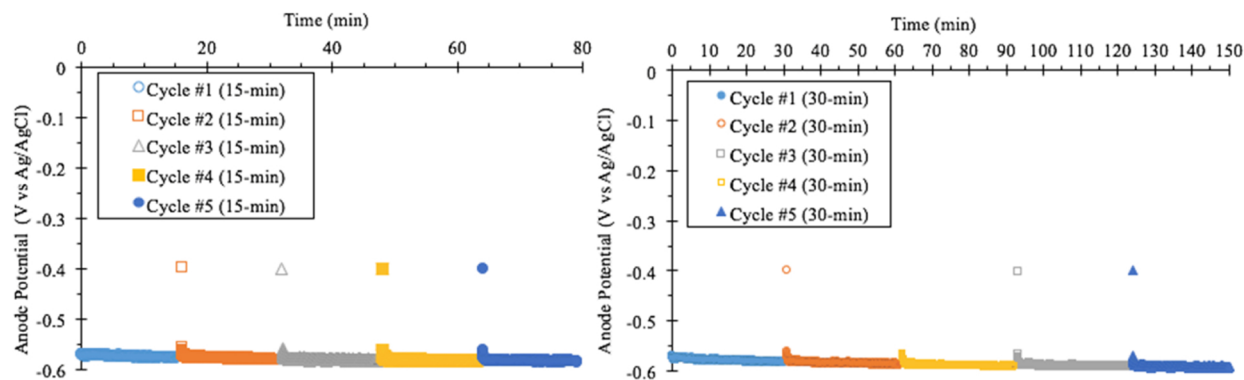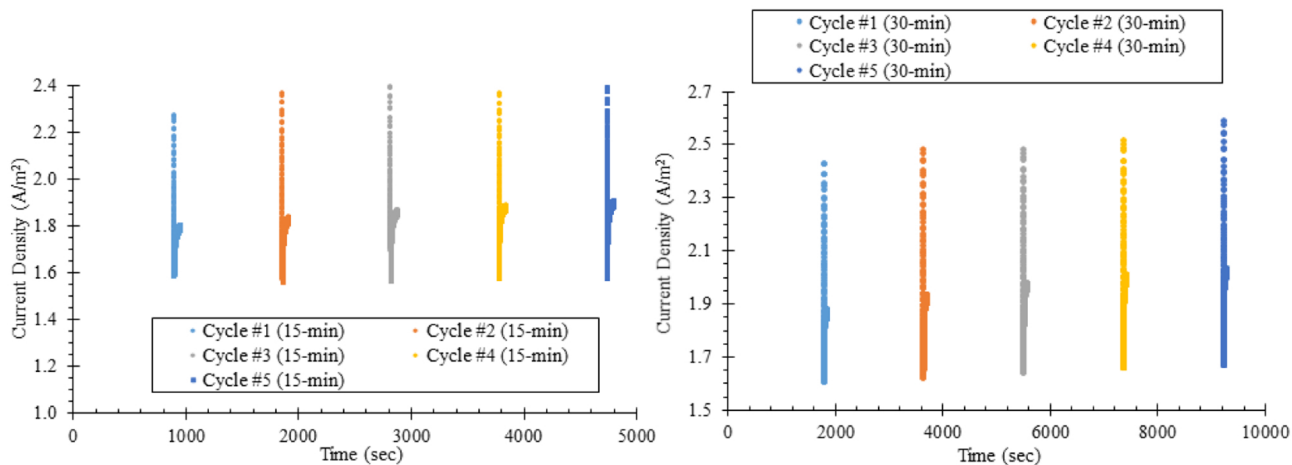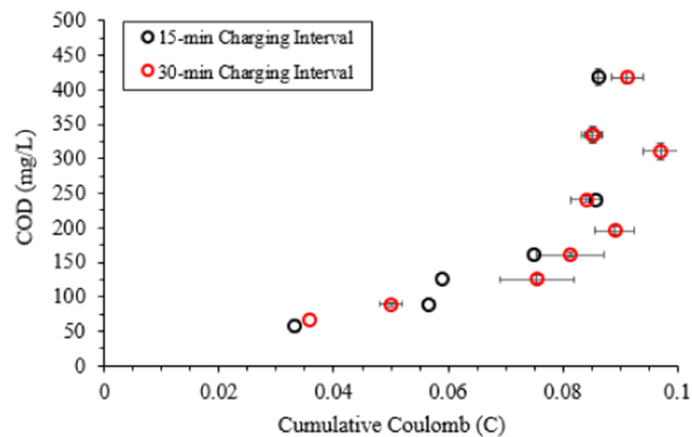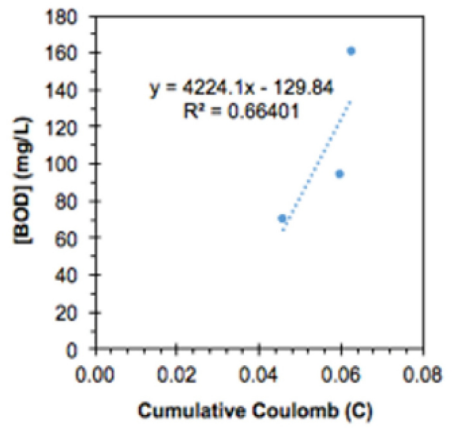
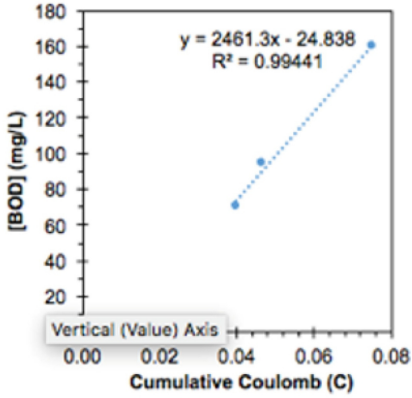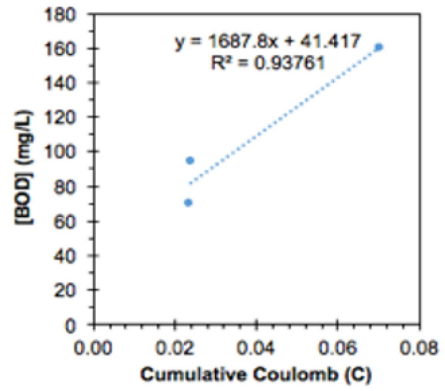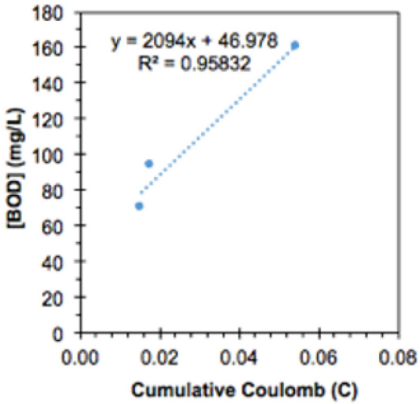Figure 1

Figure 2

(A)
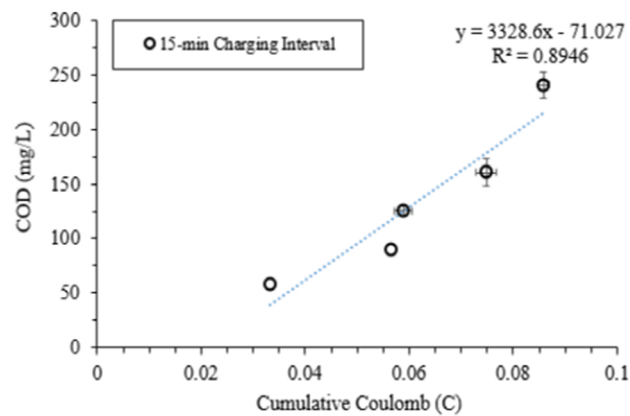


(B)
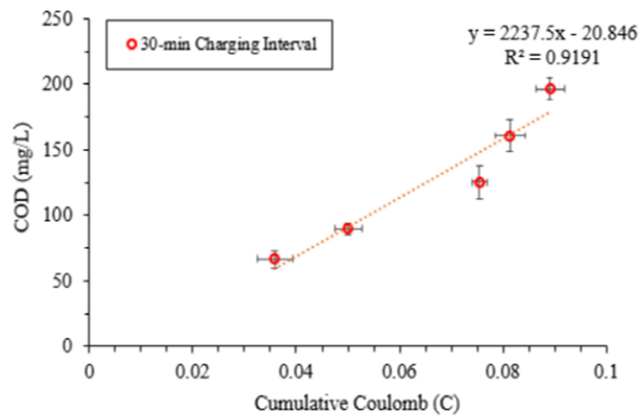


(C)

Figure 3

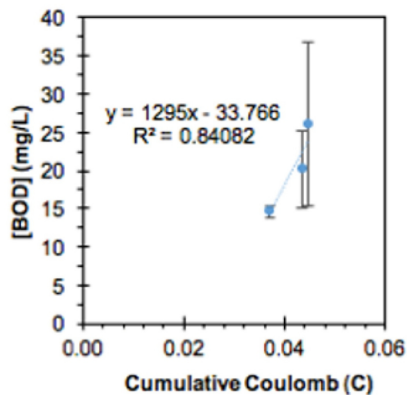(A) Charging Interval of 40 min

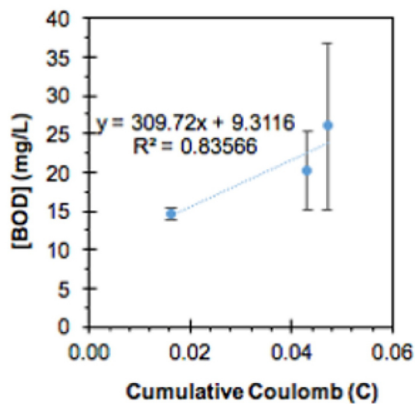(B) Charging Interval of 30 min

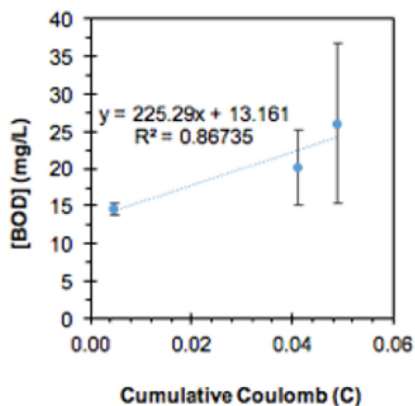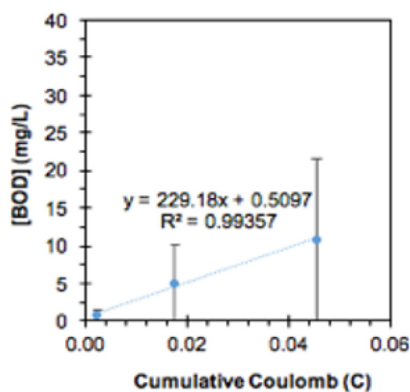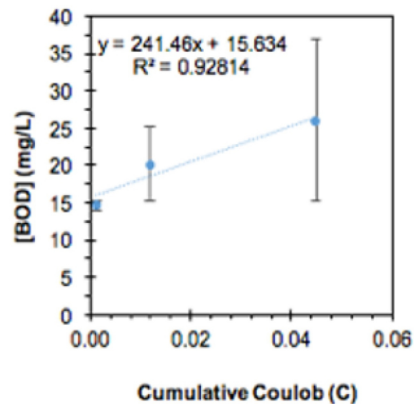(C) Charging Interval of 15 min

(D) Charging Interval of 5 min

(E) Charging Interval of 1 min

Figure 4

(A) Charging Interval of 40 min

(B) Charging Interval of 30 min

(C) Charging Interval of 15 min

(D) Charging Interval of 5 min

(E) Charging Interval of 1 min

Figure 5