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# A comparison of microbial fuel cell and microbial electrolysis cell biosensors for real-time environmental monitoring

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ABSTRACT

This study compares the biosensing performance of a microbial fuel cell (MFC) and a microbial electrolysis cell (MEC). Initial tests provided a qualitative comparison of MFC and MEC currents after the anode compartment liquid (anolyte) was spiked with acetate, or sulphates of $NH_4^+$, $Na^+$, $Mg^{2+}$, $Fe^{2+}$, or a fertilizer solution. Current measurements showed that the MFC sensor had a faster response time, higher sensitivity, and faster recovery time after the spike. Following the spike tests, the MFC and MEC were operated in a continuous flow mode at several influent concentrations of acetate, and sulphates of $NH_4^+$, $Na^+$, and $Fe^{2+}$. The continuous flow tests confirmed the better performance of the MFC sensor, which was selected for further experiments. Two MFC sensors were used for real-time (on-line) COD measurements of brewery wastewater. Regression analysis showed a strong correlation between the MFC power output and COD concentrations in the anode compartment with a coefficient of determination ($R^2$) of 0.97. Overall, results of this study suggest that an MFC-based sensor can be successfully used as a simple and cost-efficient real-time monitoring tool.



## 1. Introduction

The approach of environmental monitoring using biological systems (biosensing) is growing in popularity. The fundamental principle for all biosensors is that biological responses are converted to measurable signals, which are used to detect and validate the presence of organic and inorganic environmental pollutants (e.g. ammonium, heavy metals, hydrocarbons, etc.) or changes in the pollutant concentration. Since biosensors can be inexpensive, compact and easily deployable on-site, they provide several advantages over the traditional methods of analysis [1–3]. In particular, bioelectrochemical sensors, specifically microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) provide advantages such as a long lifespan with little or no maintenance, simplicity of the mechanical and electrical design, and a low construction cost [4].

MFC and MEC current depends on the oxidation of organic materials (electron donors) and the transfer of electrons to the anode by electroactive microorganisms [5]. A change in the anolyte concentration of the electron donors or alternative electron acceptors is reflected in the generated electrical current [6]. Accordingly, variations in the observed current can be correlated with changes in the composition of the anolyte [4]. MFCs and MECs have been used as biosensors for the monitoring of chemical oxygen demand (COD), biochemical oxygen demand (BOD) [7–9], dissolved oxygen [10], carbon monoxide toxicity [11], heavy metal toxicity [12], volatile fatty acids and ammonia in anaerobic digesters [13,14]. Although MFCs and MECs are similar in design, in an MEC an external voltage is applied to overcome thermodynamic limitations of this anaerobic system [15].

While both MEC and MEC – based biosensors have been proposed [7–14], a direct comparison of their biosensing performances is lacking. In this work, we evaluate performance of similarly designed continuous flow MFC and MEC for measuring concentrations of acetate, $NH_4^+$, $Na^+$, $Mg^{2+}$, and $Fe^{2+}$. A single chamber membraneless design is employed for both sensors to reduce construction costs and improve sensitivity as compared to the double chamber design [16,17].

## 2. Materials and methods

### 2.1. Stock solutions and analytical measurements

Solutions of ammonium sulphate (800 mg $L^{-1}$ as $NH_4^+$), magnesium sulphate (100 mg $L^{-1}$ as $Mg^{2+}$), iron sulphate (400 mg $L^{-1}$ as $Fe^{2+}$) and sodium sulphate (220 mg $L^{-1}$ as $Na^+$) were prepared by dissolving corresponding anhydrous sulphates in deionized water. Also, a commercial fertilizer with an N-P-K ratio of 20:20:20 was dissolved in deionized water to obtain a concentration of 50 mg $L^{-1}$. For the tests with $Fe^{2+}$, to reduce oxidation and precipitation before reaching the reactor, the stock solution was prepared with degassed water and delivered to the MFC using a syringe pump with the syringe replaced every 24 h.

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The concentrated solution of nutrients used during continuous sensor operation was composed of (in g $L^{-1}$): yeast extract (0.8), $NH_4Cl$ (18.7), KCl (148.1), $K_2HPO_4$ (64.0), and $KH_2PO_4$ (40.7) and a trace metal solution [18]. Sodium acetate (40 g $L^{-1}$) was added to this solution when a carbon source was required. Brewery wastewater was obtained from Fleischmann's Yeast LTD (Calgary, AB, Canada). The brewery wastewater had a COD content of 37,122 mg $L^{-1}$. Detailed characteristics of this wastewater are provided in Supplementary Information (Table S1).

Acetate concentrations were measured by gas chromatography (Agilent, Wilmington, DE, USA) as described elsewhere [19]. The concentrations of cations and anions were estimated using an HPLC Alliance (Waters Limited, ON, Canada) and HPLC ICS-3000 (Dionex, Sunnyvale, CA, USA) respectively. More details of these analytical methods can be found elsewhere [19]. Dissolved metal concentrations ($Mg^{2+}$ and $Fe^{2+}$) were measured by inductively coupled plasma mass spectrometry (ICP-MS). Total chemical oxygen demand (tCOD) of the brewery wastewater was measured according to Standard Methods [20].

### 2.2. MFC and MEC design

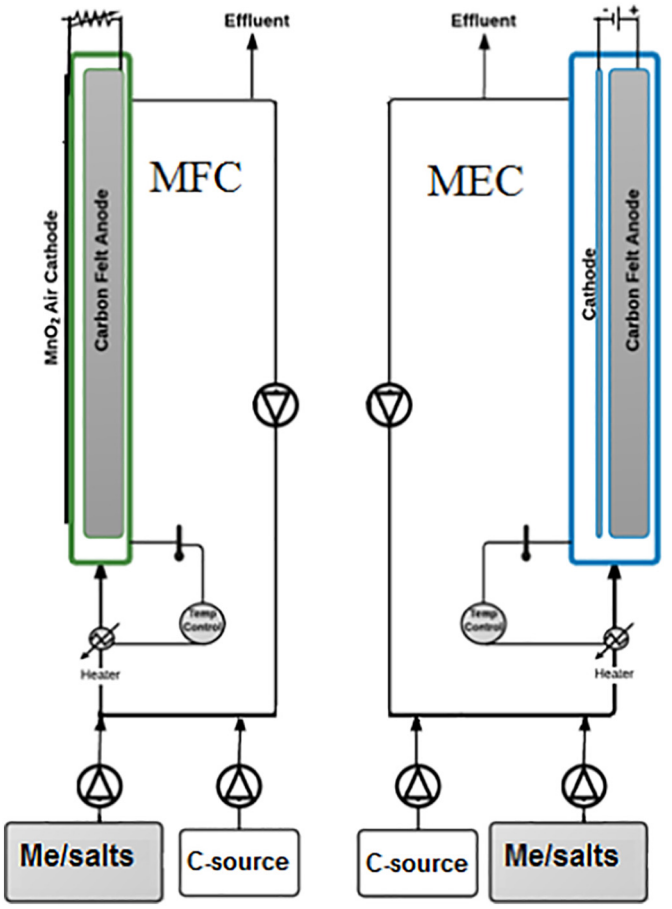
All sensors were constructed using polycarbonate plates following previously described designs of the membraneless air cathode MFC [21] and the single compartment membraneless MEC [22]. Schematic 1 shows the diagrams of both sensors. Both anode and cathode were placed in a single compartment, i.e. the MFC and MEC sensors had similar designs. Each sensor had an anode compartment volume of 50 mL. All anodes were made of carbon felt (SGL Group, Charlotte, NC, USA) with dimensions of 10 × 5 cm and a thickness of 5 mm. MFC cathode was a 10 × 5 cm manganese-based catalyzed carbon E4 electrode (Electric Fuel Ltd., Bet Shemesh, Israel) placed on one side of the anode compartment. The MEC cathode also measuring 10 × 5 cm was made of a carbon paper with electrochemically deposited Ni [23]. Overall, two MFC sensors and one MEC sensor were constructed for the tests.

In each sensor, liquid mixing was ensured using an external recirculation loop operated at a constant recirculation rate of 2 L $h^{-1}$. The external recirculation loops were equipped with flow-through heaters to maintain the temperature at 23 °C with the aid of a temperature controller (Model JCR-33A, Shinko Technos Co., Ltd., Osaka, Japan). Temperature probes were placed in anodic compartments. A pH of 7.0–7.5 was maintained by the buffering capacity of the influent nutrient solution.

### 2.3. MFC and MEC operation and electrochemical measurements

All MFC and MEC sensors used in the experimentation were inoculated with 5 mL of homogenized anaerobic sludge (Lassonde Industries, Inc., Rougemont, QC, Canada). The relative abundance (RA) of microbial families in this anaerobic sludge was previously characterized using high-throughput sequencing [24]. Dominant bacterial families were found to be *Propionibacteriaceae* (40.2%) and *Thermodesulfovibrionaceae* (29.5%), while *Methanobacteriaceae* (51.5%), *Methanosaetaceae* (15.5%), and *Methanoregulaceae* (5.7%) were the dominating archaeal families. Solutions of carbon source (acetate or wastewater) and dilution water were fed separately. The streams were combined into a single influent stream and continuously fed to the sensors to obtain a hydraulic retention time of 4.8 h (Scheme 1).

Table 1 describes the sequence of the tests and operating conditions of the MFC and MEC during each test. During phase 1 (spike tests), the MFC and MEC sensors were operated continuously with influent acetate concentration maintained at 150 mg $L^{-1}$, while inorganic contaminants (or additional acetate solution) were injected into the external recirculation loop of each sensor using 2 mL concentrated solutions. During phase 2, the compounds of interest were added to the dilution water and fed continuously, except for the tests at different acetate concentrations, where the influent acetate concentration was changed by adjusting the flow of the nutrients solution. A stabilization period of approximately 48 h was allowed after each compound or concentration change. Average values of electric current were calculated at the end of each stabilization period and used to evaluate sensor response to changes in the influent composition. During phase 1 and 2 the influent stream had a conductivity of 3.2–4 mS $cm^{-1}$. During phase 3, the nutrient solution was replaced with brewery wastewater fed at a constant flow rate. The wastewater concentration was changed by preparing diluted brewery wastewater. In all tests, the influent streams had a conductivity of 2–5 mS $cm^{-1}$. Influent and effluent samples were collected at least 48 h after each change in operating conditions.




**Scheme 1.** Schematic diagram of the microbial fuel cell (A) and the microbial electrolysis cell (B) used in this study.

During the spike and continuous tests, the MFC was operated at a fixed external resistance of 22.5 Ohm. The MEC was operated at an applied voltage of 1 V provided by a Keithley 2400 sourcemeter (Tektronix Inc., Beaverton, OR, USA). A 10 Ohm shunt resistor was used to measure MEC current. Voltage and current values of both systems were acquired using Labjack U3-LV (Labjack Corp., Lakewood, CO, USA) connected to a computer. During tests with brewery wastewater two MFC sensors were simultaneously operated. In these tests the MFCs were operated using a Perturb-and-Observe (P/O) algorithm [25], which maximized MFC power output in real time by constantly matching the internal and external resistances. Accordingly, a computer controlled digital internal resistor (4–125 Ohm) was used.

MEC current ($I$, mA) was calculated by measuring the voltage ($V$, mV) drop across a 10 Ω resistance. 1 V from a constant voltage source was applied for the operation of the MEC. A data acquisition program written in *Matlab R2014a* (Mathworks, Natick, MA, USA) was used for data logging. Correlations between the measured current and contaminant (or carbon source) concentration were computed in Microsoft Excel 2016 (Microsoft Corp, Redmond, WA, USA). The electrode potentials (vs a reference electrode) of both systems were not measured

**Table 1**
A summary of MFC and MEC tests.

| Description | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| Carbon source supply | Continuous | Continuous | Continuous |
| Contaminant introduction | Spike | Continuous | Continuous |
| Tested compounds | $CH_3COOH$<br>$(NH4)_2SO_4$<br>$MgSO_4$<br>$FeSO_4$<br>$Na_2SO_4$<br>Fertilizer (N:P:K = 20:20:20) | $CH_3COOH$<br>$(NH_4)_2SO_4$<br>$FeSO_4$<br>$Na_2SO_4$ | Brewery wastewater |
| Electrochemical parameters | MFC: $R_{ext}$ = 22.5 Ohm<br>MEC: voltage = 1 V | MFC: $R_{ext}$ = 22.5 Ohm<br>MEC: voltage = 1 V | MFC: $R_{ext}$ ~ $R_{int}$<br>(P/O algorithm) |

during the tests because of the compact nature of the anode chamber as well as the importance of avoiding oxygen intrusion into it.

The sensitivity of MFC and MEC sensors to an abrupt change in anolyte composition was evaluated during spike tests by comparing the amplitude of electric current change in response to the anolyte concentration change (normalized to the anode volume) [16]. Maximum current change after the spike was considered in the following equation.

$$Sensitivity = \frac{\Delta I}{\Delta S} \cdot \frac{1}{V} \quad (1)$$

where $\Delta I$ is the change in current (mA), $\Delta S$ is the change in the anolyte concentration (mg $L^{-1}$), and $V$ is the anode volume (L).

## 3. Results and discussion

The study was carried out in three phases. In phase 1, MFC and MEC biosensing performances were compared in a series of spike tests. In the next phase (phase 2), the two sensors were continuously fed with influent streams containing varying concentrations of compounds used in the previous set of tests. Once again, current changes were continuously measured. Steady state current values were then correlated with the corresponding concentrations. During the last set of experiments (phase 3), two MFC sensors were continuously fed with brewery wastewater. Serial dilutions of the wastewater stream were used to obtain different influent COD concentrations, then steady state values of MFC current and power output were correlated with the corresponding analytical measurements of CODs.

### 3.1. Spike tests

The spike tests were carried out to quantitatively compare changes in MFC and MEC currents in response to an abrupt change in anolyte composition caused by injecting an organic or inorganic compound of interest. Here, the sensitivity to an abrupt change in anolyte composition was expressed as a ratio of current to concentration variation according to Eq. (1). The spike tests were started by observing MFC and MEC response to acetate injection. For both bioelectrochemical systems acetate is a well documented carbon substrate. Several MFC studies demonstrated and explained rapid current increase upon acetate addition [19,26] and similar response was observed in MECs [15,27]. Not surprisingly, acetate injection (0.8 mL of stock solution containing 40 g $L^{-1}$ of acetate) to both sensors resulted in a current increase due to increased carbon source availability and therefore increased activity of anodophilic microorganisms. In the MFC sensor, currrent was increased from 2.2 to 2.6 mA, then gradually returned to its previous level due to acetate consumption and washout (Fig. 1A). In the MEC sensor, current increased from 2.7 to 3 mA and then decreased (Fig. 1B). It should be mentioned that prior to and during the acetate injection tests both sensors were operated at an influent acetate concentration of 150 mg $L^{-1}$, which explains the background current. The highest current was attained faster in the MFC (9.1 h after injection) than in the MEC (11.5 h), but near-peak values were sustained for a longer time in the MEC, apparently due to a slower acetate consumption. The sensitivity calculated based on the current peak (Eq. (1)) was higher in the MFC (50.9 A $g^{-1}$) than in the MEC (30.3 A $g^{-1}$).

The acetate injection test confirmed MFC and MEC response to changes in carbon source concentration. However, environmental monitoring often requires detection of organic and inorganic pollutants, which are not as easily degradable as acetate. A biosensor should have the potential to be used for monitoring complex industrial, agricultural, or mining effluents. The feasibility of MFC or MEC application for real-time biosensing of metal sulphates commonly found in mining waters [28], such as $Fe^{2+}$ and $Mg^{2+}$ was assesed in the next series of tests, where the two sensors were spiked with $FeSO_4$, and $MgSO_4$ as well as with sulphates of $NH_4^+$ and $Na^+$ and finally with a fertilizer. To support the anodophilic activity, continuous feeding of 150 mg $L^{-1}$ (influent concentration) of acetate was maintained for both sensors.

Injection of $MgSO_4$ and $FeSO_4$ led to a reversed response, where current was decreased immediately after the injection and then slowly recovered (Fig. 1C-F). The observed immediate drop in MFC current can be explained by a pH decrease from 6.3 to 4.8. Also, competition between the anode and metal ions as terminal electron acceptors for the metabolic activity of the anodophilic bacteria can be hypothesized. The current recovery was relatively slow and started after an approximately 5 h delay, which agreed with an HRT of 4.8 h at which the MFC was operated. This observation confirms the possibility of metal sulphates used as terminal electron acceptors by the anodophilic bacteria. Also, a higher current decrease was observed after $Fe^{2+}$ injection as compared to $Mg^{2+}$ injection, which agrees with the a higher redox potential of $Fe^{2+}$. In the MEC test, the $Mg^{2+}$ spike did not affect the current to the same extent as in the MFC, as only a small change in the current was observed (Fig. 1D). When $Fe^{2+}$ was added to the MEC, the response was similar to that observed in the MFC with a similar magnitude of current decrease and recovery period.

Interestingly, ammonium and fertilizer spikes showed similar responses. In the MFC, a spike with $NH_4^+$ resulted in an immediate current drop (Fig. 1G) followed by a subsequent current increase. The drop in current can be explained by the sudden change in the anodic compartment pH (6.2 to 5.8), while the current increase may be explained by $NH_4^+$ oxidation at the anode [29]. The subsequent decline in electricity production can be related to $NH_4^+$ washout from the anode compartment with a continuous inflow of water. In the MEC, ammonium injection led to a delayed current increase, while the initial decrease was not observed (Fig. 1H). Also, the peak was smaller. There was no significant response in MEC current upon fertilizer injection (Fig. 1J). The injection of a plant fertilizer into the MFC led to a response, which was similar to ammonium injection, i.e. after the initial current drop, a peak was observed (Fig. 1I). Overall, the higher sensitivity of MFC to $NH_4^+$ was obvious.

Injection of $Na_2SO_4$ lead to a consistent increase in the current of both sensors followed by a gradual current decline due to the salt washout after the injection (results not shown). No pH change was observed

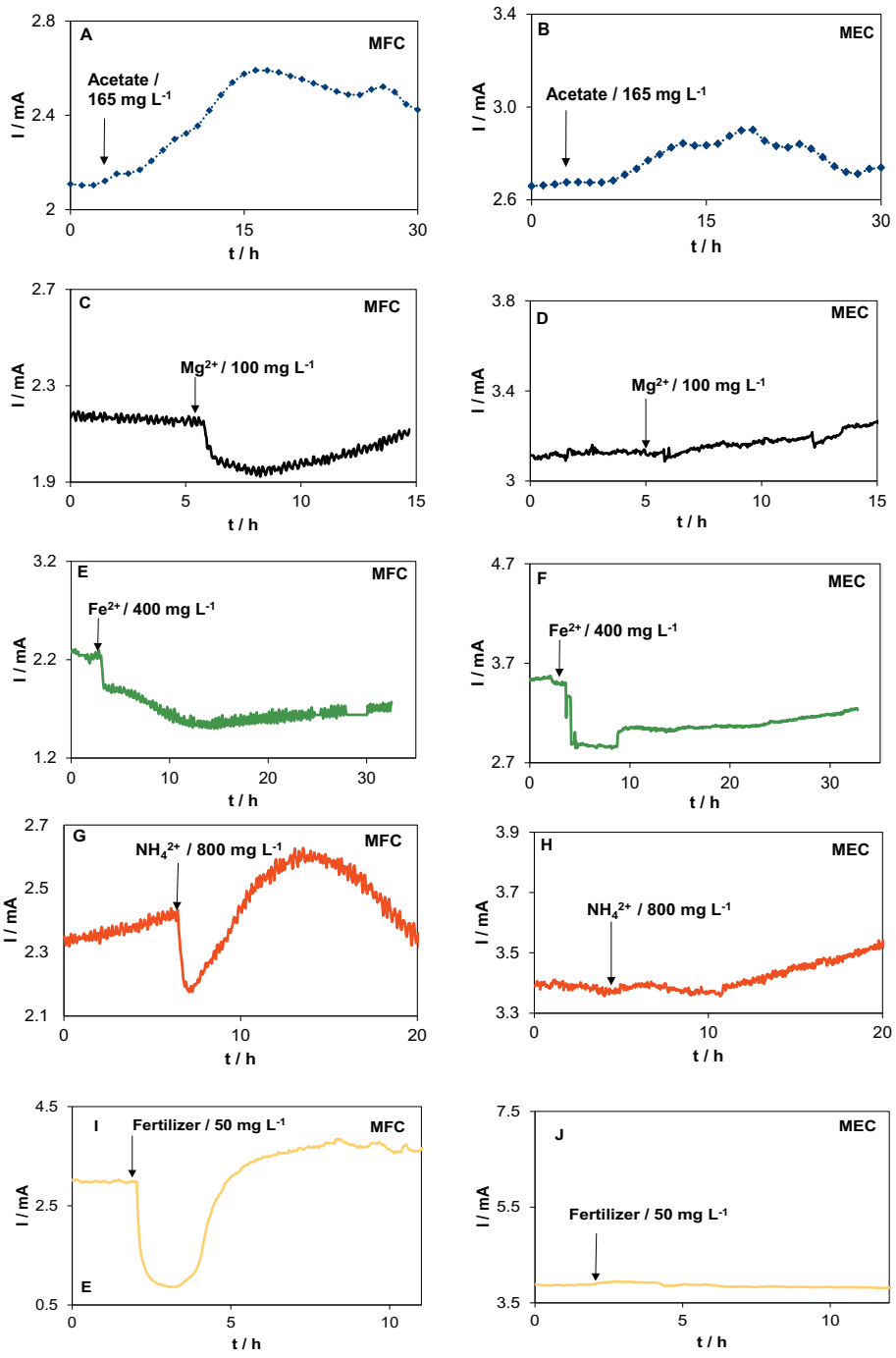



**Fig. 1.** Response of the MFC and MEC sensors to spikes of acetate (A, B), $MgSO_4$ (C, D), $FeSO_4$ (E, F), $(NH_4)_2SO_4$ (G, H), and fertilizer (I,J). Arrows indicate injection time.

with this injection, however, it might be noticed that this increase in MFC and MEC currents can be attributed both to increased conductivity (increased from 3.21 to 4.10 mS $cm^{-1}$), which decreased ohmic resistance [30] and to the presence of sulphates.

Fig. 2 summarizes MFC and MEC responses to spikes of various compounds in terms of current change. Once again, the comparison shows higher MFC sensitivity as compared to MEC. In addition, the response time of MFC was shorter. A summary of the estimated sensitivities of both sensors is presented in Table 2. Although both systems showed potential usage as a biosensor, the shorter response time and broader sensitivity of the MFC suggests that it may be more suitable for on-line biosensing, where quick detection is required. Although some performance improvements of MEC can be achieved, e.g., through anode compartment miniaturization [16], it can be concluded that the MFC had a better overall performance.

### 3.2. Continuous flow tests

Following the series of spike tests, response to changes in pollutant concentrations during continuous sensor operation was examined. The continuous flow tests provided an opportunity to establish correlations between the compounds of interest and the MFC or MEC current. Also, these tests provided further explanations on the chemical mechanisms of the MFC and MEC response. As in the spike tests, the study began with an initial evaluation of the MFC and MEC responses to changes in acetate concentration. The range of acetate concentrations

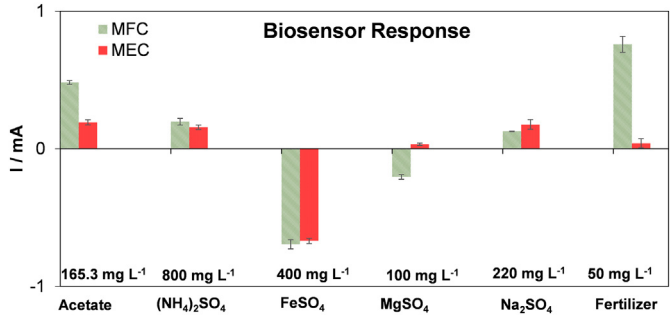



**Fig. 2.** Changes in MFC and MEC currents after injection of organic and inorganic compounds of interest.

chosen for these tests was between 50 mg $L^{-1}$ and 200 mg $L^{-1}$. Following the tests with acetate, the response to changes in influent concentrations of $Na^+$, $NH_4^+$, or $Fe^{2+}$ sulphates was evaluated. During these tests acetate concentration in the influent stream was maintained at 150 mg $L^{-1}$ to provide a sufficient amount of carbon source to anodophilic bacteria. Sensor response to concentration changes during continuous operation was expected to be somewhat different from the corresponding response obtained during the spike tests because of prolonged exposure of anodophilic bacteria to each compound.

Fig. 3 compares MFC and MEC responses in terms of steady state current values to several levels of acetate. As expected, there is a linear correlation between the current and the concentration of the acetate in the anodic compartment (Fig. 3A,B) since anodophilic activity is directly linked to the carbon source availability. At low acetate concentrations, the MFC current had a slightly better correlation ($R^2 = 0.84$) with the acetate concentration than the MEC current ($R^2 = 0.76$), but both sensors showed similar performance. Perhaps, this difference can be attributed to natural variations in microbial populations. In any case, the observed linear correlation of the MFC and MEC currents with acetate concentrations confirmed that both sensors respond positively (i.e current increased) to increases in carbon source concentration. Therefore, if the influent flow rate is fixed, the observed current can be used for estimating acetate concentration in the influent stream.

Continuous MFC and MEC operation with sodium sulphate in the influent stream at several concentrations ranging from 80 mg $L^{-1}$ to 500 mg $L^{-1}$ (as Na) showed no impact on current generation at steady state as concluded based on the determination coefficient ($R^2$) values, which were very low both for MFC and MEC (0.11 and 0.08, respectively) (Fig. 3 C,D). Notably, a slight increase in the MFC and MEC currents was observed in the spike test following the injection of a concentrated sodium sulphate solution (2 mL of 5.5 g $L^{-1}$ solution resulting in a final $Na_2SO_4$ concentration of 220 mg $L^{-1}$, Fig. 2). This increase can be attributed to temporarily increased solution conductivity, rather than a change in the anodophilic metabolic activity due to the addition of sulphate.

MFC and MEC operation with ammonium sulphate in the influent stream showed a linear correlation between the steady state current values and ammonium concentrations for both sensors, i.e., results of the continuous flow and spike tests with ammonium were in a good agreement (Fig. 3E,F). As mentioned earlier, the current increase can be attributed to bioelectrochemical oxidation of ammonium by anodophilic bacteria. Bioelectrochemically catalyzed ammonium removal was recently demonstrated both in an MFC and an MEC [29]. It might be mentioned that although statistical analysis showed a higher correlation ($R^2 = 0.85$) of ammonium concentrations with MFC current than with MEC current ($R^2 = 0.65$), these values were calculated only using three measurements and should only be considered as trends. More detailed investigation of current dependence on ammonium concentration might be required to confirm the response of bioelectrochemical systems to this compound.

**Table 2**
Estimated sensitivities of MFC and MEC biosensors. Expected concentration of each compound immediately after injection is shown in brackets.

| Compound | Sensitivity (A.g $^{-1}$) | |
|---|---|---|
| | MFC | MEC |
| Acetate (165) | 50.9 | 30.3 |
| $NH_4^+$ (800) | 11.25 | 5 |
| $Mg^{2+}$ (100) | −48 | 34 |
| $Fe^{2+}$ (400) | −35 | −30 |
| $Na^+$ (220) | 48.4 | 30.3 |
| Fertilizer (50) | 120 | 12 |

Because of consistently better overall performance of the MFC sensor, the following test, which used $FeSO_4$, was carried out only with the MFC sensor, while MEC operation was terminated. $Fe^{2+}$ readily oxidizes into $Fe^{3+}$ when dissolved in aqueous solutions, however water degassing (oxygen removal) prior to solution preparation and frequent solution change (e.g. daily) might mitigate this effect. The results of this test, shown in Fig. 4, confirm conclusions of the spike test with iron sulphate, in which a negative dependence of the MFC current on $Fe^{2+}$ concentration was observed. Based on six tested concentrations of $Fe^{2+}$, statistical analysis showed a determination coefficient $R^2$ of 0.81 (Fig. 4A). The test also demonstrated successful recovery of the MFC sensor after it was exposed to high levels of $Fe^{2+}$ leading to nearly zero current generation (results not shown). Acetate measurements in the MFC effluent showed a decrease of acetate concentration with increasing concentrations of $Fe^{2+}$ (Fig. 4B). This trend confirms the hypothesis of $Fe^{2+}$ acting as an alternative electron acceptor and therefore resulting in a decreased MFC current.

Overall, results of continuous tests were consistent with the spike test conclusions. While both sensors showed sensitivity to several common environmental pollutants, the MFC-based sensor demonstrated a higher sensitivity and responded to a larger number of pollutants. It should be mentioned that in the presence of multiple pollutants with varying concentrations, the MFC sensor output is expected to reflect a general solution toxicity, thus providing real-time toxicity estimations, which could replace standard toxicity assays, which are costly, and laborious. Also, it is worth noticing that inferior performance of the MEC sensor can be resolved by improved design, e.g. to avoid the cross flow of hydrogen from cathode to anode by implementing PEM-based design.

### 3.3. Real-time monitoring of brewery wastewater

Based on the comparison of MFC and MEC performances described above, in the brewery wastewater tests the MEC was replaced with a second MFC and the two MFC sensors were continuously fed with varying concentrations of brewery wastewater to confirm the reliability of the proposed monitoring approach for on-line COD measurements. Brewery effluents contain multiple organic and inorganic compounds [31,32]. The objective of the brewery wastewater biosensing tests was to demonstrate on-line COD measurements of a wastewater stream with a complex composition. Unlike other biosensing studies, which estimated CODs by calculating total current produced during batch MFC operation resulting in long testing times [31], the real-time monitoring needs to be fast. Accordingly, the MFC needs to be operated in a continuous, flow-through, mode at a short HRT. Limited COD degradation in the MFC anodic compartment might be expected under such conditions. To compensate for an expected small difference between the influent and effluent COD concentrations, it was decided to operate the MFCs at an optimal external resistance ($R_{ext}$). Accordingly, the on-line Perturb-and-Observe (P/O) algorithm for real-time $R_{ext}$ adjustment was employed [25]. This algorithm provided dynamic adjustment of $R_{ext}$ to match MFC's internal resistance, thus maximizing the power output at all times. The MFCs were operated at several influent COD concentrations with COD analytical measurements at the end of each testing period (approximately 48 h).

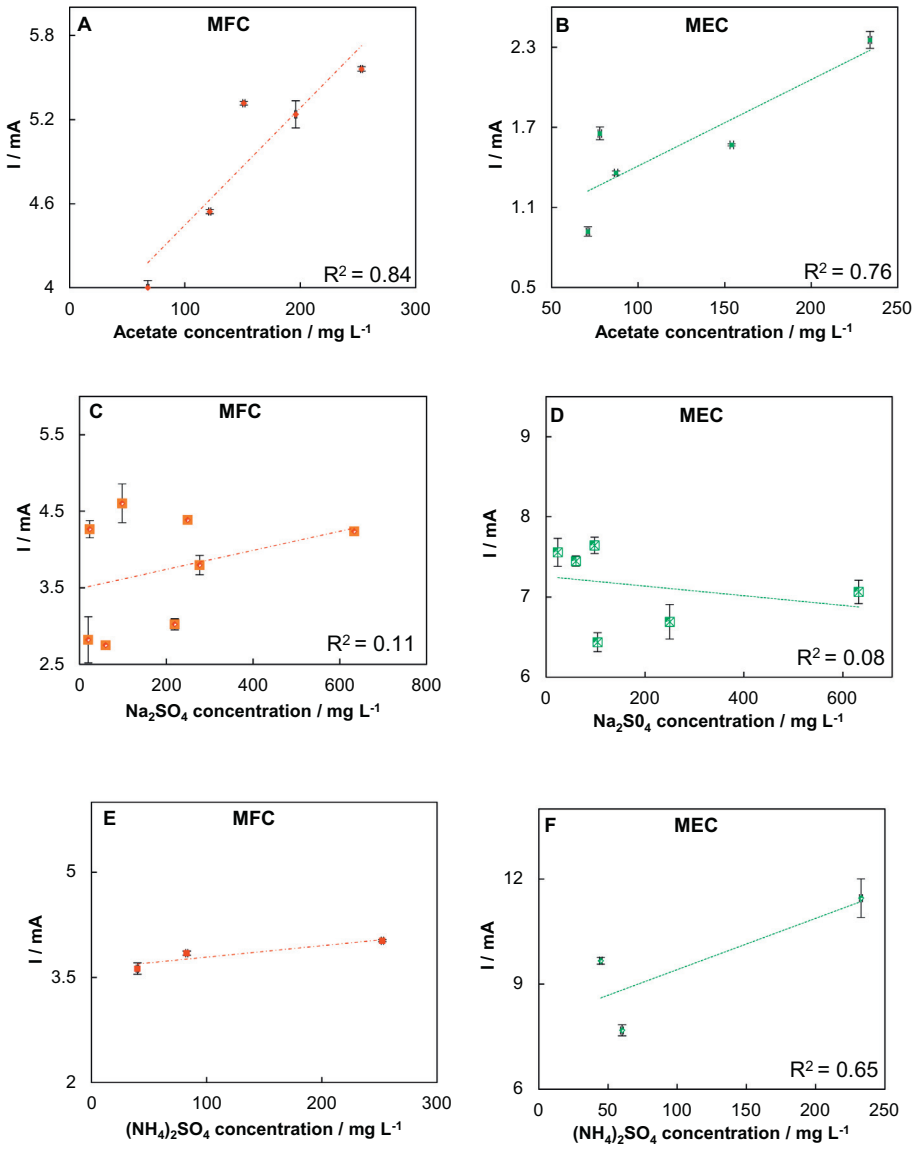



**Fig. 3.** Steady state current of the MFC and MEC sensors at different influent concentrations of acetate (A, B), $Na2SO_4$ (C, D), and $(NH_4)_2SO_4$ (E, F). Error bars represent standard deviation.

Fig. 5 shows the results of on-line COD monitoring using the two MFC sensors. Under steady state conditions, the relationship between the optimized power of the MFCs and the estimated COD concentrations in the anode compartment (Fig. 5A, D) was linear with a coefficient of determination ($R^2$) of 0.97 in MFC 1 and 0.98 in MFC 2. The linear relationship with high $R^2$ values was also obtained between the steady state current and the effluent COD concentration ($R^2 = 0.90$ for MFC 1 and $R^2 = 0.97$ for MFC 2) as shown in Fig. 5B, E). Finally, the COD concentrations were also correlated with the estimated internal resistance values (Fig. 5C, F). Here internal resistances were assumed to be equal to $R_{ext}$ values estimated by the P/O algorithm. These relationships show that once the MFC sensor is calibrated, it can be successfully used for on-line COD monitoring. Also, analysis of sensor dynamics shows that after each concentration change steady state current and power output values are attained after approximately 50 h. (Fig. 6). The approach of changing the influent COD concentration by diluting the stock brewery wastewater ensured that the response of the system was not due to volume changes or disruption of the anolyte flow in the reactor, as observed elsewhere [33]. It also explains why the current/power response is gradual rather than sharp as suggested in Chang et al. [6]. The approach of internal resistance optimization might be particularly useful in practical applications like monitoring of groundwater, lakes, mine drainages, etc. It can be suggested that the response time can be further reduced by MFC operation at a shorter HRT. The results from this test also showed that COD concentrations at least up to 500 mg $L^{-1}$ could be monitored in real time using the continuous flow MFC sensor. This range is significantly above the 100 mg $L^{-1}$ limit reported by Chang et al. [6]. It appears that the tracking of the maximum power point by the P/O algorithm might have contributed to the increase in the range of COD measurements, further highlighting the importance of MFC operation at an optimal value of $R_{ext}$. Finally, the relationship between the estimated internal resistance and COD concentration in the anode compartment (Fig. 5C, F) was found to be linear when the COD concentration was below 500 mg $L^{-1}$ ($R^2 = 0.91$ for MFC 1 and $R^2 = 0.97$ for MFC 2). This correlation shows a decrease in the internal resistance with increasing COD concentration. However, at concentrations above 500 mg $L^{-1}$ the dependence becomes non-linear.

## 4. Conclusion

This study evaluated performance of MFC and MEC – based sensors for on-line measurements of several organic and inorganic compounds including acetate, brewery wastewater, ammonium, and metal

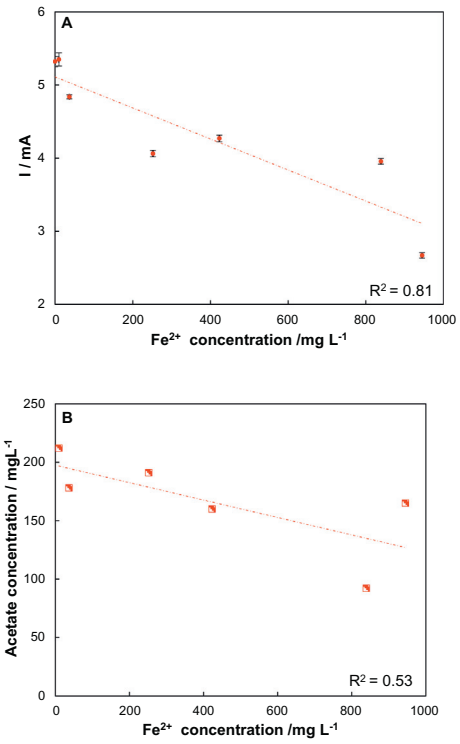



**Fig. 4.** (A) Steady state current of the MFC sensor at different influent concentrations of $FeSO_4$ (A), and (B) corresponding effluent acetate concentrations. Error bars represent standard deviation.

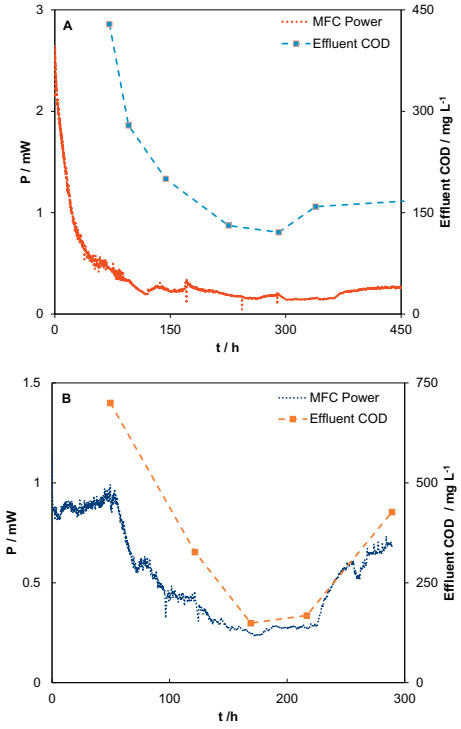



**Fig. 6.** Changes in power output of (A) MFC 1 and (B) MFC 2 operated at various concentrations of brewery wastewater.

sulphates. Although both MFC and MEC sensors showed reasonable responses to concentration changes, the MFC appeared to provide a more reliable response with a near linear dependence of current and/or MFC power output on compound concentration. Also, the MFC sensor had a faster response time, recovered faster from exposure to high concentrations, and demonstrated a broader response range.

MFC reliability as a sensor for on-line COD measurements was confirmed in a series of tests with brewery wastewater. Test results showed a high correlation between the MFC current and power output and COD concentrations. Overall, it can be concluded that, indeed, MFCs can be used for real-time biosensing of a variety of important organic and inorganic pollutants in agricultural, industrial, and mining effluents. It should be mentioned that biosensor design (e.g. membraneless or with a proton exchange membrane) and even biosensor type (MFC or MEC) might depend on particular operating conditions and the target organic or inorganic compound.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2018.11.007.

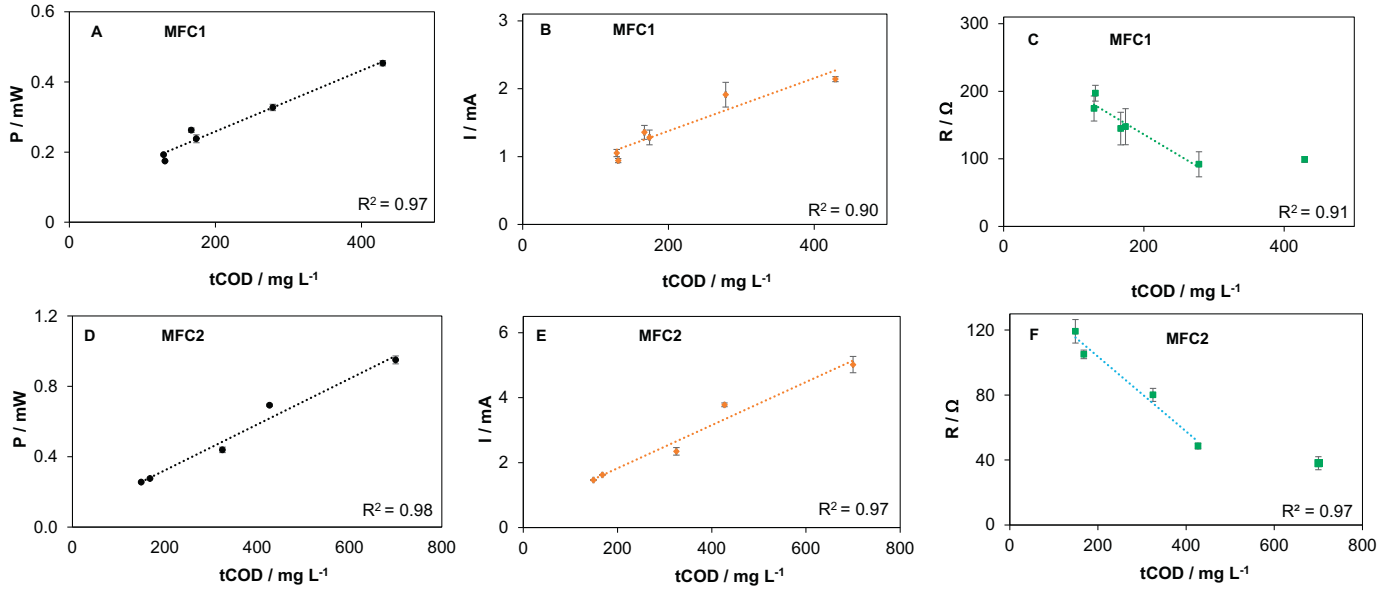



**Fig. 5.** Results of real-time brewery wastewater monitoring using two MFC biosensors (MFC 1 and MFC 2) showing concentration correlation with MFC power output (A, D), current (B, E), and internal resistance (C, F) estimated using the P/O algorithm.

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