Bioresource Technology 302 (2020) 122777

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Influence of wastewater microbial community on the performance of miniaturized microbial fuel cell biosensor

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ARTICLE INFO

*Keywords:*
Microbial fuel cell biosensor
Wastewater
Microbial community
BOD
COD

ABSTRACT

Microbial fuel cells (MFCs) based sensors had been studied in measuring biochemical oxygen demand (BOD) or the equivalent chemical oxygen demand (COD) recently. Limited attention has been paid to the effect of the microbial communities in wastewater on the responses of these sensors. This study systematically evaluated, for the first time, the effect of wastewater samples from a variety of sources on the electrical response of a microfabricated double-chamber MFC device. It was found that the response of the MFC is positively correlated with the bacterial composition, in particular electroactive bacteria. The presence of aerobic bacteria in the sample reduces the current generation. These findings indicated that the bacterial content of the water sample could be a significant interference source and must be considered in the use of μMFC-based sensors. Filtering samples may be effective in improving the reliability of these microsensors.

## 1. Introduction

The growing impact of human and animal population on the environment as well as other activities such as agriculture and resource extraction require continuous monitoring of various parameters such as BOD that could have a significant impact on the water bodies such as streams, lakes and rivers. Biosensors have been recently developed to measure some of these parameters and have favorable characteristics such as low-cost, compactness and ease of deploy ability on-site and capability for automation over other traditional methods of analysis (Bilal and Iqbal, 2019; Chaubey and Malhotra, 2002; Eggins, 2008). In particular, microbial fuel cell (MFC) based biosensor have been investigated due to its ability to directly transduce BOD/COD into electrical signal, possibility for automated continuous online monitoring and its simple reagent free operation which are ideally suited for remote environmental monitoring (Corbella et al., 2019; Di Lorenzo et al., 2009).

MFCs have been used as biosensors for the monitoring of chemical oxygen demand (COD) or biochemical oxygen demand (BOD) (Corbella et al., 2019; Jiang et al., 2018; Spurr et al., 2018) due to organic matter content on them. BOD/COD are the main parameters to determine wastewater organic matter content and the current methodologies are time consuming, produce chemical compounds that pose a threat to the environment and require qualified personnel (Kumlanghan et al., 2007). Recently, many efforts has been conducted to improve the MFC-based COD biosensor performance which has resulted in significant improvements in reducing their cost of fabrication and operation (Fraiwan et al., 2014; Hashemi et al., 2016; Yang et al., 2016), reducing the response time (Di Lorenzo et al., 2009; Moon et al., 2004), improving the detection range as well as the sensitivity (Jiang et al., 2017; Kaur et al., 2015; Yi et al., 2019). However, most of the research were conducted in lab scale and tested the sensor performance using synthetic wastewater with sole carbon source of acetate, glucose or cellulose under controlled experimental control conditions, in which the solutions used were sterilized at high temperature to eliminate the interference of unknown bacteria to the experimental results. To the best of our knowledge, only a few of them have focused on testing these sensors in real wastewater samples and none of them have done a systematic study on the influence of various bacterial species that are present in those samples on the performance of these sensors.

This lacuna is surprising as it is well known that the bacterial population on the electrode plays a significant role in the electrical response of a microbial fuel cell. For instance, studies have found that the effect of microbial sources used for inoculation during its start-up on the subsequent performance of MFCs (Tran et al., 2016) is significant. In addition, studies have also found that the anodic biofilm is dynamic in its microbial population and its composition can change even after long duration of stable operation (Paitier et al., 2017; Sciarria et al.,

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https://doi.org/10.1016/j.biortech.2020.122777
Received 16 November 2019; Received in revised form 6 January 2020; Accepted 7 January 2020
Available online 10 January 2020
0960-8524/ 

2019). Other studies have focused on effect of carbon source and its influence on the anodic bacterial population in determining the operational performance of power generating MFCs (Erable et al., 2017; Pant et al., 2010; Pham et al., 2008; Quan et al., 2013; Zhang et al., 2011). Research conducted on the use of MFCs for wastewater treatment have shown that the contaminants and organic content of the wastewater can modify the anodic microbial community of the MFC dynamically (Jia et al., 2013; Long et al., 2019; Wu et al., 2018; Xia et al., 2019). These studies and their conclusions strongly indicate that the composition of the sample provided to the MFC for biosensing purposes may also have an impact on its response. Since the electrical response of the biosensor is crucial to relate and determine the BOD/COD of the sample, such influences can be a source of interference and a detailed understanding of them is essential before MFCs can be used as biosensors in practical scenarios like in a wastewater treatment plant.

In this paper, we address this gap and systematically evaluate the response of the microfabricated MFC biosensor to various wastewater samples. We co-relate the response to the bacterial and chemical composition of the samples and clearly show that they are significant factors that must be considered. In particular, we show that the availability of electroactive bacteria and the amount of aerobic vs anaerobic bacteria in the sample can cause changes in response. Using this understanding, we also identify specific ways that the MFC biosensor can be used to accurately measure COD in wastewater samples such as calibrating it to a specific source as well as filtration of the samples to prevent any further bacterial loading onto the sensor.

## 2. Methods and materials

### 2.1. Materials and reagents

Variety of polymeric tape materials were used in xurographic fabrication of the MFC devices described in this study. The poly ethylene terephthalate (PET) film (125 μm thickness) purchased from McMaster-Carr (8567K52, Cleveland, OH) was the main component of the devices, which was first patterned using a cutter machine, FC-8600, Graphtec America (Irvine, CA) and then sandwiched layer by layer using 3M adhesive transfer tape (6038PC) to form the anode chamber and cathode chamber. The two chambers were separated by a cation exchange membrane (CMI-7000S) purchase from Membranes International, Inc. Poly dimethyl Siloxane (PDMS) from Dow Corning, Midland, MI was first casted around silicone tubing (96410-16, Cole-Parmer, Canada) and adhered on top of reservoirs cut out of the PET film by double-sided silicone tape (PIT1SD-2RL/25.4, Kapton Tapes) to form the inlets and outlets of anode and cathode chambers. The electrode material consisted of anode made of carbon cloth (330 μm, WOS1009, Cetech Co, Ltd.) and cathode made of Pt film (40 μm, Wrights of Lymm). Carbon threads were used to connect the anode to a contact pad fabricated out of adhesive copper foil tape (B07CSJMN11, ECO-FUSED®) and used to connect the MFC to external electrochemical testing system.

The anolyte used for the inoculation during start-up period was synthetic wastewater (SW), composed of sodium acetate (1.0 g per liter) in 50 mM/L phosphate buffer solution (PBS) containing (per liter): $NH_4Cl$, 0.31 g, KCl, 0.13 g, $NaH_2PO_4{\cdot}2H_2O$, 3.32 g, $Na_2HPO_4{\cdot}12H_2O$, 10.32 g), trace minerals containing (per liter): $CaCl_2$, 0.015 g, $MgSO_4{\cdot}7H_2O$, 0.2 g, $FeCl_3{\cdot}6H_2O$, 0.01 g, $MnSO_4{\cdot}H_2O$, 0.02 g, and vitamins (Selembo et al., 2009). The catholyte was composed of 50 mM ferricyanide in 100 mM PBS. The final pH of SW and catholyte was adjusted at 7.2 ± 0.2 with 0.1 M NaOH before injection. The reagents used to fill the MFC device were all purchased from Sigma-Aldrich and water were purified to 18 MX using a Milli-Q Advantage A10 (Millipore, Billerica, MA) before using.

Various wastewater samples used for biosensor performance testing were collected during different treatment periods from the Toronto wastewater treatment plant (WWTP) and the Burlington wastewater treatment plant (WWTP). The samples were stored in 4 °C refrigerator before testing. The detailed information on water quality characteristics are shown in Table1. Three wastewater samples from second clarified effluent (SCE), primary tank effluent (PTE) and sludge dewatered effluent (SDE) were collected from Toronto WWTP and their COD values were found to be 390, 381 and 1016 mg/L. Two wastewater samples from aeration tank (AET) and anaerobic tank (ANT) were collected from Burlington WWTP, with COD values of 307 and 137 mg/L. All the determination of COD in this study is based on the standard method of absorbance measurement (Method 8000, Hach Co., USA).

**Table 1**
Wastewater characteristic of sample source and water quality parameters.

| Sample name | Sample source | COD (mg/L) | $NH_4^+$-N (mg/L) | $PO_4^{3+}$ (mg/L) |
|---|---|---|---|---|
| SCE(T) | Second clarified effluent, Toronto WWTP | 390 | 4.79 | 2.8 |
| PTE(T) | Primary tank effluent, Toronto WWTP | 381 | 27.2 | 8.6 |
| SDE(T) | Sludge dewatered effluent, Toronto WWTP | 1016 | 618 | 119 |
| AET(B) | Aeration tank, Burlington WWTP | 307 | 15.4 | 1.2 |
| ANT(B) | Anaerobic tank, Burlington WWTP | 137 | 14.7 | 1.8 |

Note: "T" represent the sample come from Toronto wastewater treatment plant; "B" represent the sample come from Burlington wastewater treatment plant.

### 2.2. Devices fabrication and assembly and operation

The xurogrpahic method used for the fabrication of the MFCs has been described in detail previously (Mohammadzadeh et al., 2018, 2019). Briefly, poly ethylene terephthalate (PET) film and ion exchange membrane (IEM) were xurographically patterned and cut through the connection of a cutter machine and AutoCAD software. The MFC devices were built with chamber size of 10 mm × 10 mm and a chamber thickness of 45 μm with a volume of 4.5 μL as this configuration was found to be the optimal previously. Two layers of PET film (Fig. 1a and c) were used to form the anode chamber on top of IEM membrane (Fig. 1d) and another two layers of PET film (Fig. 1e and g) were used to form the cathode chamber below the IEM membrane. Accordingly, carbon cloth (Fig. 1b) and Pt film (Fig. 1f) were cut to 10 mm × 10 mm square as anode and cathode. 10 × 10 × 5 mm PDMS interconnect blocks was fabricated by placing a 4 mm-ID tubing into a mold (Fig. 1h) and casting a 10:1 mixture of Sylgard 184 PDMS elastomer and curing agent around it, followed by curing at 65 °C for 1 h.

The micro-MFC device was constructed by laminating a cation exchange membrane (CMI-7000S) and anode chamber (with carbon cloth electrode inside) and cathode chamber (with Pt film inside) as shown in Fig. 1(i). The transfer adhesive tape (7951-3M™) was used to cold laminate these layers to each other tightly. Anolyte (SW or testing wastewater) and catholyte (50 mM ferricyanide in 100 mM PBS) were injected into the corresponding chambers through interconnects, perfused to ensure that there is no leakage.

### 2.3. Experiment set up and methods

The experimental setup consists of the device connected to a fluidic control module, an electrical measurement module and data acquisition module. The fluid control module is composed of two syringes containing the anolyte (wastewater samples) and catholyte. The syringes were connected to the inlets of the cathodic and anodic chambers of the MFC device while two effluent collectors were connected to the outlets. The electrical measurement unit consisted of a load resistor with an ammeter in series to measure the current flow the MFC. The current signal was measured and recorded as a function of time (10/min), via a

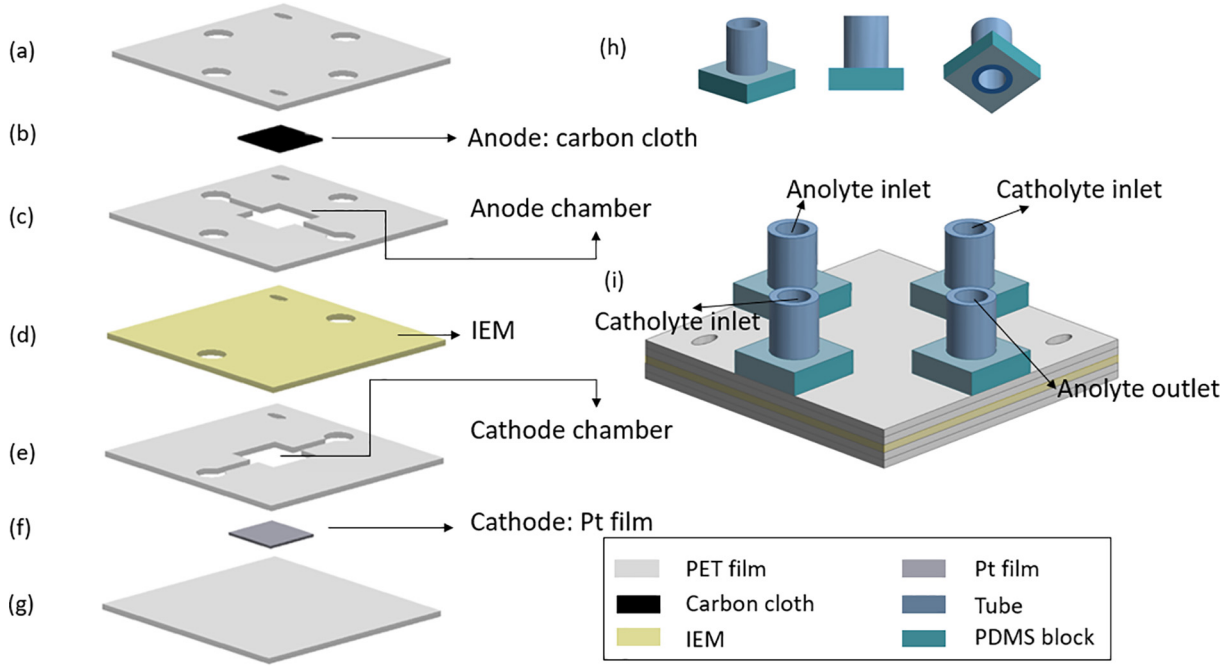


**Fig. 1.** Fabrication and assembly of microfluidic MFC devices. The fabricated devices have 10 mm × 10 mm anode area and a 4.5 μL reaction volume.

data acquisition unit using eDAQ Pod-Vu software (Edaq Pty. Ltd., NSW, Australia), connected to a PC for data visualization. Peak current value was used as the value representative of the concentration of the organic species in the testing wastewater samples. All the experiments and tests were conducted at room temperature (25 °C) and repeated three times.

The chemical oxygen demand (COD), ammonia nitrogen ($NH_4^+$) and orthophosphate ($PO_4^{3+}$) of the wastewater samples were determined by the standard method of absorbance measurement (Method 8000, Method 10205 and Method 8048, Hach Co., USA).

Wastewater samples were collected in polypropylene bottles and transported in an insulated and chilled container. 16S rDNA sequencing technology was used to analyse the microbial community in wastewater samples (50 mL) within 24 h of collection and was performed at the Mobix facility of McMaster University. Genomic DNA was extracted from the water samples using a modified DNA isolation method as described in Stearns et al. (2015). Purified DNA was used to amplify the variable region 4 of the 16S rRNA gene by PCR using Illumina adapted primers as described in Bartram et al. (2011). The primers were modified to amplify 515f (GTGYCAGCMGCCGCGGTAA) and 806r (GGACTACNVGGGTWTCTAAT). PCR was performed using 50 ng of template with 1U of Taq, 1× buffer, 1.5 mM MgCl2, 0.4 mg/mL BSA, 0.2 mM dNTPs, and 5 pmol of each primer. The reaction was carried out at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s, with a final extension of 72 °C for 10 min. Resulting PCR products were checked by gel electrophoresis and positive amplicons were sent for sequencing. Sequence variants were then resolved from the trimmed raw reads using DADA2, an accurate sample inference pipeline from 16 s amplicon data (Callahan et al., 2016). DNA sequence reads were filtered and trimmed based on the quality of the reads, error rates were learned and sequence variants were determined by DADA2. Bimeras were removed and taxonomy was assigned using the RDP classifier against the SILVA database version 1.2.8.

### 2.4. Biosensor start up and wastewater testing

The bacterial inoculum was obtained from effluent of an acetate-fed microbial electrolysis cell (MEC) mother reactor that had mixed bacterial culture from anaerobic digester sludge. Sequential batch mode was used for the biosensor start-up process. After the inoculum and anolyte (with 1 g/L NaAc) were mixed in a 1:1 vol ratio and added to the anode chamber, the current generation from the micro MFC device was measured over the next 24 h. As the amount of organic matter (food for the bacteria) added was fixed, the current will increase and reach the peak value and then fall as the organic matter is depleted. This characteristic response, indicates that the microorganism has completed a growth cycle and formed biofilm on the carbon cloth electrode. Next, a 0.2 mL bolus of anolyte (with 1 g/L NaAc but without the inoculum) and catholyte was added to the MFC and the response of the MFC monitored to ensure that the biofilm is stable and the characteristic response can be repeated. The bolus was fed 3 times. An external resistor of 10 Ω was used as the load as it has been found to be optimal previously (Xiao et al., 2019).

After successful start-up of the micro MFC device, different source wastewater samples were fed as anolyte to measure the current response and evaluate the performance of biosensor with real wastewater samples. The testing mode was similar to the start-up process. A 0.2 mL bolus of anolyte (with real wastewater sample) and catholyte was perfused through the MFC to completely flush away and replace the previous solution and the current generated was recorded as function of time (10/min) by data acquisition unit. Between each wastewater sample, a control anolyte with 1.0 g/L NaAc was introduced to the anode chamber to calibrate the sensor by checking if the current returns to the baseline value. Diluted samples were also prepared in order to test the sensor performance when the sample source was the same but the COD values are different. For this purpose, the original samples was diluted to 38% of its original concentration by addition of 62 mL of deionised water into 38 mL of sample SDE to get a testing sample of "diluted SDE". In case of municipal sewage, due to the absence of refractory industrial organic pollutants, the COD and BOD values are co-related and normally in a ratio of about 2.5 (Henze and Comeau, 2008), which could be used to co-relate with the BOD of the samples.

## 3. Results and discussion

### 3.1. Biosensor response to wastewater samples from various sources

In order to evaluate the applicability and robustness of the

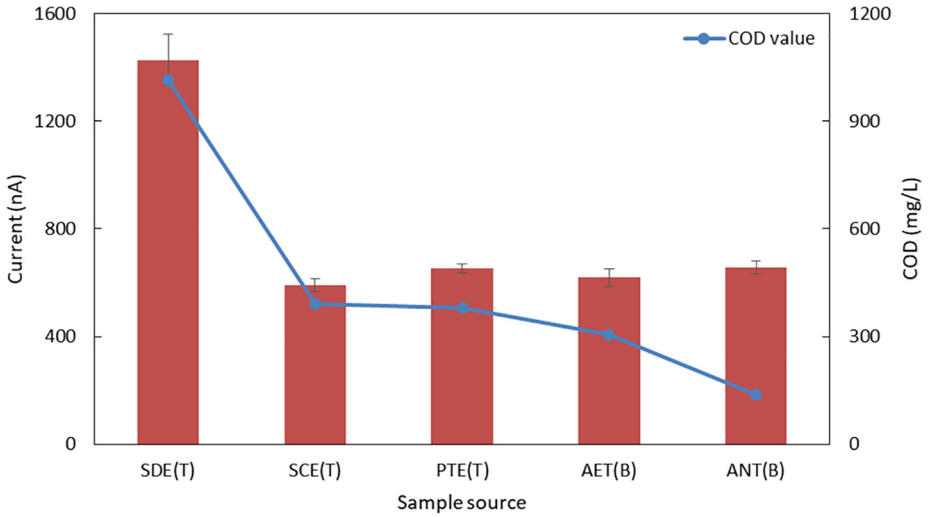


**Fig. 2.** Current generated (red) by the sensor when the miniaturized microbial fuel cell biosensor was fed with various wastewater samples whose COD is shown in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

xurographically fabricated MFC device, various wastewater samples were fed into the anode chamber to measure its electrical response to the different COD concentration of wastewater samples from various sources. Wastewater samples of second clarified effluent (SCE), primary tank effluent (PTE) and sludge dewatered effluent (SDE) from Toronto WWTP and aeration tank (AET) and anaerobic tank (ANT) from Burlington WWTP were introduced into the device one after another (with calibration anolyte between different testing samples) and the resulting peak current values were measured to evaluate the biosensor performance. Each concentration was measured three times. Before testing in the micro MFC device, the characteristics of the wastewater samples were determined using standard method (Table 1). The sensor response (shown in Fig. 2), demonstrates that the micro MFC biosensor is responsive to various sample and there is qualitative agreement between the sensor response and the COD of each of these samples measured through chemical means. It should be noted that the measurement is not an absolute measurement of the COD value and can only be co-related with BOD though appropriate calibration with various samples from the same source.

As Fig. 2 showed, sample SDE with a highest COD concentration of 1016 mg/L generated the highest current peak value of 1428.4 ± 95.22nA. Samples of SCE, PTE and AET with relatively lower values in COD concentration generated proportionately lower current peak values when these samples were fed into the sensor. It was interesting to note that sample ANT which had a much lower COD value of 107 mg/L still had a response that was similar (618.6 vs. 656.7nA) to sample AET which had a COD value of 307 mg/L. This interesting result suggests that the COD value of tested samples is not the only determining factor in the sensor response and other aspects of the sample showed also be analyzed. This is in contrast with the response of the sensor simulated samples were water is spiked with a model organic matter such as sodium acetate. In these experiments the sensor shows a proportional response to the COD values. Since the water samples were obtained from various locations within two different wastewater treatment plants, they could have different bacterial compositions in addition to organic matter. Some of these bacteria, especially those that are electroactive, could affect and influence the response of the MFC.

### *3.2. Biosensor response to wastewater samples with same COD and different sources*

In order to understand the factors that influence the sensor response to various samples from different wastewater treatment plants, experiments were conducted on those samples with same COD values but were from different sources. The DNA from the wastewater samples were extracted to analyze the types of bacteria in the microbial population in order to provide a deeper insight into the electrical response of the sensors. Samples SCE, PTE and a diluted SDE sample (38% dilution of SDE in order to obtain a COD value in the same range as the other samples) with 390, 381 and 386 mg/L in COD values, obtained from second clarified effluent, primary tank effluent and sludge dewatered effluent were used.

The measured peak current response of the MFC biosensor when exposed to various wastewater samples (n = 3) and corresponding COD values obtained from standard method is shown in Fig. 3a. In a μMFC, majority bacteria present in the feed can potentially interact with the electrode due to the configuration of the anode chamber which has a height of only 45 μm. The interaction of the bacteria in the feed with the electrode and its biofilm can produce synergistic effect which could enhance the conversion of organic matter in the feed to electrical current and affect the performance of the sensor. It can be seen that there are significant differences between the electrical responses of the biosensor to the various samples even as their COD is about the same. The peak current for PTE sample was found to be the highest at 653.1 ± 14.99 nA while the SCE sample had a current of 589.7 ± 23.76 nA and the diluted-SDE sample was the lowest at 470.4 ± 5.66 nA. Since the bacterial population on the electrode was largely affected by the feeding wastewater, the population in the feed was investigated as a potential source for this variation in response. *Geobacter* and *Shewanella* are considered as the most significant electroactive bacteria and the relative abundance and activity of these two genus should be correlated with the current generation (Okamoto et al., 2014; Shi et al., 2007). Molecular analysis of the sample for these two specific bacteria, as shown in Fig. 3b, reveals that there was no electroactive bacteria in the SCE sample, while similar abundance of *Geobacter* was found in PTE and diluted-SDE samples. Interestingly, *Shewanella* was found in greater abundance to *Geobacter* in PTE samples while it was present but not as significant in diluted SDE samples. The presence of additional electroactive species may explain the higher current generated in the biosensor when PTE samples were measured as compared with SCE. The additional electroactive bacteria could add to and displace other bacterial species on the biofilms in proximity to the current collectors in the electrode leading to a higher current generation (Shi et al., 2019). However, this does not explain the lower current obtained when diluted-SDE sample is measured with the biosensor as compared with the SCE sample. Therefore, the entire microbial population was analyzed for all the three samples and shown in Fig. 3c which list the top 20 genus in each. It can be seen that the microbial

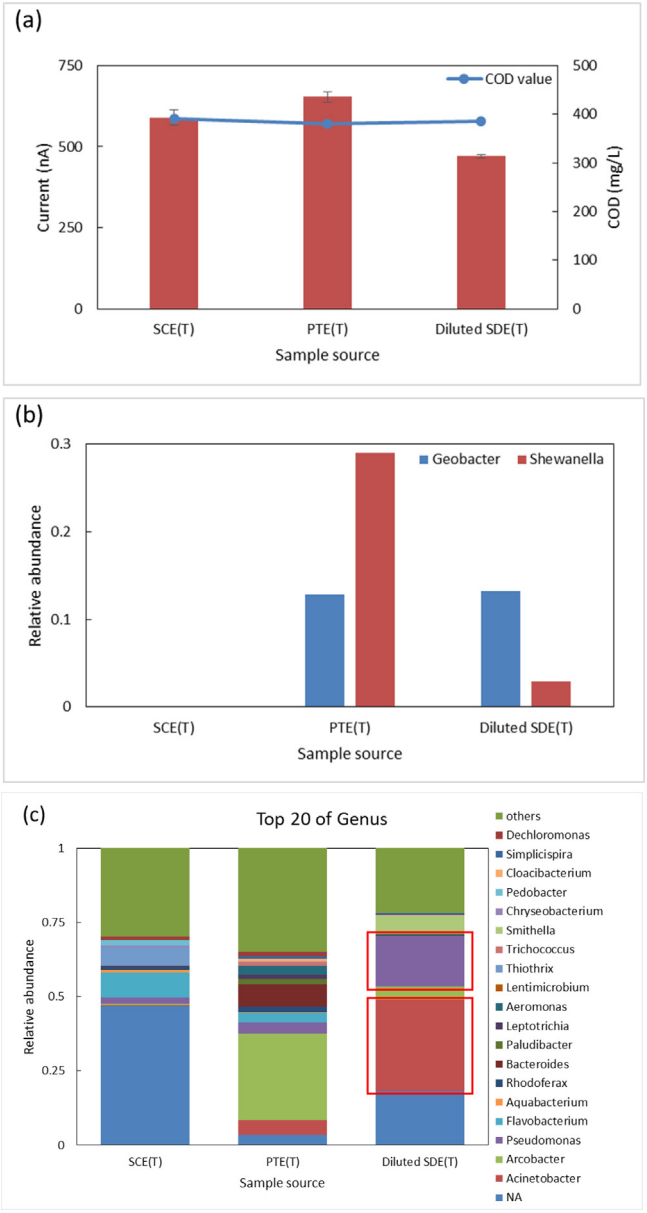


**Fig. 3.** The comparison of wastewater samples with same COD value and different sources. The sample "diluted SDE" was obtained by diluting the original sample SDE by 38% (addition of 62 mL of deionised water into 38 mL of sample SDE to get a testing sample of "diluted SDE"). (a) The measured peak current (red column) and the COD value obtained from standard method (blue line). (b) DNA analysis of the sample for *Geobacter* and *Shewanella*. (c) The top 20 genus present in the tested wastewater samples. Red wireframe marked are aerobic bacteria. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

population is very different in each of the samples. In the diluted SDE samples, *Acinetobacter* and *Pseudomonas* which are aerobic (Yang et al., 2019) are the major components of the population (~48%). Since the anodic chamber in a MFC is generally anaerobic (Chaudhuri and Lovley, 2003) especially under static conditions, many of them may be inactivated and unable to participate in the degradation of large organic matters to smaller one for the consumption of electroactive bacteria (Lovley, 2006). In contrast, the SCE samples does not have any significant amount of aerobic bacteria (Fig. 3c) and therefore the entire population may be able to participate in the organic matter degradation. Based on this reasoning, not only is the electroactive bacterial content of the sample important, but the proportion of the aerobic bacteria is also critical in electrical response of the MFC based biosensor and its co-relation with the COD. Concurrently, analysis of the proportion of nutrients present in the samples (Table 1) shows that the diluted SDE samples are carbon restrictive to the growth of bacteria as compared with the SCE and PTE samples. It is generally recognized that the ratio of nutrients needed for the metabolism of anaerobes should be 200:5:1 of COD:N:P (Talarposhti et al., 2001) for optimal growth of bacteria and the significant deviation from this value could be another factor in the lower current generation capability of the diluted SDE sample. These findings, in combination, indicate that the electrical current obtained from the MFC biosensor is influenced by a variety of factors including the COD, the amount of electroactive and aerobic bacteria in the samples as well as the nutrient ratios present that allow growth and metabolism of the bacteria. It is interesting to note that many of these factors are important in the BOD and the biosensor could provide a complex and nuanced picture of the biological processes and parameters in the sample in an amalgamated form. Due to the sensitivity to the sample type, the biosensor is to be calibrated to the specific sample type and location and cannot be used as a universal sensor to provide absolute COD or BOD measurements.

### 3.3. Biosensor response to wastewater samples containing bacteria with different metabolic type

In order to understand the effect of metabolic type of the bacterial population on the sensor response, the samples from aeration tank (AET) and anaerobic tank (ANT) were compared. The aeration tank will primarily have aerobic bacteria while the anaerobic tank sample is expected to have anaerobic bacteria.

The measured peak current response of the MFC biosensor when exposed to various wastewater samples and corresponding COD values obtained from standard method is shown in Fig. 4a. It can be seen that the electrical responses of the biosensor to the two samples is very similar even as their COD are significant different. The peak current for AET sample was found to be 618.58 ± 31.51 nA and the ANT sample had a current of 656.7 ± 23.01 nA, while the COD value of AET sample was 301 mg/L and ANT sample had a different COD value of 137 mg/L. These results further indicated the microbial community composition and its activity in these two samples could differ and influence the current generated. The relative abundance of aerobic and anaerobic (contains facultative anaerobic) bacteria were classified according to the 16S DNA analysis data and the information from (Moat et al., 2002), and shown in Fig. 4b. The aerobic bacteria constituted about 43.14% of the AET sample while it was 26.38% of ANT sample. Similarly, anaerobic bacteria constituted 26.57% and 41.76% of the AET and ANT sample, respectively, as shown in Fig. 4b. Since the anodic chamber of the MFC will be anaerobic, the relatively larger composition of anaerobes in the ANT sample will be able to break down complex organic matter in the sample into simpler ones that are then consumed by the electrogenic bacteria and could result in a more efficient current production. The aerobic bacteria become inactive under the same conditions and do not participate in the organic matter breakdown. This results strongly emphasizes that the metabolic type of the bacteria in the sample feed is also an important consideration in addition to the amount of electroactive species that may be present.

### 3.4. Biosensor response to wastewater samples from single source with different COD

In order to evaluate the biosensor performance in measuring the COD levels of the wastewater sample from the same source, a panel of diluted samples was prepared and measured using the biosensor. The dilutions provide different COD levels while still maintaining the complexities of the organic matter content as well as the other chemical and biological parameters in the sample. Samples of SCE, AET and ANT were diluted by half, one quarter and one eighth to form the sample

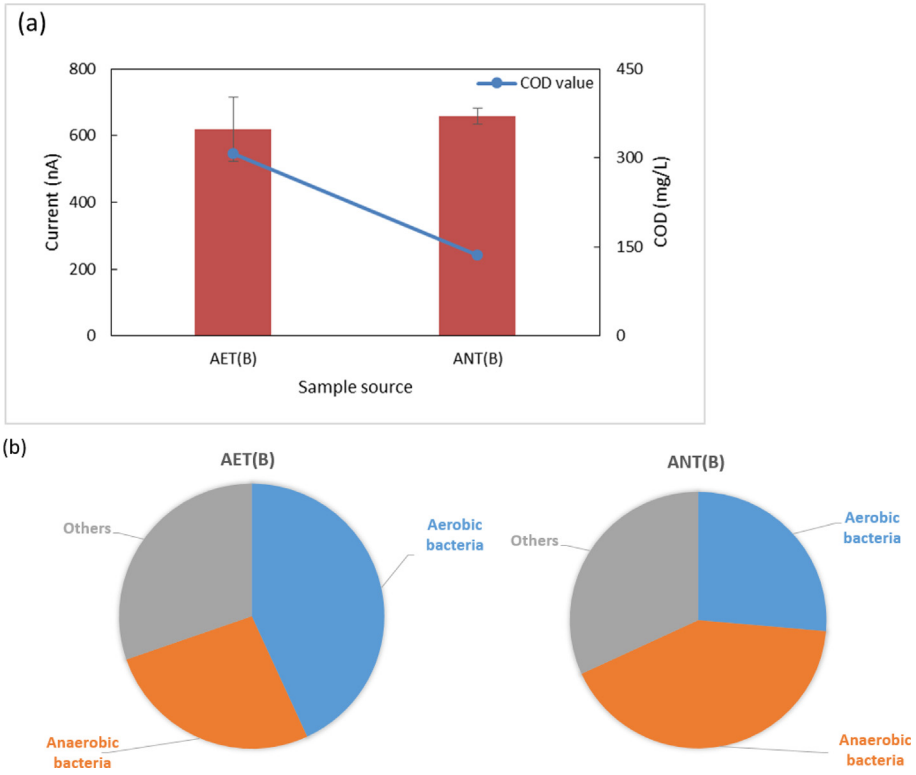


**Fig. 4.** The comparison of wastewater samples from aerobic vs. anaerobic environments. (a) The measured peak current (red column) and the COD value obtained from standard method (blue line). (b) DNA analysis for *aerobic and anaerobic bacteria* of 20 genus of the tested wastewater samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

panel and then introduced into the anode chamber of the MFC device one by one when it is in a stable operation mode. The peak current values were recorded and plotted to corresponding COD values. Each diluted sample was tested for three times at room temperature.

The biosensor performance, shown in Fig. 5, demonstrates a reasonably linear co-relation between the current generated and the COD of the samples. After dilution, the conductivity changes and will also have an effect on the current (albeit small) generated in addition to the change in concentration of the organic matter in the diluted samples. The linearity is significant with the ANT and SCE samples while less so with the AET samples. For instance, ANT sample and its dilutions has higher $R^2$ of 0.9594 and higher slope of 2.65 even though the COD values of ANT is relatively lower than that of corresponding SCE and AET samples. As discussed in the previous section, the abundance of anaerobic microorganisms in the sample ANT efficiently degrade the complex organic matter into simpler ones that facilitate the electroactive bacteria on the anode to efficiently convert them and produce a current response. Similarly, the aerobic microorganisms in the sample AET play the opposite role, which not only results in reducing the current generated by the biosensor, but also reduces the linearity of its response ($R^2$ value to only 0.8938). This is further supported by the sensor response to the diluted SCE samples. The linearity of SCE sample panel is better than AET and worse than ANT ($R^2$ value is 0.9504 but the slope is relatively lower) as its bacterial population is not dominated by well-known aerobic or anaerobic species. As expected the various sample sources had different slopes and the determination of this slope is the calibration that is required to customize the sensor for intended use at a particular location. Since the microorganisms in the sample have a significant role to play in the response, filtration of them prior to analysis could be undertaken to obtain a much more standardized response from these sensors. The exposure of the MFC biosensor to wastewater samples with different chemical compositions can in turn influence the bacterial population of the biofilm and its distribution over long exposure durations. This could cause a drift in the response of the sensor which may require periodic calibrations against standard samples. These effects need to be carefully studied and the long term performance of the biosensor in field conditions and the drift associated with exposure to multiple sources assessed.

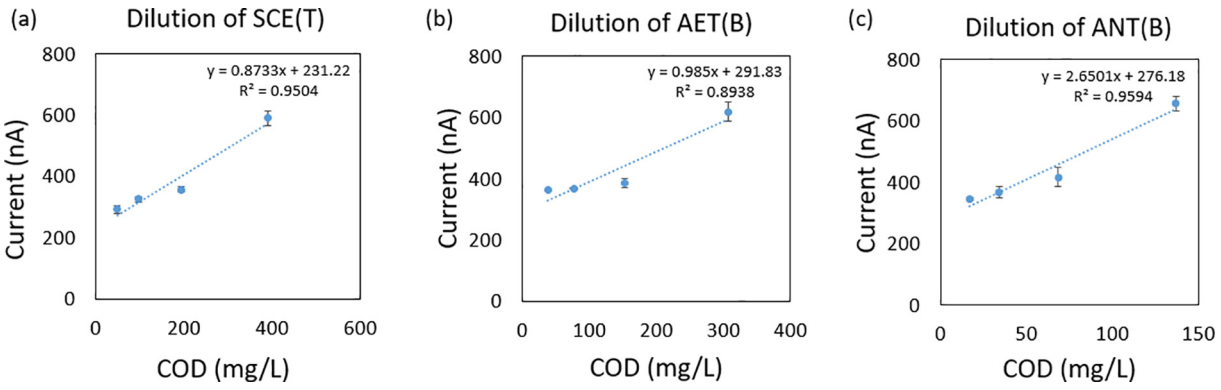


**Fig. 5.** The relationship between the current generated and the COD of the samples. Linear fits were obtained for the various samples SCE(T), AET(B) and ANT(B) that were serially diluted indicating that when calibrated to a sample type the sensor could be used to measure COD.

## 4. Conclusions

This study demonstrated that the micro-MFC biosensor is responsive to a broad range of COD concentrations. Interestingly, the peak current was sensitive to not only COD concentrations but also electroactive and aerobic bacteria as well as the nutrient ratios in the water samples. MFC based biosensor should be developed and proposed for a specific type of wastewater. This study provided a nuanced understanding of the bacterial population of a sample on the response of a MFC based biosensor. The sensor with appropriate calibration can be used to indirectly measure BOD given that the ratio of the bacteria consumable to non-consumable organic content remains the same.

## CRediT authorship contribution statement

**Nan Xiao:** Conceptualization, Methodology, Software, Investigation, Writing - original draft. **P. Ravi Selvaganapathy:** Writing - review & editing, Supervision, Data curation. **Rong Wu:** Validation, Formal analysis, Visualization, Software. **Jinhui Jeanne Huang:** Writing - review & editing, Supervision, Data curation.

## Acknowledgements

This research was financially supported by the National Key Research and Development Program of China (2016YFC0400709), Ministry of Science and Technology of the People's Republic of China; the program of China Scholarships Council (No. 201806200149). It is also supported by the Canada First Research Excellence Program through the Global Water Futures Project, Canada. Ponnambalam Ravi Selvaganapathy wishes to acknowledge support from the Canada Research Chairs Program and the Ontario Research Fund. Finally, we would like to thank Prof. Younggy Kim at McMaster University for the bacterial source provided for supporting this study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.122777.

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