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Enhancing the robustness of microbial fuel cell sensor for continuous copper(II) detection against organic strength fluctuations by acetate and glucose addition

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Abstract

Microbial fuel cell sensors have shown great promise for continuous monitoring of toxic substances in wastewater, but a persistent problem is the signal interferences due to fluctuations in organic strength. An approach to eliminate the interferences is to saturate the sensor with an added organic substrate. In this study, signal stabilization using acetate and glucose addition (150, 300 and 500 mg COD/L) to domestic wastewater was examined. Addition of acetate (500 mg COD/L) gave the best performance, increasing the robustness of the sensor by reducing signal interference (decrease in baseline current) from 65% to 15% for a sudden 75% decrease in organic strength. The sensor sensitivity remained unchanged at current drop of 0.16%/(mg/L Cu(II)) for a toxicity event (300 mg/L Cu(II)). Addition of acetate (300 mg COD/L) and glucose (150, 300 and 500 mg COD/L) also resulted in increased robustness but led to a reduced sensitivity to Cu(II).

Keywords: Microbial fuel cell, Biosensor, Copper, Substrate addition, Acetate, Glucose

1. Introduction

Toxicants present in wastewater could adversely inhibit the activity and viability of microorganisms in biological treatment systems in wastewater treatment plants (Madoni et al., 1999). Hence, a sensor is needed to detect the toxicants before the wastewater is allowed to enter the treatment process. Manual sampling and analysis of wastewater are laborious and costly, and produce non-real-time data due to the time lag between sampling and analysis.

Recently, the use of a microbial fuel cell (MFC) sensor has been proposed as a viable alternative technology for online detection of toxicant in wastewater as it allows for real time bio-monitoring of toxicants. In a toxic event, the electrical current produced by the electroactive biofilm in the MFC sensor decreases quickly and triggers an early warning system so that preventive measures can be taken by the plant operators immediately. The results of earlier studies on MFC sensor (Di Lorenzo et al., 2014; Du et al., 2017; Jiang et al., 2015; Li et al., 2016; Shen et al., 2013; Wang et al., 2013a) showed that the microbial fuel cell sensor exhibits distinct advantages over traditional biosensors such as high sensitivity, quick response time, low maintenance and online monitoring (Sun et al., 2015; Xiao et al., 2015).

To date, the studies on MFC sensors only focused on investigating the types of toxicants that can be detected and optimizing the sensor architecture or operating conditions to increase the sensitivity of the sensor. The robustness of the sensor is a critical factor that has been overlooked by previous studies. For the sensor to be robust, the sensor signal must not only be sensitive to toxicants, but also not be affected by non-toxic events. In field application of the MFC sensor, however, the characteristics of the streams to be monitored will exhibit a high level of diurnal variations, causing

perturbation on the sensor signal recorded. A pertinent issue is the susceptibility of the MFC sensor to false positives due to a decrease in the organic strength of the wastewater (Chang et al., 2004; Di Lorenzo et al., 2009; Kim et al., 2003; Liu & Logan, 2004). The electrical current recorded by the MFC sensor will decrease for both a toxic event and an event of a sudden decrease in the organic strength of the stream.

This study investigated the addition of acetate and glucose as model supplementary substrates to domestic wastewater to enhance the robustness of the MFC sensor signal for the detection of Cu(II) against fluctuations in organic strength of the wastewater. Acetate and glucose are electron donors which could be utilized by the electroactive bacteria in the sensor. The addition of substrates would enable the microorganisms in the sensor to grow in a substrate saturated environment so that the variation in the organic strength of the wastewater would have minimal effect on the sensor signal (Jiang et al., 2016). The response of the sensor without the addition of acetate and glucose was first investigated and the results were compared to the results of subsequent tests with acetate and glucose addition (150, 300, and 500 mg/L added COD). The focus of this study is to investigate the response of the MFC sensor to the model toxicant Cu(II) at different concentrations (i.e., 50, 100, 200, and 300 mg/L) and their response to organic strength fluctuations (i.e., 25%, 50%, and 75% decrease in organic strength).

The aim of the study was to determine the optimum substrate type and the substrate concentration to be added to increase the robustness of the MFC sensor against organic strength fluctuations. The effect of the substrate addition on the sensitivity of the MFC sensor to Cu(II) was also investigated.

2. Material and Methods

2.1. MFC sensor design and operation

The MFC sensor design was adapted from a previous study (Shen et al., 2013) and consisted of a single chamber air-cathode MFC with a serpentine flow path anode chamber (net volume of 43.2 mL) with carbon cloth (Fuel Cell Earth, USA) as anode (43.2 cm^2). The anode chamber was separated from the Pt-loaded carbon cloth cathode (43.2 cm^2, 0.5 mg/cm^2 20% Pt, Fuel Cell Earth, USA) by a proton exchange membrane (Selemion HSF, Asahi Glass Co., Japan).

The MFC sensor was operated at an optimum HRT of 2 min (Shen et al., 2012) in a continuous-fed mode with recirculation of the wastewater. An external resistance of 5Ω was used. The MFC sensors were inoculated with domestic wastewater for 4 to 5 weeks before any experiment was carried out. Triplicates were used for both the wastewater dilution tests and the Cu(II) dosing experiments to ensure accuracy of results. The domestic wastewater (pH = 7.06 ± 0.24, COD = 315 ± 49 mg/L) was collected from the Ulu Pandan Water Reclamation Plant, Singapore, and kept in cold room at 4^oC to inhibit degradation. The wastewater was brought to room temperature before being fed to the MFC sensors.

2.2. Experiments using Cu(II) and organics at different concentrations

As shown in Table 1, experiments using different Cu(II) (i.e., 50, 100, 200, and 300 mg/L) and organics concentrations (i.e., 25%, 50%, 75% wastewater (v/v)) were carried out without any addition of substrate after the inoculation phase was complete. Cu(II) dosing tests were carried out by challenging the MFC sensor with the model toxicant – Cu(II) – by adding $CuCl_2$ into the wastewater feed to achieve different Cu(II)

concentration of 50, 100, 200, and 300 mg/L. The Cu(II)-laden wastewater was fed continuously to the MFC sensors for 2 h before the feed was reverted to fresh wastewater without $CuCl_2$ added.

To simulate an event of sudden decrease in organic strength of domestic wastewater, the domestic wastewater was diluted with tap water to achieve 25%, 50%, and 75% wastewater (v/v). To ensure that the current drop was not caused by the decrease in conductivity due to the dilution of the wastewater, the conductivity of the diluted wastewater was adjusted to the original conductivity using sodium chloride. To ensure the accuracy of results, the initial current must be stable for more than 10 min before any test was conducted (Fig. 1).

After the Cu(II) dosing and organic strength decrease tests for control (no substrate addition) were completed, the influent was switched to domestic wastewater with additional 150 mg/L of COD added as sodium acetate and the MFC cells were allowed to acclimate for 4 d before the next set of Cu(II) dosing and organic strength decreases tests were carried out. The same acclimation procedure was applied before Cu(II) dosing and organic strength fluctuation tests for the remaining substrate additions using acetate at concentration equivalent to COD concentration of 300 or 500 mg/L. The added COD concentrations of 150, 300, and 500 mg/L were selected based on the saturation concentration calculated from the reported half saturation constants in previous studies. The two sets of tests were repeated using glucose as the substrate subsequently. All chemicals used in the experiment are reagent grade and were purchased from Sigma Aldrich, Singapore.

[Table 1]

2.3. Cu(II) complexation tests

The substrates used for the Cu(II) complexation experiment were selected from the list of substrates used in previous MFC studies (Pandey et al., 2016; Pant et al., 2010) based on the power output and cost of substrate. Acetate and citrate were used to investigate the effect of the carboxyl group on the complexation with Cu(II). Citrate contains three carboxyl groups while acetate contains only one carboxyl group. Glucose, sucrose and starch were selected as a model monosaccharide, disaccharide and polysaccharide for the study.

Free Cu(II) concentration was measured using an ion selective electrode (HI4108, Hanna Instruments, Italy), and an ionic strength adjuster (HI4000-00, Hanna Instruments, Italy) was added to the sample at a ratio of 1:50 and mixed thoroughly before the reading was taken. The electrode was calibrated before each daily use to eliminate any drift in the readings.

2.4. Analysis

The voltage was measured using a digital multimeter (M3500A, Array Electronics, Taiwan) every 1 min and the data was recorded by a software using a computer. The voltage recorded by the digital multimeter was converted to current by applying the Ohm's Law, $V = IR$, where V is the voltage recorded by the digital multimeter, I is the current, and R is the external resistance (5Ω). The current drops due to both Cu(II) dosing and BOD fluctuations were calculated by $Current\ drop\ (\%) = (I_{2h} - I_0)/I_0 \times 100$, where I_0 is the initial baseline current and I_{2h} is the current measured after 2 h of Cu (II) dosing or BOD change. The experiments were conducted for 2 h because previous tests have shown that the current would stabilize within a cutoff time

of 2 h. Wastewater ratio (v/v) was the volumetric ratio of the domestic wastewater in the final diluted wastewater.

3. Results and Discussion

3.1. Signal stabilization by addition of acetate

3.1.1. Effect of acetate addition on signal stabilization

Figure 2 shows that the addition of acetate as a supplementary substrate increased the robustness of the MFC sensor against BOD fluctuations. The perturbation of the current after 2 h was greatly reduced from 65% (control with no acetate addition) to 12%, 16% and 15% (150, 350 and 500 mg/L COD added as sodium acetate, respectively) when the organic strength of the original domestic wastewater was lowered by 75% (i.e., wastewater ratio (v/v) of 25%). Similarly, the magnitude of current drop was reduced from 21% (control) to 9%, 11% and 10% when the organic strength was decreased by 50%. The magnitude of current drop was reduced from 15% (control with no acetate addition) to 6%, 10% and 7% when the organic strength of the original domestic wastewater was decreased by 25% (i.e., wastewater ratio (v/v) of 75%). It was evident that the addition of acetate stabilized the signal for an event of sudden decrease in organic strength in the original wastewater. The results also showed that the addition of acetate reduced the current drop by 5 times for a 75% decrease in the organic strength of the original wastewater. When the MFC sensor was subjected to a lower decrease in the organic strength of the original wastewater by 50% and 25%, the magnitudes of the current drop were reduced by 2 times. Clearly, the stabilization effect was more pronounced for a larger drop in the organic strength of the original feed wastewater.

[Figure 1]

The maintenance of the robustness of the MFC sensor against a drop in the organic strength of the domestic wastewater was due to the supplementary of COD from the added acetate, which resulted in an overall smaller percentage drop or increase in COD in the feed wastewater. In addition, acetate is the preferred substrate by the electroactive bacteria and acetate would be preferentially utilized over the complex substrates in the wastewater. Hence, the reduction in the amount of complex substrates in the wastewater did not adversely affect the activity of the electroactive bacteria as the reduced substrate level was compensated by the addition of acetate (10.0 W/m^3 for 100% wastewater (v/v) + 0 mg/L COD from acetate, compared to 1.14, 40.7, 81.5 and 88.1 W/m^3 for 25% wastewater (v/v) + 0, 150, 300 and 500 mg/L COD from acetate, respectively). On the other hand, when acetate was not added, there was no readily available substrate for the electroactive bacteria. As the level of the complex substrates decreased, the amount of fermentation products, which are the preferred substrates of the electroactive bacteria, available would decrease according to the decrease in the organic strength of the wastewater. Therefore, the added acetate ensured that there would always be readily available electron donors for the electroactive bacteria.

[Figure 2]

In this test, acetate was added as an additional substrate to domestic wastewater and the MFC sensor was tested for its robustness against fluctuations in changes in organic strength. The most extreme possibility of a drop in organic strength, which is a sudden drop in organic strength, was specifically tested. Although the event of a gradual organic strength was not tested, it was expected that the drop in electrical current in this scenario would be smaller in magnitude than the results obtained in this test. Hence, the

results in this test represent the largest possible signal drop for the case of sudden drop in the organic strength of wastewater.

The stabilizing effect of acetate addition into the feed wastewater suggests that the MFC sensor could be applied in 2 scenarios for heavy metals monitoring: (1) wastewater streams with low organic strength such as the electroplating industry where the illegal discharge of heavy metals is a pertinent problem and the organic strength of the wastewater is not sufficient to support microorganism growth; and (2) wastewater streams with diurnal organic strength fluctuations.

3.1.2. Effect of acetate addition on MFC sensor sensitivity

While it has been revealed that the addition of acetate increases the robustness of the MFC sensor against organic strength fluctuations, the MFC sensor must retain its functionality of sensing Cu(II) with high sensitivity. In the experiment without any acetate addition, the current of the MFC sensor dropped by 28% and 48% in the presence of Cu(II) concentrations of 200 and 300 mg/L, respectively (Fig. 3). Acetate addition (150 mg/L COD) decreased the sensitivity of the MFC sensor as the current drop was lowered to 12% and 25% in the presence of Cu(II) concentration of 200 and 300 mg/L, respectively. Similar results were obtained for 300 mg/L COD of acetate addition as the current drop was decreased to 17% and 48% in the presence of Cu(II) concentration of 200 and 300 mg/L, respectively. For 500 mg/L COD of acetate addition, the drop decreased to 21% and 48% in the presence of Cu(II) concentration of 200 and 300 mg/L, respectively. The results showed that acetate addition decreased the sensitivity of the MFC sensor for Cu(II) detection. The results also clearly revealed the

tradeoff between the increased robustness of the MFC sensor against organic strength fluctuations and the sensitivity of the MFC sensor for Cu(II) detection.

[Figure 3]

There are three possible reasons for the reduction in MFC sensor sensitivity for Cu(II) detection. First, acetate could form soluble complexes with Cu(II) ions, thereby reducing free Cu(II) concentration in the influent and reducing the Cu(II) toxicity to the bacteria (Azenha et al., 1995). Second, the addition of acetate increases the pH of the wastewater, causing more Cu(II) to precipitate. Nevertheless, the effect of pH was less pronounced in this study because the amount of acetate added only caused a slight increase of pH (i.e., less than 0.1). Third, the increased extracellular polymeric substance production by the biofilm and the increased ATP production for detoxification by efflux could also lead to lower sensor sensitivity (Nies, 2003).

The small change in pH was not sufficient to cause significant change in the current. The complexation of Cu(II) is the only factor which can be easily controlled by changing the substrate type, and complexation has the most direct effect on Cu(II) toxicity. Hence, the complexation of Cu(II) with substrates was studied to identify an alternative substrate which ideally does not complex with Cu(II) so that the robustness-sensitivity tradeoff could be overcome, and both high robustness and sensitivity could be achieved.

3.2. Complexation of Cu(II) with different substrates

As the complexation of Cu(II) to acetate was proposed as the main reason for the reduced sensitivity of the MFC sensor for Cu(II) detection, it would be intuitive to increase the free Cu(II) to total Cu(II) ratio in the feed to achieve a higher sensor

sensitivity for Cu(II) detection. The correlation between free Cu(II) concentration and the sensitivity of the MFC sensor could be explained by (i) bioavailability; and (ii) abiotic reduction. First, the free ion activity model (FIAM) and biotic ligand model (BLM) are two commonly used models to quantify metal uptake by organisms. These models describe the correlation between the bioavailability of metals and the free metal ion concentration. In the former model, the bioavailability of metal is directly correlated to the free metal ion activity. In the BLM, however, the biomass could be modelled as an organic ligand which could complex with metal ion. In this model, the metal bioavailability is quantified by the amount of Cu(II) bound to the active sites of the organism (biotic ligand). The amount of bound Cu(II) to the biotic ligand is a function of several parameters, one of which is the free Cu(II) concentration. Hence, both models suggest that increased concentration of free Cu(II) would increase the bioavailability of Cu(II) and the apparent Cu(II) toxicity to the biofilm, thereby increasing the response or sensitivity of the MFC sensor. Second, abiotic reduction of free Cu(II) at the anode could contribute to the total current drop (Jiang et al., 2015). An increase in free Cu(II) concentration would increase the rate of the abiotic Cu(II) reduction and lead to a larger current drop. Hence, the objective of maximizing the response or sensitivity of the sensor is achieved by maximizing the free metal concentration. To increase the free metal concentration, the complexation of metals with the added substrates need to be minimized.

In this study, five substrates – acetate, citrate, glucose, sucrose and starch were evaluated for the degree of complexation with Cu(II) to identify the correct substrate that can be supplemented as a source of organics to maintain or improve the response/sensitivity of the MFC sensor. The results, as shown in Fig. 4, suggest that

acetate is not the optimum substrate which can maximize the free Cu(II) concentration in the water. Only 53%, 34% and 23% of the total Cu(II) added was present in free Cu(II) form when acetate was added at equivalent COD of 150 mg/L, 300 mg/L and 500 mg/L (0.78 g COD/g anhydrous sodium acetate), respectively. The low free Cu(II) concentration was due to the presence of a carboxyl group in acetate that could bind with Cu(II) to form a Cu(II)-acetate complex.

[Figure 4]

In order to confirm the complexation effect of the carboxyl group, another electron donor – citrate, which contains three carboxyl groups, was investigated for its complexation with Cu(II). The results clearly showed that the free Cu(II) concentration was lower with citrate than acetate, confirming that the carboxyl group was the main cause of the reduction in free Cu(II). The free Cu(II) concentration remained fairly constant for glucose, sucrose and starch, all of which containing no carboxyl group. The results suggest that there is no complexation of Cu(II) with these substrates.

While the Cu(II) complexation was investigated using deionized (DI) water to compare the relative complexation effect of different substrates, the actual free Cu(II) concentration when using domestic wastewater with the added substrates need to be investigated to understand the actual complexation effects in domestic wastewater. The results of the domestic wastewater experiments (Fig. 4) clearly revealed that the free Cu(II) concentrations were lower in domestic wastewater than DI water. This observation was also true for domestic wastewater with no additional substrate added, suggesting the presence of ligands, both organic and inorganic, in domestic wastewater which could bind to Cu(II) and reduce the free Cu(II) concentration drastically. In addition, free Cu(II) could also be reduced due to precipitation and adsorption to the

suspended solids in the wastewater, thus explaining the higher detection limits of the MFC sensor in this study compared to previous studies (Sun et al., 2015). Therefore, only 20%, 17% and 14% of the total Cu(II) added remained as the free Cu(II) form when acetate was added at 150, 300 and 500 mg COD/L to domestic wastewater.

In contrast, when glucose was used, 98%, 99%, and 99% of the total Cu(II) added remained in the free Cu(II) form when DI water was used, and 26%, 25% and 24% of the total Cu(II) added remained in the free Cu(II) form when domestic wastewater was used. It was evident that Cu(II) does not form complexation with glucose. Hence, it is intuitive to investigate glucose as an alternate substrate to be added to improve the sensitivity and robustness of the MFC sensor.

3.3. Signal stabilization by addition of glucose

3.3.1. Effect of glucose addition on signal stabilization

Glucose has been commonly used as the sole electron donor for synthetic wastewater in previous studies (Rabaey et al., 2003). In this study, glucose was added into domestic wastewater as a supplementary substrate to stabilize the baseline signal against changes in the organic strength of the feed. Similar to acetate, the results in Fig. 2 showed that glucose increased the robustness of the sensor as shown from the decrease in the current drop. The original current drops were 15%, 21% and 65% without any glucose addition when the organic strength was reduced to 75%, 50%, and 25% wastewater (v/v), respectively. However, when glucose was added at 150 mg COD/L, the current drop was reduced to 7%, 11% and 11% for 75%, 50%, and 25% wastewater (v/v), respectively. Similarly, the current drops were reduced to 11%, 17% and 15% when glucose was added at 300 mg COD/L, and 11%, 16% and 14% when

glucose was added at 500 mg COD/L to 75%, 50%, and 25% wastewater (v/v), respectively.

Acetate and glucose were both able to increase the robustness of the MFC sensor against fluctuations in the organic strength of the feed wastewater, reducing the current drop from 65% to less than 16% and 15% (Fig. 2) for a 25% wastewater (v/v) shock event for the respective substrates. Comparing the current drop results from Fig. 2, acetate generally performed better than glucose in buffering the signal against changes in organic strength.

The other difference between acetate and glucose addition is the difference in the power output of the two substrates, with acetate addition producing a higher power and current output than glucose addition. Addition of glucose only increased the power output slightly from 10 W/m^3 to 15 W/m^3 when glucose was added at 500 mg COD/L while addition of acetate increased the power output from 10 W/m^3 to 85 W/m^3 for the same COD added. It should be noted that the power density of the electroactive bacteria acclimated to glucose remained relatively low and did not increase beyond 15 W/m^3 even after prolonged acclimation. Acetate gave a higher power output as acetate was the preferred substrate for the electroactive bacteria, while glucose could not be directly oxidized (Freguia et al., 2008) and has to be fermented before some fermentation products could be utilized by the electroactive bacteria. The increased power output from acetate would reduce the noise to signal ratio as the baseline signal produced with acetate addition is higher, while the artifacts in the signal recorded by the measurement device remained the same.

[Figure 5]

The higher power output from acetate was also observed in previous studies where acetate and glucose were utilized as the sole carbon source in synthetic wastewater (Martin et al., 2010; Yuan et al., 2011). In the previous studies, it was shown that the power output follows single substrate growth kinetic models such as the Monod and the Andrews model. In this study, however, the influent consisted of both complex substrates originally present in domestic wastewater and the added substrates – acetate or glucose. Nonetheless, the results in Fig. 5 suggest that the power output followed a single substrate growth kinetics model despite the plethora of substrates present in the domestic wastewater. Eliminating the effects of complex substrates present in the wastewater, the half saturation constant for acetate was 173 mg/L, while the half saturation constant for glucose was 154 mg/L. The magnitudes of the half saturation constants in this study are similar to the values obtained in other studies. The well fit of the power output data to the single substrate growth kinetics model suggests that the added substrates were preferred to the complex substrates in the wastewater by the electroactive bacteria in the MFC.

The preference of the electroactive bacteria for the added substrate is key in increasing robustness of the sensor as it introduces a degree of freedom in controlling the signal of the sensor. While the sensor signal was completely susceptible to fluctuations in the organic strength previously, the sensor signal could now be controlled by setting a fixed concentration of acetate or glucose addition to obtain the desired power output. Using the information in this study, the desired substrate type and concentration could be chosen, and the signal fluctuation limits could be set using the results. If the signal fluctuation for a 2-hour period is less than the current drop limit

obtained in this study, a decrease in organic strength would not cause a false positive alarm to be sounded off.

3.3.2. Effect of glucose addition on sensor sensitivity

Similar to acetate addition, the addition of glucose reduced the sensor's sensitivity to Cu(II). Without any substrate addition, the 2-hour current drops were 16% for 50 ppm Cu(II), 14% for 100 ppm Cu(II), 28% for 200 ppm Cu(II) and 48% for 300 ppm Cu(II). When glucose was added at 150 mg/L COD, the current drops reduced to 4% for 50 ppm Cu(II), 7% for 100 ppm Cu(II), 14% for 200 ppm Cu(II) and 33% for 300 ppm Cu(II). For glucose at 300 mg/L COD, the current drops decreased to 6%, 5%, 15% and 32% for the respective Cu(II) concentrations. When glucose was added at 500 mg/L COD, the current drops were 5%, 6%, 15%, and 32%, respectively.

Even though glucose was found to exhibit lower level of complexation with Cu(II) than acetate (Fig. 4) and would supposedly lead to higher free Cu(II) concentration, glucose addition caused the sensor to be less sensitive to Cu(II) than acetate addition. As shown in Fig. 3, when 50 ppm Cu(II) was dosed to the influent, the current drop was 4%, 6%, and 5% for glucose addition at 150, 300, and 500 mg/L COD. The current drops for acetate addition for the same Cu(II) concentration were 4%, 8% and 10% respectively. For dosing of 100 ppm Cu(II), the current drops for glucose were 7%, 5% and 6% for 100 ppm of Cu(II) compared to 11%, 11% and 11% for acetate, respectively. For 200 ppm Cu(II), the current drops were 14%, 15% and 15% for glucose compared to 12%, 17% and 21% for acetate. Lastly, when 300 ppm Cu (II) was dosed, the current drops were 33%, 32% and 32% for glucose while the current drops for acetate were 25%, 48% and 48%. These results were not expected as a supposed

higher free Cu(II) concentration with glucose addition did not result in a higher sensitivity of the sensor to Cu(II).

This meant that while the sensitivity of the sensor to Cu(II) should theoretically be increased due to the increased free Cu(II) ion concentration, other parameters changes caused by glucose addition resulted in a reduction in the sensitivity of the sensor to Cu(II). Interestingly, previous studies also reported reduced Cu(II) toxicity due to the presence of glucose (Menkissoglu, 1991). There are several explanations for the reduced sensitivity by the addition of glucose. First, the reduction in current drop could be due to a change in the microbial matrix due to the different substrate added. It is known that acetate could be directly utilized by electroactive bacteria while glucose could not be directly oxidized by most anodic bacteria (Freguia et al., 2008). Glucose is fermented to fermentation products such as acetate and propionate, before being utilized by the electroactive bacteria (Min & Logan, 2004). The addition of glucose promoted the growth of the glucose fermenters and increased the non-electroactive biomass density (Lee et al., 2008). The presence of a higher level of non-electroactive biomass resulted in a biofilm rich in extracellular polymeric substances (Kato et al., 2013), thereby reducing the apparent free Cu(II) concentration to the electroactive bacteria due to the increased immobilization of Cu(II) by the extracellular polymeric substances. Second, since fermentation products such as acetate and propionate were formed, the free ionic Cu(II) concentration was lowered due to complexation with these fermentation products. Due to diffusion limitations in the biofilm, these organic ligands would accumulate in high concentrations in the inner layers of the biofilm, further complexing with the remaining free ionic Cu(II) present and reducing the bioavailability of Cu(II) to the biofilm. In a previous study, it was found that 13% of the COD fed as

glucose was converted to acetate and 4% of the COD was converted to propionate (Min & Logan, 2004). Hence, the local concentrations of acetate and propionate in the biofilm, while not directly measurable, would be higher than in the bulk solution.

While the free Cu(II) concentration in the bulk solution was high for glucose addition, the local free Cu(II) concentration in the biofilm was low due to (i) adsorption to non-electroactive biomass and (ii) complexation to fermentation products such as acetate and propionate. Acetate addition, on the other hand, did not result in an increase in adsorption to non-electroactive biomass as acetate promoted the growth of electroactive bacteria, as seen from the increase in the power output. Although the complexation of Cu(II) with acetate was inherent due to the added acetate present in the bulk solution, the local acetate concentration was lower in the biofilm than the bulk solution due to diffusion limitations and utilization of acetate as the substrate by the biofilm. This could have resulted in a higher local concentration of Cu(II) in the biofilm for acetate addition compared to glucose addition. Recent developments in the field of in situ analysis of intra- and extracellular metal concentrations in biofilms have shown great promise in revealing the distribution of metals ions in biofilms. The state-of-the-art imaging technique, which is based on highly specific fluorescent probes binding to the target metal ion, could prove useful in quantifying the distribution of Cu(II) in the biofilms subjected to the different substrate conditions in future studies (Huang et al., 2018; Tao et al., 2017; Xue et al., 2017).

In addition to the different chemical properties of the two substrates, the shift in the biological or chemical matrix of the biofilm could have altered the bioavailability of Cu(II) in the biofilm (You et al., 2015). The spatial distribution of intra- and extra-cellular Cu(II) concentration within the biofilm could have been affected by factors such

as the different pH, E_h, competing ions, metal-specific attributes, temperature and sorption behavior of the extra-cellular polymeric substances in the biofilm (Wuertz et al., 2000).

Compared to previous MFC toxicity sensor studies, the detection limit of Cu(II) for the control (without acetate or glucose addition) in this study is more than an order of magnitude higher. This is likely due to the high concentration of organic ligands already present in the domestic wastewater (Fig. 4) and the level of complexation is further increased when the wastewater was amended with acetate addition. While the MFC sensor exhibits high sensitivity to Cu(II), it should be noted that a limitation of the MFC sensor is the low selectivity as the sensor is sensitive to other toxic metals such as Zn(II), Cd(II) and Ni(II), and organic toxins such as formaldehyde and p-nitrophenol (Abrevaya et al., 2015; Zhou et al., 2017). Therefore, MFC sensors should be used in early warning systems rather than a method for toxicant identification and toxicant concentration determination. Genetic engineered electroactive bacteria could be used to overcome the low selectivity limitation (Webster et al., 2014), but the tradeoff would be operational complexity to maintain the single bacteria strain and the resulting sensor being able to detect only a single toxicant in real operation.

Another potential limitation of the MFC is the acclimation of the bacterial community to toxicants (Kim et al., 2011). The microbial adaptations could potentially reduce the inhibitory effect of the toxicant on the electroactive bacteria in the MFC sensor, causing a smaller current drop to be registered. In this study, the current drops were observed for the tested Cu(II) concentrations despite the sensor being subjected to daily Cu(II) spiking (2-hour toxicant spiking followed by 22-hour recovery cycle). Hence, the sensor can be installed as an early warning system where the occurrence of

an illegal or accidental discharge event would be expected to be less frequent than the daily experimental testing frequency. In the unlikely event of an illegal or accidental discharge of high Cu(II) concentration over an extended period, the surge in the elevated Cu(II) concentration would cause the sensor to register a continuous low current. The low current would clearly show the presence of toxicants in the wastewater. Moreover, current drop was observed upon toxicant spiking in a previous study with elaborate acclimation of biofilm along a toxicant concentration gradient over an extended period (Wang et al., 2013b). Hence, it is evident that current drop could be observed despite possible acclimation of the electroactive bacteria to the toxicants. The MFC sensors have both the advantage of being able to detect a myriad of toxicants due to its low selectivity and the resilience against acclimation to the toxicants.

4. Conclusions

Acetate addition is a simple and cost-effective method to increase the robustness of the sensor against fluctuations of the organic strength of the water. Although acetate addition resulted in reduced bioavailable Cu(II) in the bulk solution through complexation with Cu(II), acetate addition promoted the growth of a desirable biofilm matrix which is more sensitive to Cu(II) than glucose addition. To the author's knowledge, this is the first experimental study to investigate the tradeoff between the robustness of the MFC sensor against organic strength fluctuations and the sensitivity of the MFC sensor by background addition of acetate and glucose.

E-supplementary data for this work can be found in e-version of this paper online.

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Figure Captions

Figure 1. Current profiles showing the decrease in current from the baseline current due to decrease in organic strength of the feed wastewater (top). The currents recovered the respective baseline currents at the end of the 2-hour experiment. The current profile for the sensor subjected to Cu(II) toxicity (bottom) showed great resemblance to the current profile recorded for the decrease in organic strength event.

Figure 2. Current drop of the sensor fed with acetate-amended and glucose-amended domestic wastewater at added COD of 150, 300, and 500 mg/L when subjected to an organic strength decrease in the feed wastewater. The points on the graph indicate the mean of the triplicates and the error bar indicates the standard deviation of the samples at each experimental condition.

Figure 3. Current drop of the MFC sensor fed with domestic wastewater with acetate and glucose addition (added COD of 150, 300, and 500 mg/L) when subjected to Cu(II) toxicity. The points on the graph indicate the mean of the triplicates and the error bar indicates the standard deviation of the samples at each experimental condition.

Figure 4. Free Cu(II) concentration reduced due to complexation of Cu(II) with acetate, citrate, glucose, sucrose and starch in domestic wastewater (top) and deionized water (bottom).

Figure 5. Power output of microbial fuel cell sensor when fed with acetate-amended and glucose-amended wastewater.

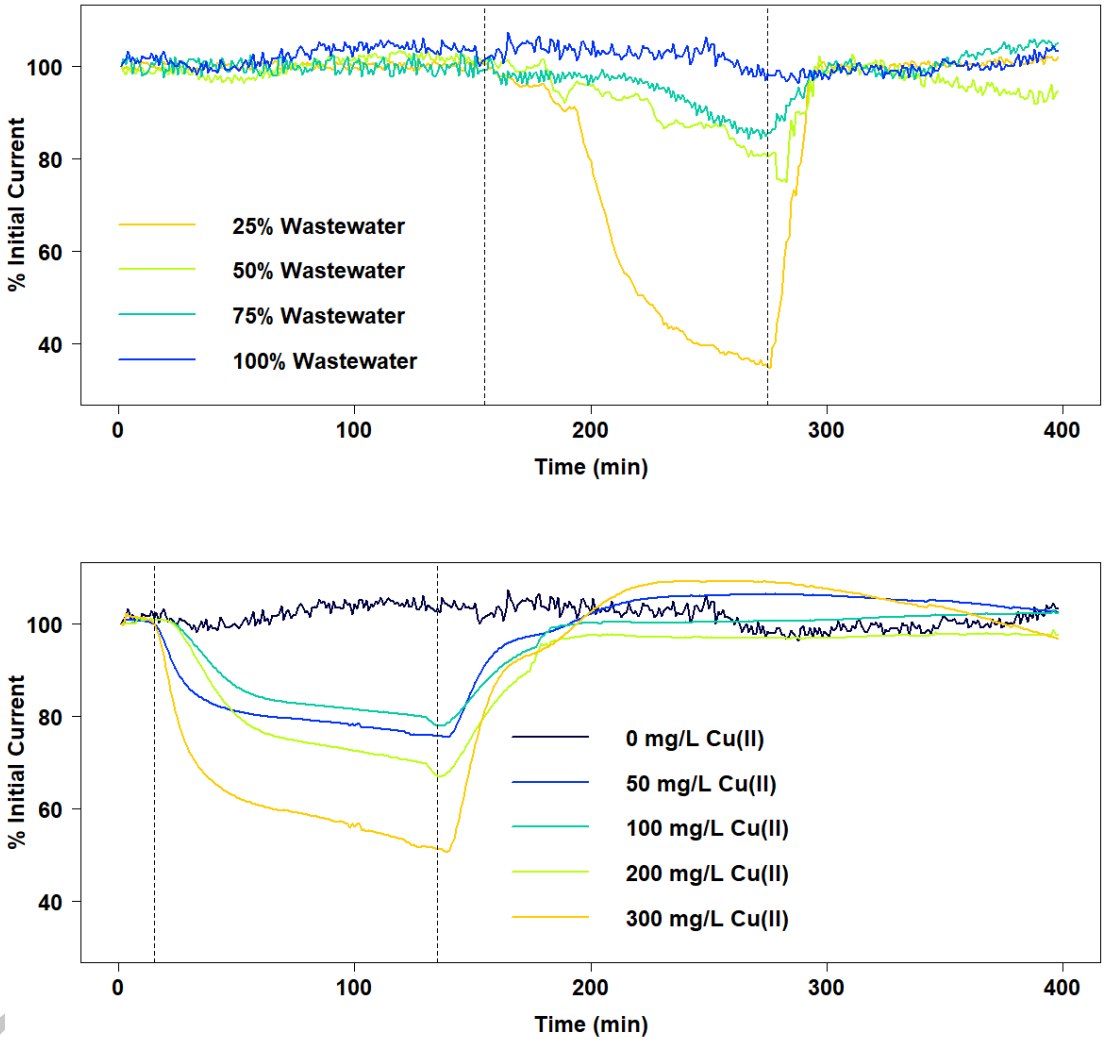

Figure 1.

[Electronic Version]

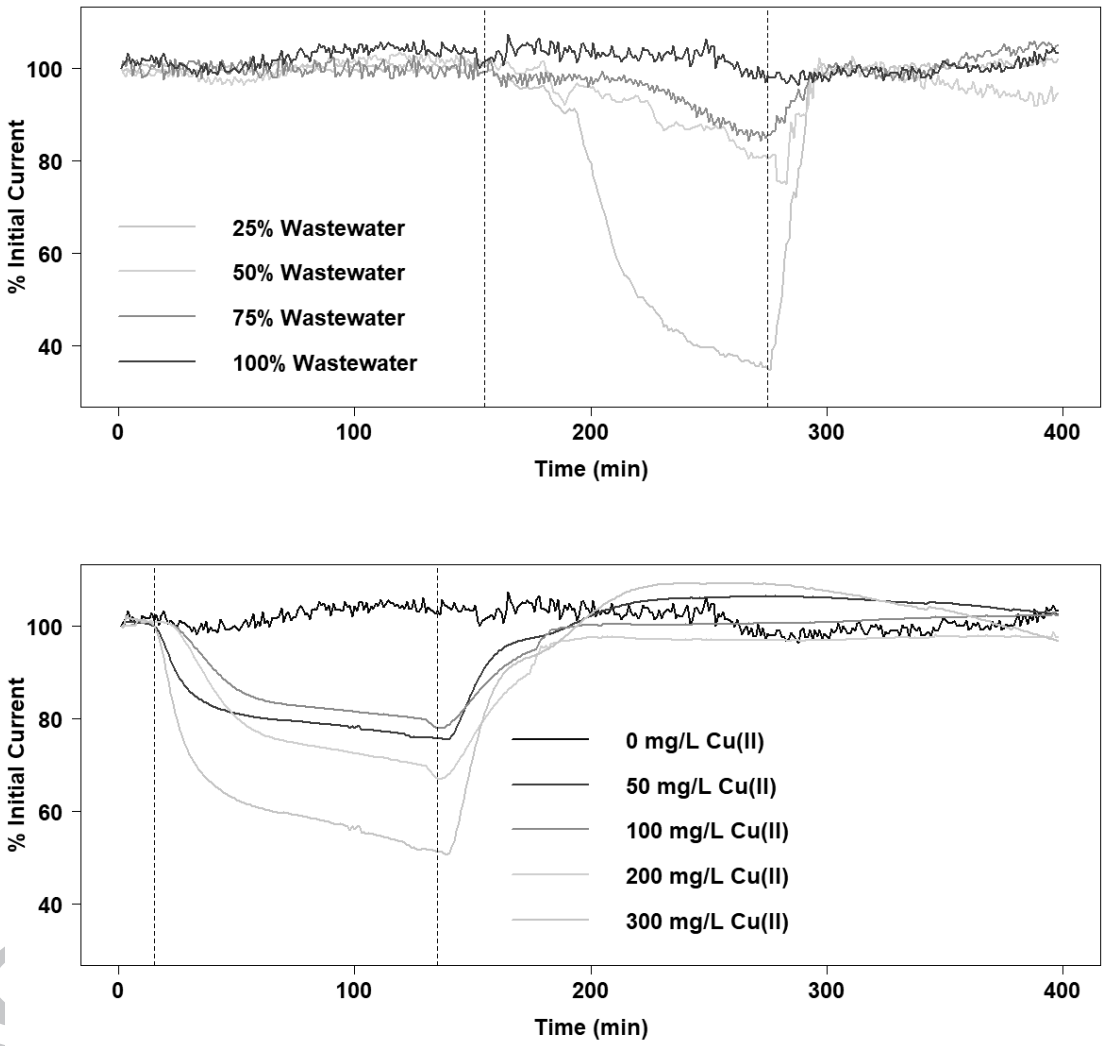

Figure 1.

[Print Version]

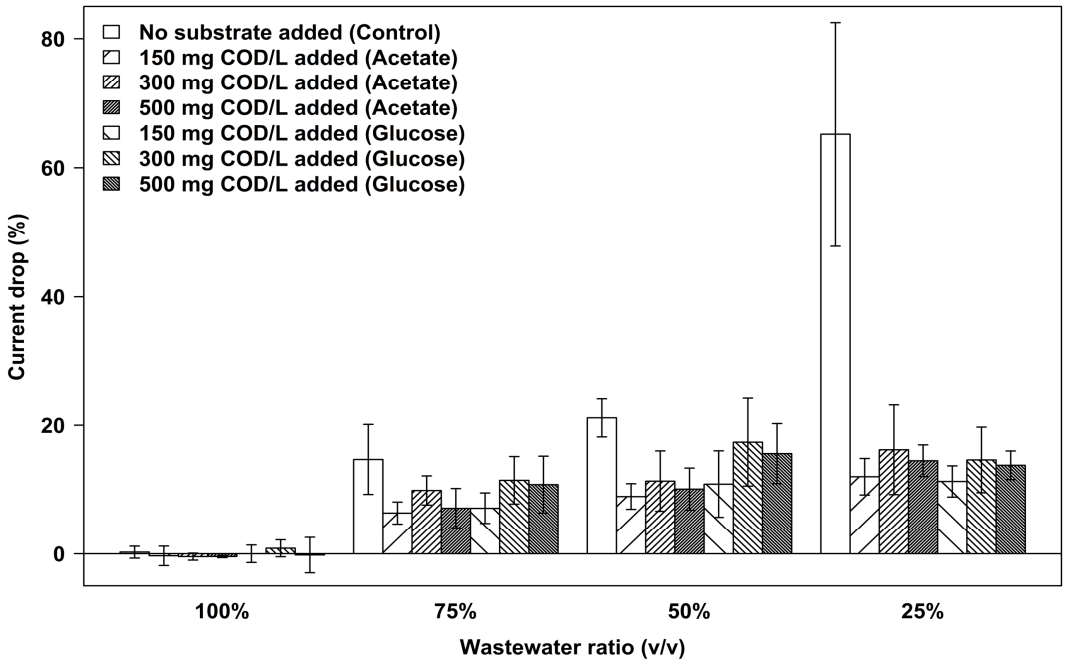

Figure 2.

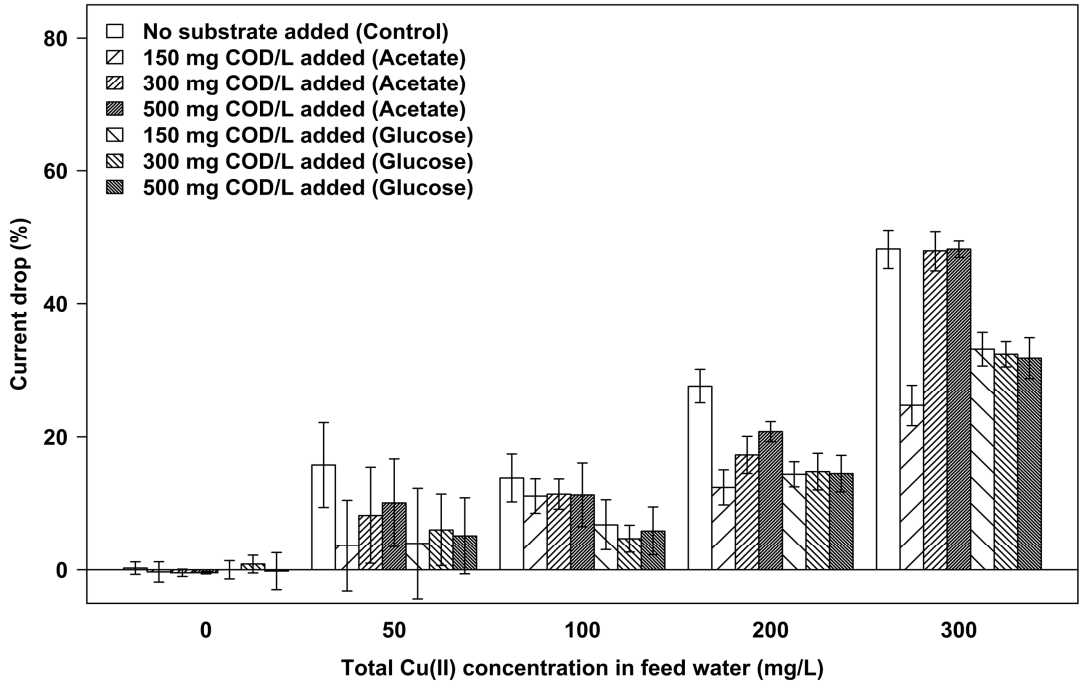

Figure 3.

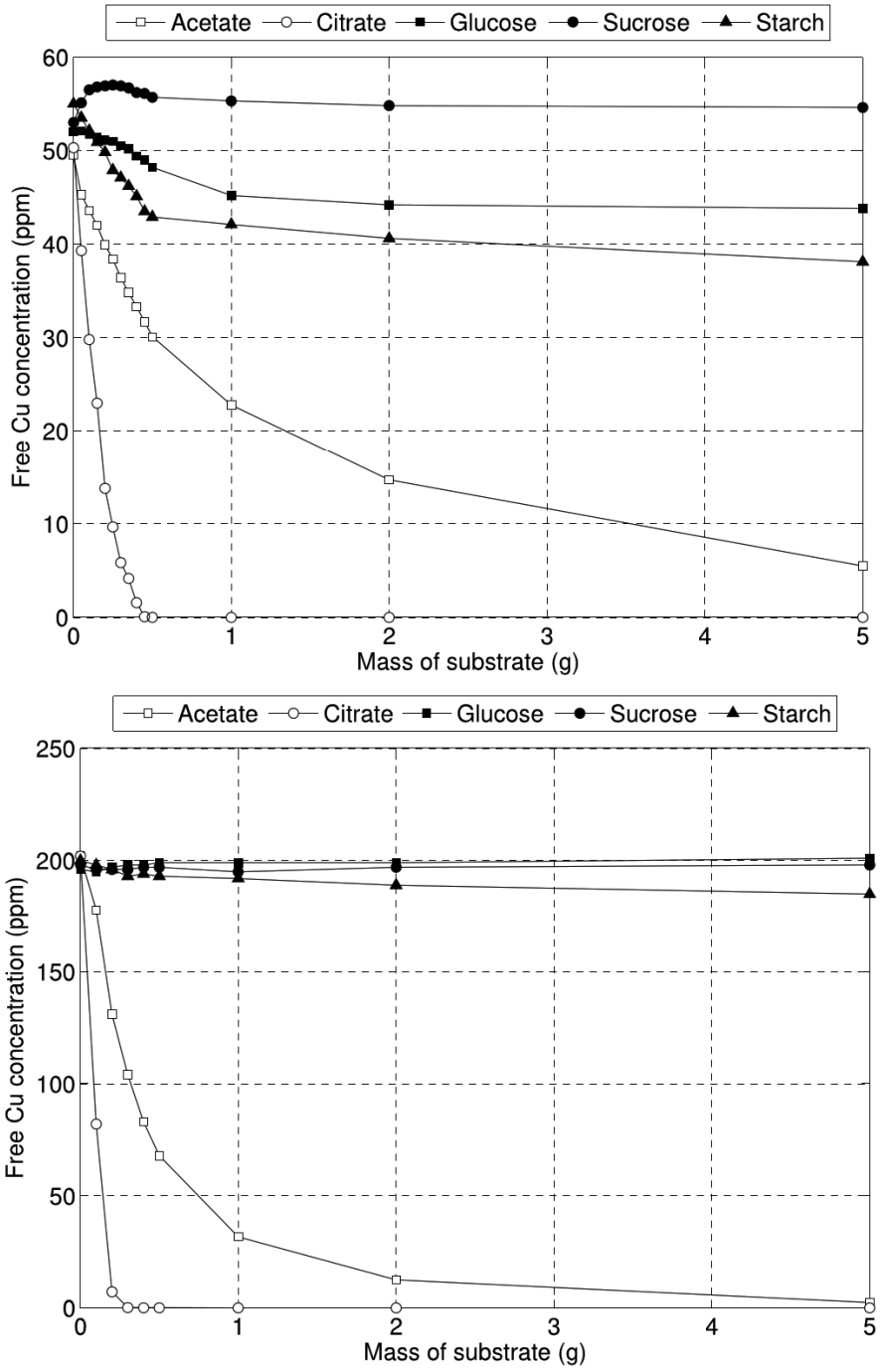

Figure 4.

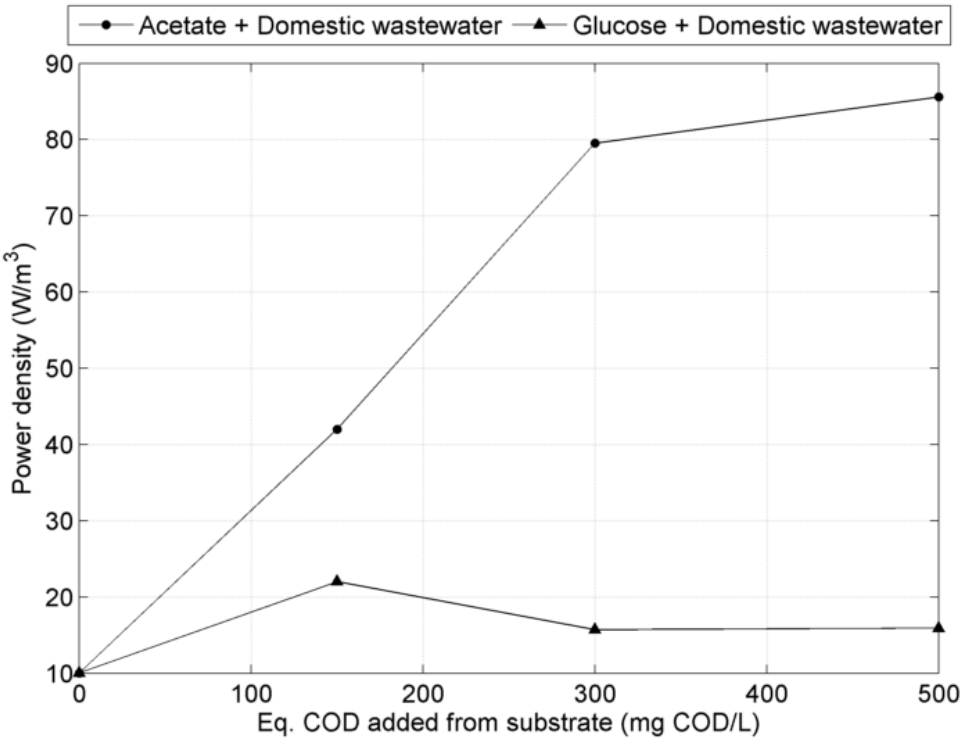

Figure 5.

Table 1. Operational conditions of the microbial fuel cell sensor for different phases of the experiment.

			Baseline Condition		Experimental Condition	
Substrate added	Equivalent COD of substrate added (mg COD/L)	Test	Wastewater percentage in feed water (v/v)	Cu(II) concentration in feed water (mg/L)	Wastewater percentage in feed water (v/v)	Cu(II) concentration in feed water (mg/L)
No substrate (control)	-	Inoculation	100%	-	-	-
		Cu(II) dosing	100%	-	-	50, 100, 200, 300
		Organic strength decrease	100%	-	25%, 50%, 75%, 100%	-
Acetate	150	Acclimation	100%	-	-	-
		Cu(II) dosing	100%	-	-	50, 100, 200, 300
		Organic strength decrease	100%	-	25%, 50%, 75%, 100%	-
	300	Acclimation	100%	-	-	-
		Cu(II) dosing	100%	-	-	50, 100, 200, 300
		Organic strength decrease	100%	-	25%, 50%, 75%, 100%	-
	500	Acclimation	100%	-	-	
		Cu(II) dosing	100%	-	-	50, 100, 200, 300
		Organic strength decrease	100%	-	25%, 50%, 75%, 100%	-
Glucose	150	Acclimation	100%	-	-	
		Cu(II) dosing	100%	-	-	50, 100, 200, 300
		Organic strength decrease	100%	-	25%, 50%, 75%, 100%	-
	300	Acclimation	100%	-	-	
		Cu(II) dosing	100%	-	-	50, 100, 200, 300
		Organic strength decrease	100%	-	25%, 50%, 75%, 100%	-
	500	Acclimation	100%	-	-	
		Cu(II) dosing	100%	-	-	50, 100, 200, 300
		Organic strength	100%	-	25%, 50%,	-

	decrease	75%, 100%

Highlights

- Acetate and glucose addition increased sensor's robustness against COD decrease
- Tradeoff for the increased robustness of the sensor was the lower Cu(II) sensitivity
- Cu(II) complexation with acetate resulted in reduced sensor's sensitivity to Cu(II)
- Cu(II) complexation with glucose fermentation products reduced sensor's sensitivity
- Acetate addition (500 mg COD/L) provided the optimal condition for sensor operation