A dual chamber microbial fuel cell based biosensor for monitoring copper and arsenic in municipal wastewater

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Abstract

This study investigated a dual-chamber microbial fuel cell-based biosensor (DC-MFC-B) for monitoring copper and arsenic in municipal wastewater. Operational conditions, including pH, flow rate, a load of organic substrate and external resistance load, were optimized to improve the biosensor's sensitivity. The DC-MFC-B's toxicity response was established under the electroactive bacteria inhibition rate function to a specific heavy metal level as well as the recovery of the DC-MFC-B. Results show that the DC-MFC-B was optimized at the operating conditions of 1000 Ω external resistance, COD 300 mg L^{-1} and 50 mM $K_3Fe(CN)_6$ as a catholyte solution. The voltage output of the DC-MFC-B decreased with increasing in the copper and arsenic concentrations. A significant linear relationship between the maximum voltage of the biosensor and the heavy metal concentration was obtained with a coefficient of R^2=0.989 and 0.982 for copper and arsenic, respectively. The study could detect copper (1-10 mg L^{-1}) and arsenic (0.5-5 mg L^{-1}) over wider range compared to other studies. The inhibition ratio for both copper and arsenic was proportional to the concentrations, indicating the electricity changes are mainly dependent on the activity of the electrogenic bacteria on the anode surface. Moreover, the DC-MFC-B was also recovered in few hours after being cleaned with a fresh medium. It was found that the concentration of the toxicant efftected on the recovery time and the recovery time was varied between 4-12 hours. In short, this work provided new avenues for the practical application of microbial fuel cells as a heavy metal biosensor.

Keywords: Microbial fuel cell, biosensor, voltage output, inhibition ratio, copper, arsenic

1.Introduction

Environmental pollution due to heavy metals has been regarded as one of the critical issues to its persistence, toxicity and bio-accumulation. Heavy metal ions such as Cu^{2+}, Cd^{2+}, Cr^{6+}, Pb^{2+}, As, etc. can be accumulated in wastewater and directly threaten human health and the ecosystem (Bashir et al., 2019). World Heal Organization (WHO) has been indicated that arsenic is one of the critical contaminants in the aquatic system (Jiang, 2001; Smith et al., 2000), which causes a high risk to the human body, such as destroy chromosomal stability, cause neuropathy, damage the liver. Copper is one of heavy metal which plays a vital role in many processes including electron transfer, oxidation and oxygennationof substrates. However, the high level of copper can lead harmful to the ecosystem and human being, such as vomiting, diarrhea, tremors, rigidity, nausea, abnormality of the brain, and/or even death. According to the United States Environmental Protection Agency, the maximum contaminant level standard is 0.25 mg L^{-1} for copper and 0.05 mg L^{-1} for arsenic (Arshad et al., 2019).

The conventional methods for heavy metals measurement are high performance liquid chromatography (HPLC) coupled with electrochemical (Shimizu et al., 2019), inductively coupled plasma-mass spectrometry (ICP-MS) (Ravikumar et al., 2012) flame atomic absorption spectroscopy (FAAS), inductively coupled plasma optical emission spectroscopy (ICP-OES) (Escudero et al., 2010). Despite of high accuracy and sensitivity of these methods, they still rely on complex and expensive equipment, high skilled analysis, time intensive procedures and unsuitable for online and onsite measurement (Cui et al., 2015; Quinn et al., 2018). Therefore, a simple, rapid and accurate system for heavy metals detection in wastewater is very essential.

Microbial fuel cells (MFCs) have gained much attention in the last decade as biosensors for wastewater quality management, such as monitoring BOD, COD, and toxicity (Abrevaya et al., 2015; Do et al., 2020; Ren et al., 2021; Shen et al., 2013; Stein et al., 2012c; Xu et al., 2021). MFCs are a bioelectrochemical device that can generate a current through a microorganism's

metabolism (Choi et al., 2011; Lu et al.2020; Mukherjee et al., 2013). Typically, microbial fuel cells include anaerobic anode and aerobic cathode compartments, separated by a proton exchange membrane (PEM). Organic matters in wastewater oxidized under the function of electrochemically active bacteria produce electrons and protons. Protons are transported through PEM to the cathode chamber, where they are combined with an electron acceptor such as oxygen or ferricyanide to form water. Electrons are transferred to the anode electrode and then flow to the cathode via external resistance to generate an electrical current.

The current generated in MFC can play a role as a measurable signal for monitoring the quality of wastewater as it directly depends on electrogenic bacteria's metabolic activity. Any disturbances such as pH, organic substrate, dissolved oxygen, or the sudden presence of toxic substances will affect the mechanism and transfer rate of electrons to the anode (transducer), and subsequently cause current production to change (Jiang et al., 2015).

Recently, the concept of microbial fuel cell-based biosensor for toxicity detection has attracted much attention due to its simple compact, high stability and fast response time (Stein et al., 2012a; Xiao et al., 2015). Kim et al. (2007) investigated a system utilizing MFC as a biosensor for monitoring toxicity substances. The inhibition ratios were 61%, 46%, 28% and 38% with 1 mg L^{-1} in, respectively, organophosphorus compound, lead, mercury and polychlorinated biphenyls. In another study by Liu et al. (2014), Cr^{6+} and Fe^{3+} were selected to represent of acute-toxic heavy metal and low-toxic heavy metal in wastewater and monitered by a single-chamber MFC. This research showed that an MFC's ability to recognize different heavy metals through the change of the voltage signal. Tran et al. (2015) has been developed a MFC-based Fe^{2+}/Mn^{2+} biosensor by inoculating iron-oxidizing bacteria consortia. Researchers illustrated a good linear relationship between the current generated and the Fe^{2+} concentration varied from 168 to 1120 mg L^{-1} and response of the Mn^{2+} was 165 mg L^{-1}. Recently, Yu et al. (2020) investigated a self-powered MFC biosensor containing bioanode and Prussian blue (PB)

cathode to monitor toxic substances. The toxicity inhibition absorbances (IAs) were 28.4%, 11%, 33.8% and 66.6% for the respective samples of 1 mg L^{-1} Cd^{2+}, 1 mg L^{-1} Co^{2+}, 1 mg L^{-1} Pb^{2+} and 1 mg L^{-1} Cu^{2+}. Stein et al. (2012c) established a microbial fuel cell-based biosensor for detecting nickel, while a few years later, Deng et al. (2015) devised a method based on MFC to measure the effect of Cu^{2+} on soil microorganisms. These studies have led to a promising MFC system for real-time, self-powered toxicity biosensors to efficiently manage wastewater quality. However, virtually all MFC systems are complicated by having to employ the single-chamber method. Complicating this situation is the fact that the self-powered MFC-based heavy metal biosensor with complete response has not yet been systematically investigated.

In this study, a dual-chamber microbial fuel cell used for monitoring copper and arsenic in municipal wastewater was optimized. This research aims to: (i) determine the optimal operating conditions of microbial fuel cell-based biosensor for accurate monitoring; (ii) establish the correlation between copper and arsenic concentrations and the cell voltage production of the biosensor; (iii) evaluate the inhibition ratio of the heavy metal ions to the cell voltage; and (iv) investigate the recovery of MFC-based biosensor.

2. Experimental details and methods

2.1. Configuration of the MFC biosensor system

The double chamber MFC-B was made from plexiglass and constructed in a rectangular shape with the working volumes of anode and cathode chamber being 300 and 400 mL, respectively (Figure 1). Nafion 117 Dupont, USA (5cm x 5 cm) served to separate anode and cathode compartments. Carbon felt (3 cm in diameter, 6 mm in thickness) was placed in the anode chamber, while a carbon fiber brush that was 3 cm in length and 4 cm in diameter functioned as the cathode electrode. Titanium wire was selected to combine two electrodes through external resistance.

2.2. Inoculation process

Anaerobic sludge served as the inoculum at the anode chamber and the sample was taken from the Cronulla wastewater treatment plant, and in the meantime artificial wastewater acted as an enrichment source. The components of the artificial wastewater are summarized in Table 1 below. 100 ml anaerobic sludge and 200 ml wastewater were put into the anodic compartment. The microbial fuel cell with an external resistance of R 1000 was fed in a continuous mode. The anode compartment was kept in an anaerobic state by flushing it with nitrogen for 30 minutes to remove oxygen. The cathode chamber remained in an aerobic state with potassium ferricyanide $K_3Fe(CN)_6$ as the catholyte solution. When the potential between two electrodes was stable, this meant that the anode biofilm was mature enough for the next toxicity experiment to be conducted.

Table 1: Composition of artificial wastewater used in this study

Compounds	**Concentration (mg L^{-1})**
Organics and nutrients	
Glucose ($C_6H_{12}O_6$)	300
Ammonium chloride ($(NH_4)Cl$	19.11
Potassium phosphate (KH_2PO_4)	4.39
Trace nutrients	
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.368
Magnesium sulphate ($MgSO_4.7\ H_2O$)	5.4
Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	0.44
Ferric chloride anhydrous ($FeCl_3$)	1.45
Cupric sulphate ($CuSO_4 \cdot 5H_2O$)	0.39
Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	0.42
Sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$)	1.26
Yeast extract	30

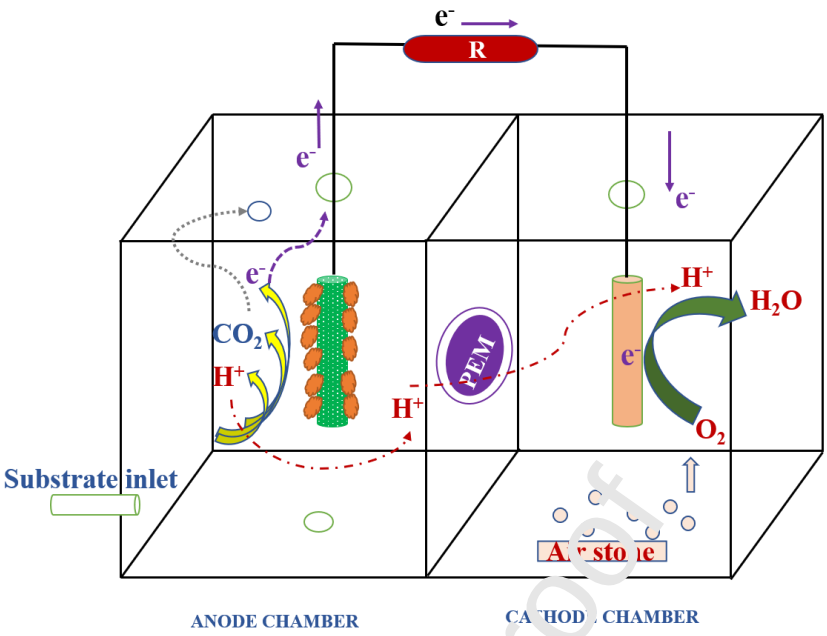


Figure 1. Schematic diagram of the MFC biosensor

2.2 Experiment process

The fuel feeding process was prepared with artificial wastewater in a 1 L bottle was pumped at 0.3 mL min^{-1} (HRT 24h) into the anode chamber. The feeding solution was sparged with nitrogen for 30 minutes before using to ensure no oxygen in the solution. Firstly, operational parameters involve pH, temperature, external resistance R, organic loading rates, and catholyte solution were optimized to get the biosensor's high sensitivity. After optimization, a toxicity test was established with varying concentrations of heavy metal ions (2-10 mg L^{-1} of copper and 0.05 to 5 mg L^{-1} arsenic).

The toxic substance used in this study was Cu^{2+} solution 500 mg L^{-1} and arsenic solution 100 mg L^{-1}. The effect of copper and arsenic on the performance of the MFC-based biosensor was as follows. Firstly, the anolyte solution without toxicant was continuously pumped into the anode compartment. When the voltage output remained constant in 10-12 hours, the influent was fed with a medium having a desired concentration of toxicant and operated for 4-6 hours. Secondly, the toxic anolyte was drained and replaced with clean artificial wastewater without toxicant to remove any redundant toxicant substance still left inside the anode chamber. Each

toxicity test was conducted in duplicate. The biosensor's inhibition ratio was calculated as equation 4 below. V_{nor} and V_{tox} were measured at the stable stage of the biosensor's operation with and without the toxicant, respectively.

2.3. Analysis method

The biosensor's voltage output was automatically recorded every 5 minutes via a voltage data logger 101A (MadgeTech) connected with a computer.

The current is calculated according to the Ohm law: $I = U/R$ (mA) (1)

The following two equations calculate the power density and current density:

$$P = \frac{U*I}{A} \quad (mW/m^2) \quad (2)$$

$$i = \frac{I}{A} \quad (mA/m^2) \quad (3)$$

A: surface area of the anode electrode

The following formula makes it possible to document the toxicity inhibition rate (I):

$$I = \left(\frac{Vnor - Vtox}{Vnor}\right) * 100 \quad (\%) \quad (4)$$

V_{nor}, V_{tox}: the voltage output of the biosensor with and without the toxicant, respectively (V).

COD was analyzed according to the standard method (APHA et al., 1995). The concentration of heavy metal ions were measured using Inductively Coupled Plasma mass spectrophotometry (ICP-MS). The pH of the sample was monitored using a pH meter (Hanna Instruments, HI9025), while the morphology of the anode biofilm was observed by utilizing a scanning electron microscope (Zeiss Supra 55VP, Carl Zeiss AG).

3. Results and discussion

3.1 Enrichment efficiency

As noted previously, the biosensor was inoculated with anaerobic sludge and enriched by wastewater, where glucose acts as a continuous carbon source. The MFC's voltage output generation increased with time and reached a peak at 0.48 mV after nearly two months. There

was no further increase in the voltage output following this acclimation period, which confirmed that the anode biofilm was fully acclimated with electrochemically-active bacteria. It was stable and ready for the following experiment process.

3.2 Optimization of MFC-B operating conditions

The growth of the electrochemical bacteria in an anode chamber depends on the energy supplied with micro-and macro-nutrients. Operating conditions such as pH, temperature, catholyte solution, external resistance, and organic substrate are also critical to the growth of bacteria. For this reason, these elements should be kept in the best possible conditions to get a more accurate biosensor.

3.2.1 Effect of pH and temperature

pH and temperature are the two crucial operational parameters that affect the mechanism of electrons transfer to the anode electrode and the biofilm stability, and consequently do influence the performance of the DC-MFC-B. A level of pH ranging from 5 to 9 was established to determine variation in the MFC biosensor's voltage output. The pH of feed solution was adjusted by utilizing HCl and $NaHCO_3$. The MFC biosensor's performance was assessed using temperatures ranging from 20 ^{0}C to 40 ^{0}C. Meanwhile, 300 mg L^{-1} COD, external resistance R 1000 Ω and DI water as the catholyte were maintained throughout the whole experiment.

As shown in Figure 2, the biosensor's maximum voltage was 456.5 mV at pH value variation from 7.0-8.0. When pH dropped to 5.0 or rose to 9.0, the maximum voltage output decreased to 345.3 mV and 325.6 mV, respectively. These outcomes indicated that neutral pH is the favored value for the biosensor's voltage generation because it is the best environment for: firstly, the growth of electroactive bacteria; and secondly, biofilm development in MFCs (Behera et al., 2010; Patil et al., 2011; Yuan et al., 2011). Upper or lower optimal pH will inhibit the electron mechanism and stability of the anode biofilm, so the voltage production diminished. These present results agree with what has been previously (Xu et al., 2016; Zhang et al., 2011; Zhuang et al., 2010). .

). The reason might be that the activity of electrogenic bacteria was inhibited in the acidification environment, affecting the biofilm formation of the anode surface and the stability of the MFC biosensor

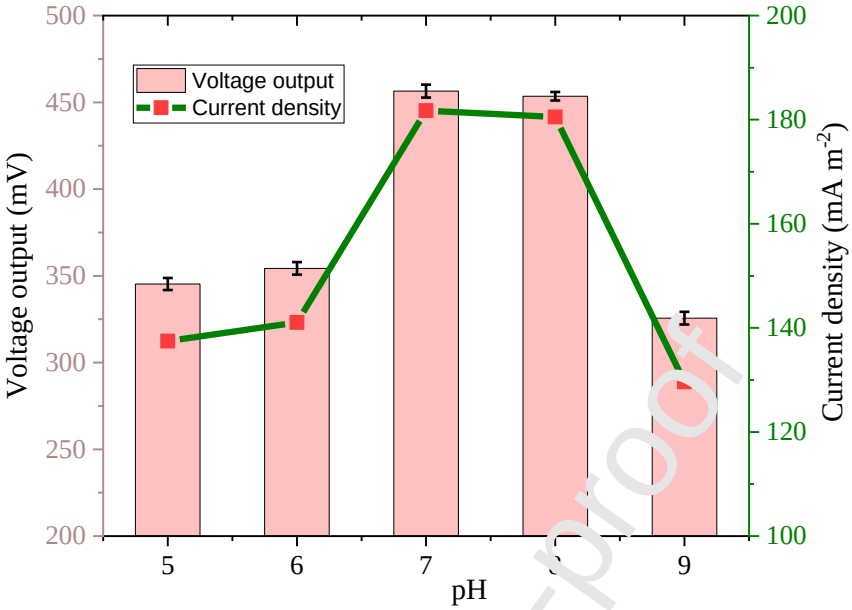


Figure 2: Effect of pH on the voltage output of the DC-MFC-B (COD 300 mg L^{-1}, R 1000 Ω, temperature 25^{0}C)

When the pH was kept stable at 7.0, the voltage generated was observed at temperatures ranging from 20 ^{0}C to 40 ^{0}C. As indicated in Figure 3, the voltage output of the biosensor increased with the rising of temperature from 20^{0}C to 25^{0}C and the highest voltage output (445.5mV) was obtained at 25 ^{0}C. There was an approximately 30% reduction in the voltage output when the temperature reached 40 ^{0}C. This result demonstrated that the ideal temperature for a developing community of electrogenic microorganisms was 25 ^{0}C. A too high temperature may benefit the growth of methanogens, which is harmful to the MFC biosensors' ability to create electricity. Therefore, pH 7.0-8.0 and 25^{0}C were selected as the optimal values for devising microbial fuel cell-based biosensors.

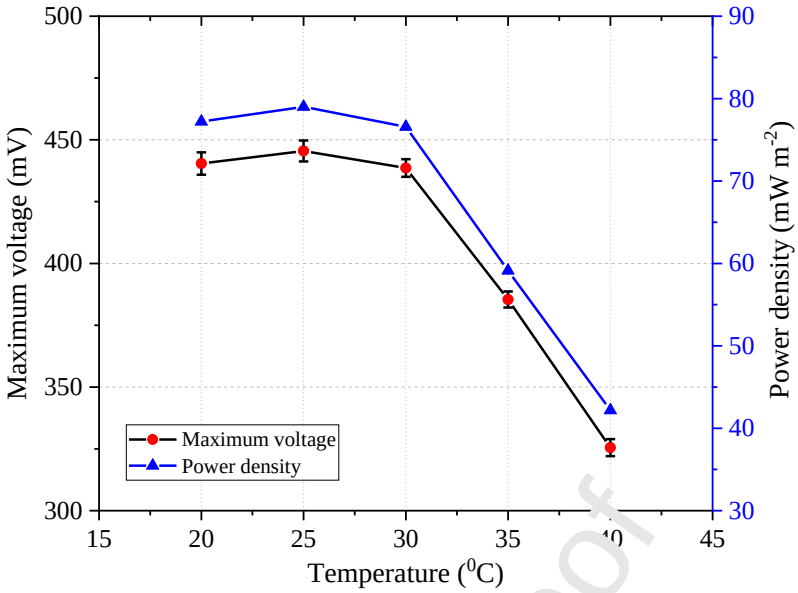


Figure 3: Effect of different temperature on the voltage output of the DC-MFC-B (COD 300 mg L^{-1}, R 1000 Ω, pH 7)

3.2.2 Effect of different resistance R

External resistance greatly influences the anode potential, microbial activity, and in turn, affects the sensitivity of the DC-MFC-B. Different R from 100 Ω to 10000 Ω was established to examine the voltage generation, current density and power density of the MFC biosensor. Figure 4 depicts the DC-MFC-B's polarization and power curves at the stable stage of power generation. The voltage produced in the MFC biosensor was increased when R rose up to 1000 Ω, but higher external resistance leads to a great reduction in voltage creation. The maximum voltage output was 456.6 mV at 1000 Ω. As seen from Figure 4, the power density increases with external resistance and reaches the highest value of 82.9 mW m^{-2} at R 1000. However, increasing R causes a reduction in the power density. One possible explanation is the transfer rate of electrons to the anode surface being inhibited at high external resistance and consequently diminished the voltage output. These results are consistent with several other studies (Xie et al., (2017). Therefore, the best external resistance 1000 Ω was selected for the biosensor experiment.

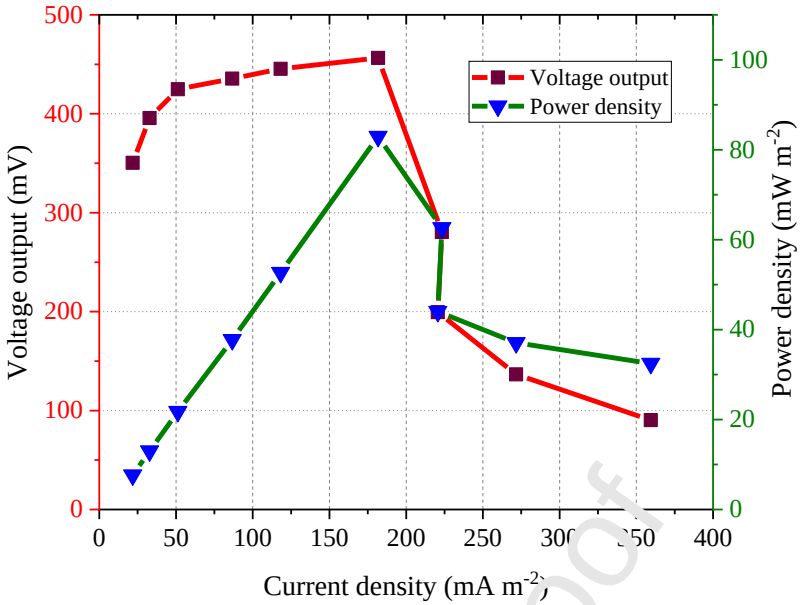


Figure 4: Polarization and power density curves

3.2.3 Effect of the catholyte solution

Different catholyte solutions, including distilled water (DI), NaCl 100 mM and 100 mM PBS, and $K_3Fe(CN)_6$ with different concentrations of 50 mM and 100 mM, were investigated as electron acceptors to test the ability to generate electricity. At the same time, COD 300 mg L^{-1} and R 1000 Ω were utilized for the experiment. The voltage output and current density experiments were assessed.

As seen from Figure 5, the maximum current density was observed at 185.3 mA m^{-2} with $K_3Fe(CN)_6$ 50 mM as a catholyte solution, whereas the cell produced 13.4% and 5.4% lower in current generation with NaCl 100 mM +100 mM PBS and DI water, respectively. Furthermore, the load voltage only increases about 1.5% when increasing the concentration of $K_3Fe(CN)_6$ from 50 mM to 100 mM. It can be concluded there was no significant increase in the load voltage output at a higher concentration of ferricyanide. The reason may be that if the $K_3Fe(CN)_6$ concentration is too high, there could be a penetration through PEM to the anode chamber, which can harm microorganism activity however, there was no seeing the sudden

change in the voltage creation in such a short time of penetrating through the proton exchange membrane.

These present results are consistent with other papers' findings. Oh et al. (2004) discovered that the power produced in the MFC when using ferricyanide was higher than that of oxygen because effective mass transfer and cathode potential are superior. In their work, Fan et al. (2016) investigated the production of electricity with different catholytes encompassing NaCl and $K_3Fe(CN)_6$, and subsequently, the highest current density was obtained at 8.5 mA m^{-2} with $K_3Fe(CN)_6$ solutions. For this reason, $K_3Fe(CN)_6$ 50 mM was selected as the catholyte solution for the following toxicity experiments.

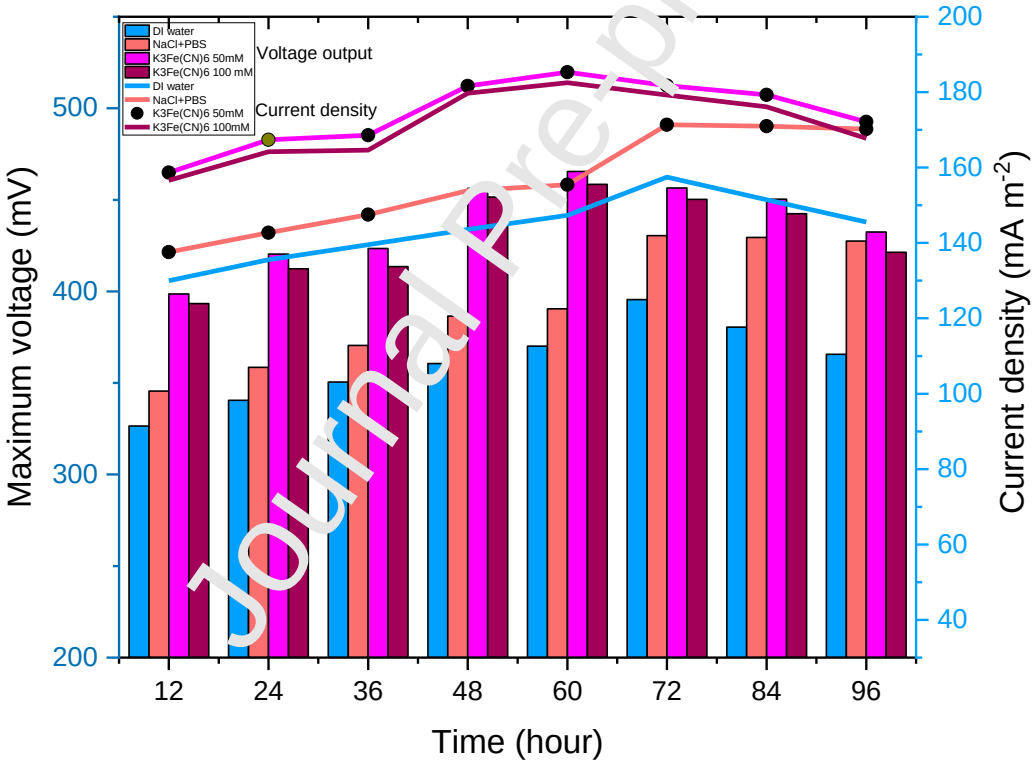


Figure 5: Cell voltage and current generation with different catholyte solution

3.2.4 Effect of organic substrate concentration

A suitable substrate concentration is also determined to enable fast and sensitive toxicity detection. Different COD concentrations from 100 to 500 mg L^{-1} were employed to detect the change in voltage generation in the DC-MFC-B. During the experiment, external resistance was

kept at 1000Ω, $K_3Fe(CN)_6$ 50 mM was cathode solution. Furthermore, pH 7.0-8.0 and 25^0C was selected as the optimal values for devising microbial fuel cell-based biosensors.

As seen in Figure 6, the DC-MFC-B's voltage output increases gradually with rising COD concentration from 100 mg L^{-1} to 300 mg L^{-1}. However, there was a drop-off in the voltage generation when the COD concentration rose to 400 and 500 mg L^{-1}. The highest voltage achieved was 445.5 mV at 300 mg L^{-1} COD and its value fell to 395.6 and 356.5 at 400 and 500 mg L^{-1} COD, respectively. The reason for this may be that the lower organic substrate (100 to 300 mg L^{-1}) favored the metabolism of the electrogenic bacteria (Hiegemann et al., 2016). A higher organic concentration leads to the development of methanogenic microorganisms, which does not facilitate electricity production. In a study by Juang et al. (2011), the authors concluded that the MFC biosensor could reach its maximum value of voltage production at a specific organic concentration. Furthermore, they also illustrated that the voltage output would decrease with the increase in the organic concentration. Morover, Elakkiya and Matheswaran (2013) revealed that membrane fouling occurs due to a high organic loading rate, further affecting the proton's transport mechanism from anode to the cathode. Consequently, this reduced the voltage generation of MFC biosensor. A decade ago, Juang et al. (2011) concluded that the MFC could produce the highest amount of electricity at the optimal substrate concentration. In this study, the best value of organic substrate concentration - 300 mg L^{-1} - was selected.

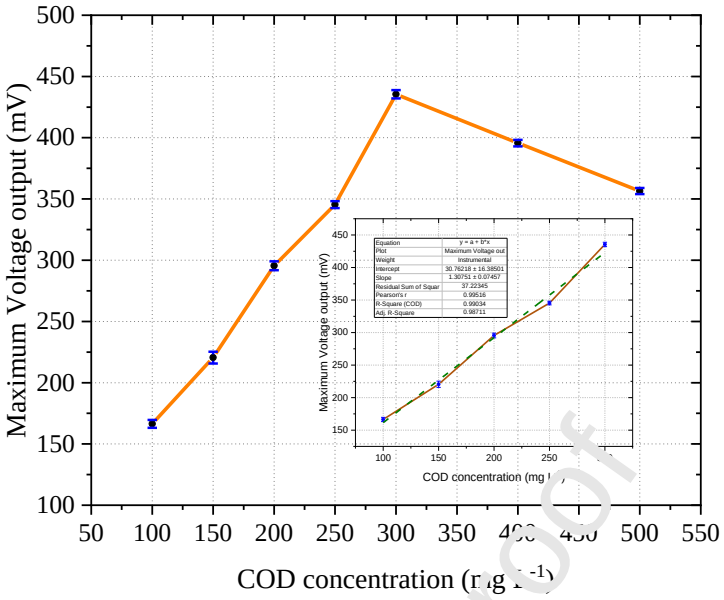


Figure 6: Maximum voltage generation of MFC biosensor with COD concentrations

3.3. Heavy metals – Toxicity inhibition test

Heavy metals pollution is a very serious and ongoing issue due to their toxicity, persistence and non-biodegradability (Ferati et al., 2015). In this study's experiment, copper and arsenic at different concentrations were added to the MFC biosensor in a steady state of voltage output to simulate toxicity inhibition. Adding copper and arsenic at various concentrations led to a drop in the voltage output generation and the sensor experiencing a drop in the current. The inhibition ratio was calculated by the difference between the peak of voltage output before and after the presence of a heavy metal per unit time.

3.3.1 Response of MFC biosensor to copper (1-10 mg L^{-1})

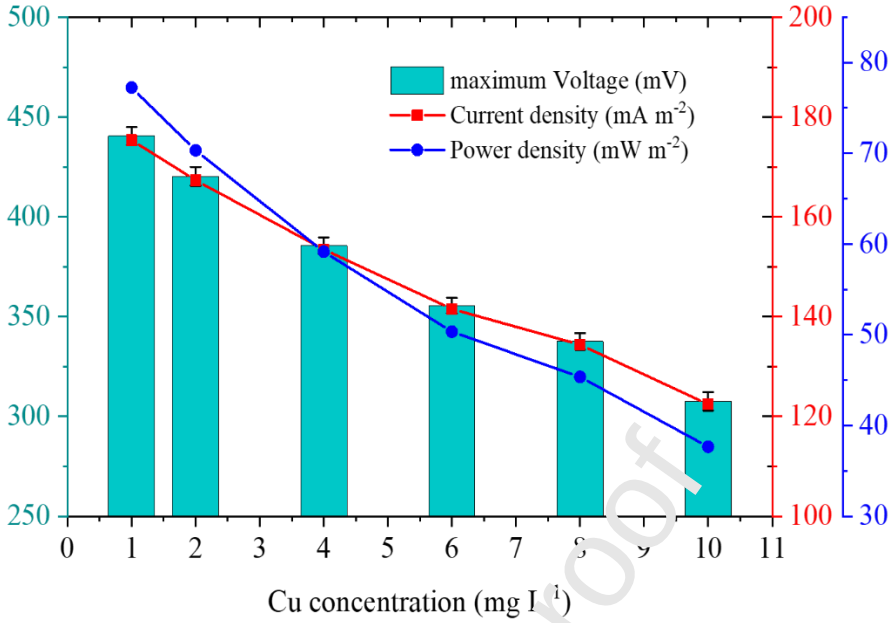
As can be seen in Figure 7(a), it is generally the case that an increasing copper concentration leads to a decline in the biosensor's voltage output. The maximum voltage of the biosensor was 440.5 mV at 1 mg L-1, which represented a slight change (2.22%) compared to this when the sensor was in a normal state. The normal state of the MFC biosensor is considered at the optimum conditions of operating parameters, including pH 7, temperature 250C, R 1000 Ω,

COD 300 mg L-1 and $K3Fe(CN)_6$ 50 mM as the catholyte solution and the maximum voltage generated is 450.5 mV.

It means there was no significant effect of the electrogenic bacteria of the biosensor on microbial activity at a low concentration of Cu^{2+}, resulting the biosensor working normally. However, the voltage generated by the MFC-biosensor diminished significantly from 420.3 mV at 2 mg L^{-1} of Cu^{2+} to 307.6 mV at 10 mg L^{-1} of Cu^{2+}, which confirmed that the poisoning effect of copper on the electrogenic activity becomes more critical. It in turn decreases the amount of voltage being generated. Figure 7(b) illustrates the correlation between Cu^{2+} and the maximum voltage of the cell with R^2=0.989.

Recovery time of the biosensor after being washed with fresh medium varied according to different concentrations of Cu^{2+}. As shown in Figure 8(c), when the toxic substance was at a smaller concentration (2-4 mg L^{-1}), the cell voltage can reach a new steady-state after 4 hours (nearly 90% compared to the initial state). However, a larger concentration of copper requires a longer recovery time (10-12 hours) to establish a new stable voltage. The inhibition ratio of the MFC biosensor by copper is illustrated in Figure 7(d). The inhibition ratio at 2 mg L^{-1} of copper was 6.68% and the inhibition ratio increases gradually with an increasing copper concentration. The highest inhibition (nearly 30%) to the toxic substance was observed at 10 mg L^{-1} of Cu^{2+}. Figure 7(c) clearly depicts an excellent linear correlation (R^2=0.983) between inhibition ratio and copper concentrations. These observations agree with previous research such as that by Yue et al. (2015), who discovered an excellent linear relationship between Cu^{2+} concentration from 1- 10 mg L^{-1}, and inhibition ratio was obtained.

(a)



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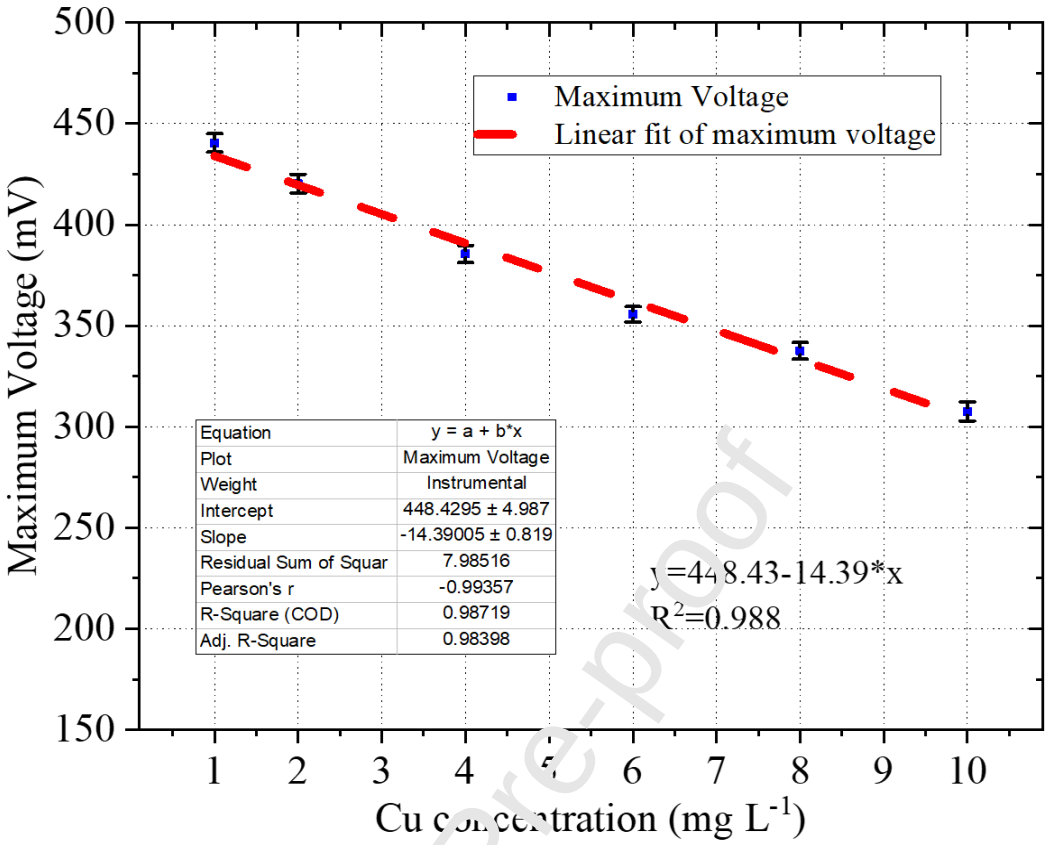

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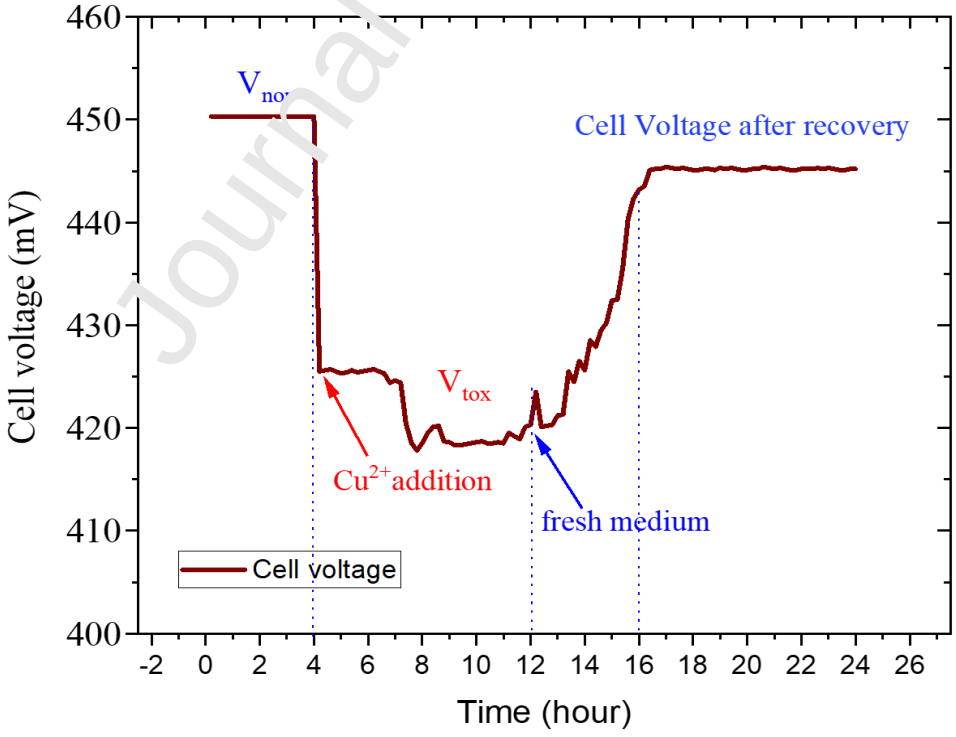


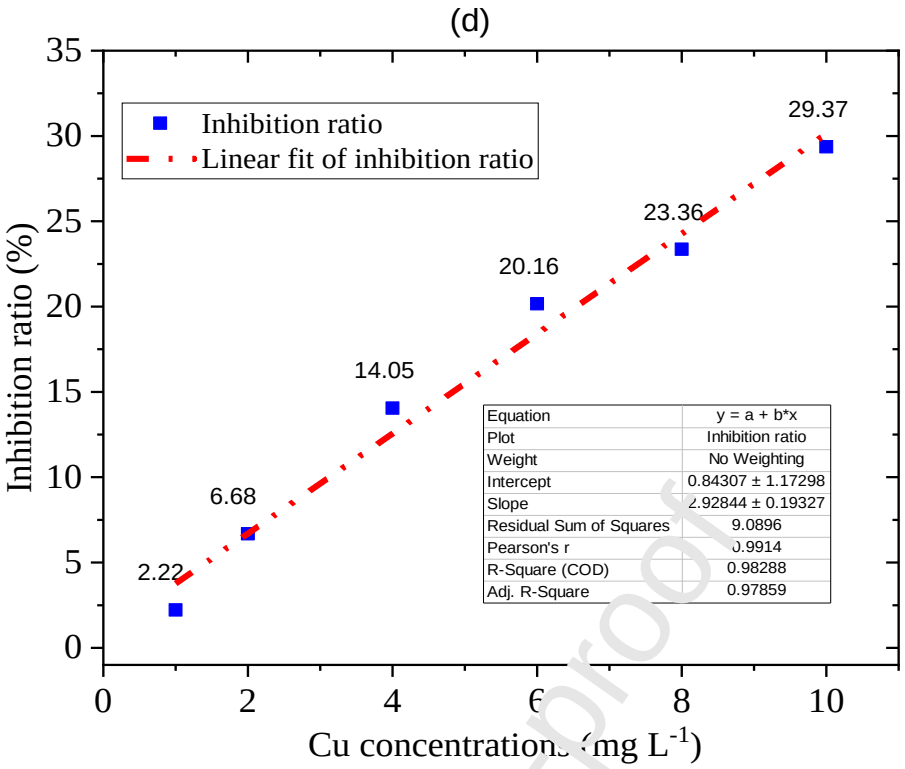


Figure 7: (a) Maximum voltage, current density and power density with different copper concentrations; (b) Linear relationship between Cu^{2+} and the maximum voltage of biosensor; (c) Variation in cell voltage of the biosensor following the addition of 4 mg L^{-1} copper;

(d) The inhibition ratio of the sensor by different copper concentrations

Table 2: MFC inhibition by copper

Cu^{2+} concentrations (mg L^{-1})	*V_{nor} (mV)*	*V_{tox} (mV)*	*Inhibition ratio (%)*
1	450.5	440.5	2.22
2	450.4	420.3	6.68
4	448.5	385.5	14.05
6	445.4	355.6	20.16
8	440.5	337.6	23.36

10	435.5	307.6	29.37

Results from this study have some competition compared to previous researches on the broader detection range of copper. For example, in a study by Yu et al. (2017), 1 - 4 mg L^{-1} of copper was detected by a self-power MFC. Shen et al. (2013) developed a toxicity MFC system for a quick response with copper with 5-7 mg L^{-1}. Literature research also reveals that a concentration of 12 mg L^{-1} Cu^{2+} was detected in the study by Wu et al. (2018). Different results might come from the difference in configurations (single or dual chamber MFC), the type of inoculation and the operating parameters of the biosensor.

Table 3: Comparison of the detection range of copper and arsenic

Configuration of MFC	*Anode/Cathode material*	*Inoculun*	*Inhibition ratio (%)*	*Detection range (mg L^{-1})*	*Reference*
Single chamber	Carbon felt	Domestic wastewater	30-85	5-7	(Shen et al., 2013)
Double chamber	Grafite felt	Aanerobic sludge	7.9-18.48	1-4	(Yu et al., 2017)
Double chamber	Grafite felt/Carbon fiber brush	Mixed culture	Not available	2-6	(Jiang et al., 2017)
Double chamber	Grafite felt/Carbon fiber brush	Mixed culture	Not available	2	(Jiang et al., 2015)
Single	Carbon cloth	Activated	7.5-22.5	1-10	(Yue et al.,

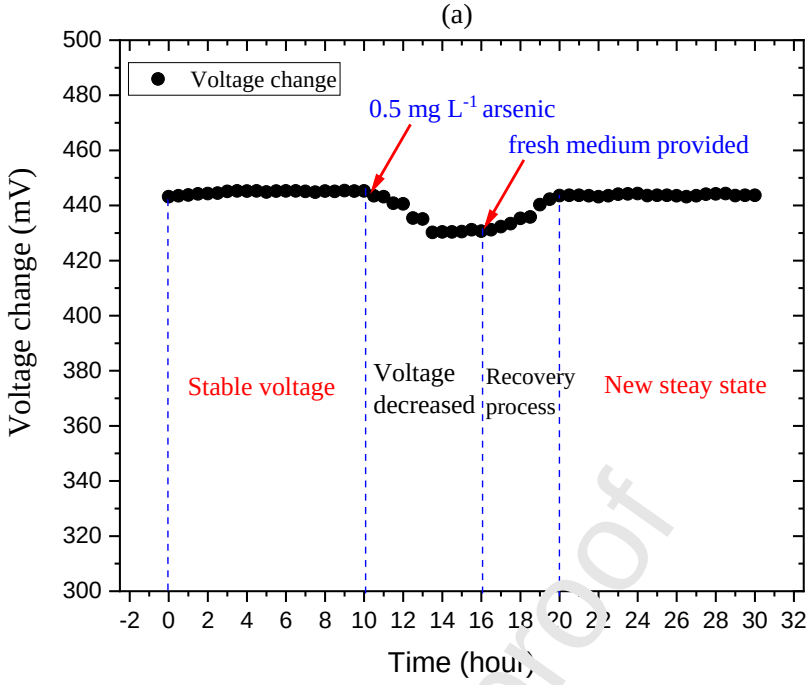
chamber		sludge			2015)
Single chamber		*S. oneidensis*	N/A	Arsenite 0-100 µM	(Webster et al., 2014)
Double chamber MFC	Carbon felt/Carbon cloth	LB media (tryptone, yeast, solium chloride)	N/A	Arsenite: 0-0.5 mM Arsenate: 0-0.44 mM	(Rasmussen and Minteer, 2015)

3.3.2 Voltage responses of MFC biosensor to arsenic (0.05-5 mg L^{-1})

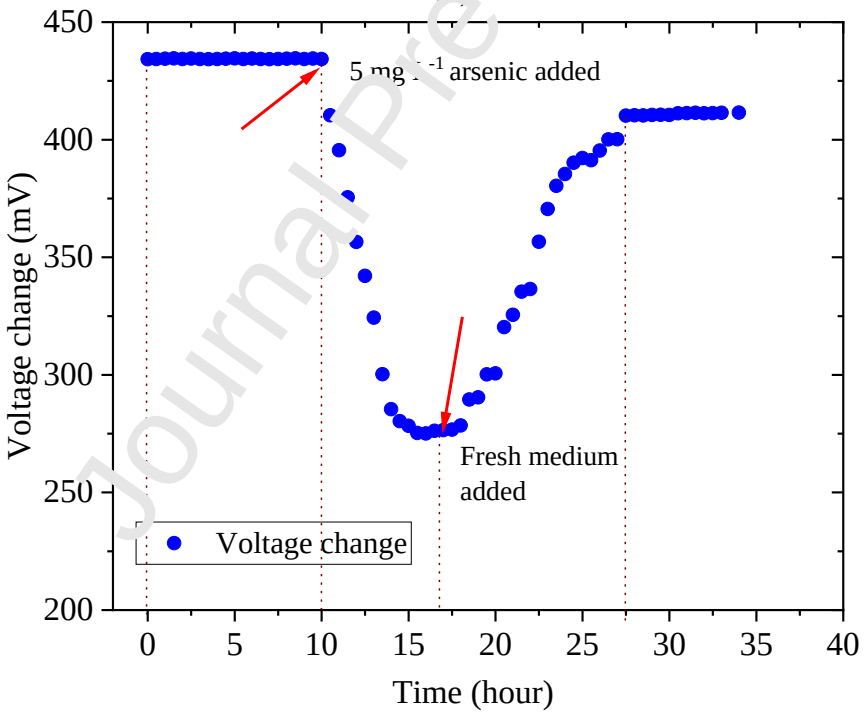
The calibration curve between MFC biosensor voltage and arsenic concentration was investigated by spiking arsenic (0.5-5 mg L^{-1}) in the medium of the MFC biosensor. In general, the voltage decreased in proportion to the concentration of the toxic substance, which indicated arsenic is biologically toxic to the electrogenic bacteria in the anode chamber. There was a minor change in the voltage output of the MFC biosensor (standing at 430.5 mV) after dosing at 0.5 mg L^{-1}. Higher concentrations of arsenic lead to a more significant drop in the biosensor's voltage generation. Nearly 40% reduction in the cell voltage was observed at 5 mg L^{-1} of arsenic compared to the regular operation conditions. The reason could be that the higher the arsenic concentration, the fewer electrons were transferred to the cathode, so the cell voltage was expected to decrease with rising arsenic concentrations.

Figures 8(a) and 8(b) illustrate the DC-MFC-B's response at two typical concentrations of 0.5 and 5 mg L^{-1} of arsenic. These figures clearly show that the fall in voltage at the very beginning (1-2 h) was not significant due to the protection strategy of the mature biofilm itself. With the increase in contact time (4-6 h), the cell voltage decreased gradually, which illustrates that the toxic arsenic needs time to diffuse into the biofilm towards cells and inhibit the activity of bacteria.

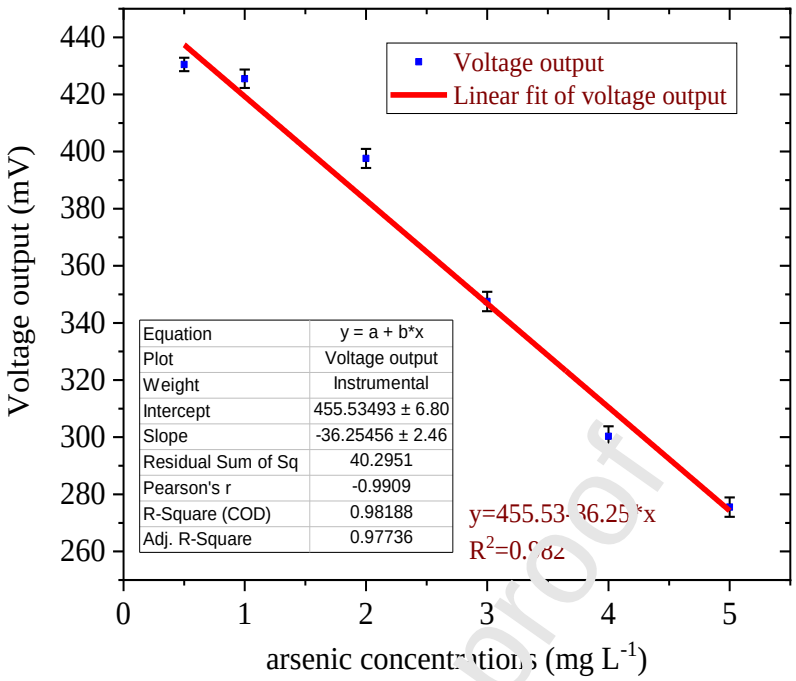
(a)



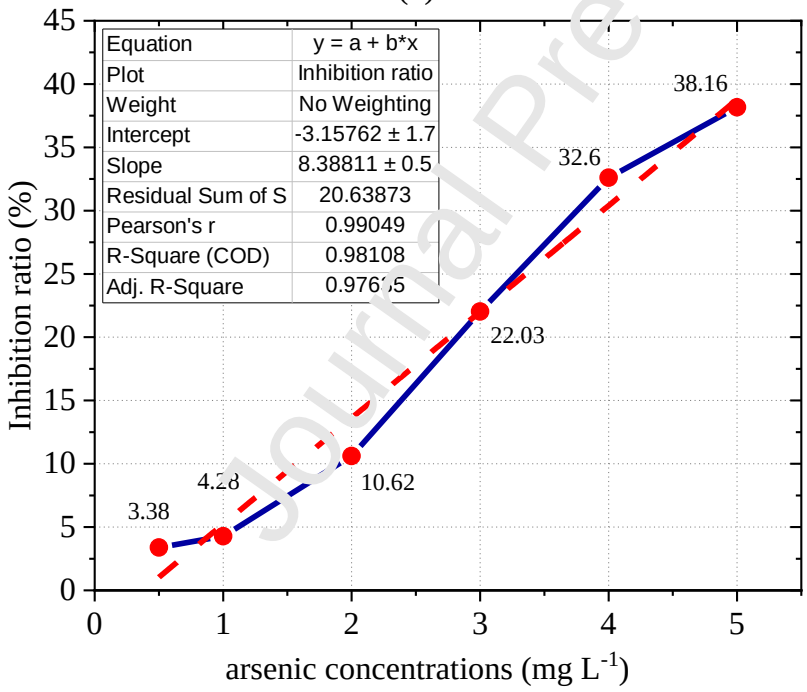
(b)



(c)



(d)



(D)

Figure 8: (a), (b): Response of the biosensor with time after dosing 0.5 and 5 mg $L^{-1}$of arsenic; (c): Influence of arsenic on the voltage output of MFC biosensor; (d): Inhibition ratio of MFC biosensor at different arsenic concentrations

As shown in Figure 8c, a strong linear relationship could be obtained when the arsenic was in the 0.5 mg L^{-1} to 5 mg L^{-1} range. The regression equation was determined as y=459.05-37.25*x (R^2=0.982). When the arsenic concentration was under 0.5 mg $L^{-1,}$ there was no significant change in the cell voltage, while a dramatic change at a high concentration of arsenic over 5 mg L^{-1} did occur. Subsequently, 0.5 to 5 mg L^{-1} served as the detection range of arsenic concentration by the MFC biosensor in this study. A good correlation between the inhibition ratio of different concentrations of arsenic to the biosensor voltage generation was observed with R^2= 0.981 (Figure 8d). Our results are an improvement over previous studies regarding the limit of arsenic detection (Rasmussen & Minteer, 2015; Webster et al., 2014).

Arsenic was cleaned out by feeding the fresh medium without any toxic material following exposure to the toxicant, which helped the biosensor to recover. Recovery is one of the key requirements for a biosensor. If the sensor cannot recover, the biosensor is virtually useless for urgently required real-world applications. Recovery time is the time needed for the cell voltage or current to reach a new steady-state after the effects of toxicity. With fresh media being added after exposure to the toxic substance, the anode bacteria activity could recover (nearly 90%) from the toxic samples. The recovery time varied at different arsenic concentrations, ranging from 4 to 12 hours. A higher toxic concentration requires a longer recovery time. In this study, the cell voltage can recover after 12 hours at the highest concentration of arsenic (5 mg L^{-1}) which means that an MFC-based biosensor can be applied for monitoring heavy metals. These recovery times' results are consistent with what other studies have documented (Stein et al., 2012b; Stein et al., 2012c).

4. Conclusion

This study successful developed a DC-MFC-B for monitoring copper and arsenic in wastewater. The operating conditions, including pH, temperature, organic loading rate and external resistance, were optimized. The MFC-B could monitor Cu^{2+} (1-10 mg L^{-1}) and arsenic (0.5-5 mg

L^{-1}) by measuring the decrease in the cell voltage after adding copper or arsenic to the anolyte solution. Results indicate that only a minor change occurred in the voltage output for both heavy metal ions at a low concentration because the activity of the bacteria in the anode chamber was not affected at a low concentration. Furthermore, a linear decrease in the cell voltage and heavy metal concentration was observed with R^2 = 0.989 and 0.982 for copper and arsenic, respectively. In the meantime, the inhibition ratio of the MFC biosensor was proportional to the concentration of both copper and arsenic. The recovery of the MFC-B was obtained after a feed with a fresh medium containing no heavy metal ions, and the MFC-B could recover in a few hours. Finally, the linear response was observed over a larger detection limit of Cu^{2+} and arsenic than what has been reported in other MFC-related analyses. These results indicate that our proposed system can function well as a heavy metal biosensor indicator.

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