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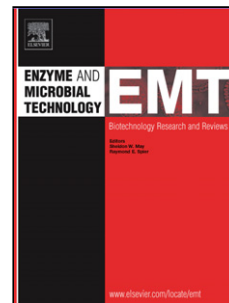
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Decolourisation of Acid orange 7 in a microbial fuel cell with a laccase-based biocathode: Influence of mitigating pH changes in the cathode chamber.

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Highlights of Research

1. First Study to observe the influence of mitigating pH changes and its effect on laccase activity in a microbial fuel cell (MFC).
2. Decoupling pH and salinity in the cathode chamber of MFC.
3. Correlation of laccase activity with performance of the system.
4. Comparison on efficiency of Nafion and CEM membrane in a MFC relative to laccase activity.

Abstract

Biocathodes may be a suitable replacement of platinum in microbial fuel cells (MFCs) if the cost of MFCs is to be reduced. However, the use of enzymes as bio-cathodes is fraught with loss of activity as time progresses. A possible cause of this loss in activity might be pH increase in the cathode as pH gradients in MFCs are well known. This pH increase is however, accompanied by simultaneous increase in salinity; therefore salinity may be a confounding variable.

This study investigated various ways of mitigating pH changes in the cathode of MFCs and their effect on laccase activity and decolourisation of a model azo dye Acid orange 7 in the anode chamber. Experiments were run with catholyte pH automatically controlled via feedback control or by using acetate buffers (pH 4.5) of various strength (100 mM and 200 mM), with CMI7000 as the cation exchange membrane. A comparison was also made

between use of CMI7000 and Nafion 117 as the transport properties of cations for both membranes (hence their potential effects on pH changes in the cathode) are different.

Results show that using Nafion 117 membrane limits salinity and pH changes in the cathode (100 mM acetate buffer as catholyte) leading to prolonged laccase activity and faster AO7 decolourisation compared to using CMI7000 as a membrane; similarly automatic pH control in the cathode chamber was found to be better than using 200 mM acetate buffer.

It is suggested that while pH control in the cathode chamber is important, it does not guarantee sustained laccase activity; that salinity increases affect the activity and could be mitigated using a cation selective membrane.

Key words: microbial fuel cell, azo dye decolourisation, laccase, biocathode, pH, salinity, acid orange 7

1. Introduction

Microbial fuel cells (MFC) are a promising technology in the treatment of wastewater and simultaneous electricity generation. However, there are operational and technological challenges that prevent the use of MFCs for practical applications. The major hurdles are coulombic losses, high internal resistances, poor reaction kinetics of the oxygen reduction reaction (ORR) at the cathode and development of pH gradients across the membrane all of which affect the performance of the system [1].

The efficiency of the oxygen reduction reaction (ORR) at the cathode is affected by high overpotentials at the electrode and oxygen mass transfer limitation. Although platinum is currently the most effective and commonly used catalyst, its high cost and unsustainability hinders the scaling up of MFCs. Transition metal based catalysts such Mn, Co, V and their oxide forms have been used as cathode catalysts in MFCs as a replacement for platinum [2–4]. These catalysts have produced power comparable to Pt but they are also unsustainable and are not stable. The possible leaching of the metals into the environment and their toxic effects is a concern [5].

To improve the cathode reaction and reduce the cost, bio-cathodes such as microorganisms and enzymes have been investigated for their catalytic activity. Oxidoreductase enzymes such as manganese peroxidase (MnP), bilirubin oxidase and laccase have been utilised as catalysts

at the cathode of MFCs [6–8]. Enzymes are sustainable and their production is more environmentally friendly compared to metal-based catalysts. Enzymes (e.g. laccase) have an advantage over microorganisms as a biocathode in the sense that they do not need a carbon source (and other nutrients) to maintain them. The use of a carbon source would not only add to the operational costs but also add COD to a system whose objective is partly to remove COD from wastewater.

Laccase is a multi-copper containing oxidoreductase enzyme. It is capable of one electron oxidation of other substrates and four electron reduction of O_2 to H_2O [9]. Laccase from the fungi *Trametes versicolor* is thermodynamically favourable for oxygen reduction at the cathode due to its high redox potential (780 mV vs NHE). The cost of commercially available laccase is only a fraction of platinum on a mass basis.

Luo et al., 2010 [3] generated a maximum power density of 160 mW m^{-2} from a MFC with an immobilised laccase (*T. versicolor*) with ABTS as mediator, comparable to 190 mW m^{-2} for a MFC with a platinum-based catalyst with acetate as substrate at the anode. A maximum power density of 37 mW m^{-2} was obtained from laccase immobilised in hydrogel with ABTS as mediator on a platinum based electrode [10].

The use of enzymatic cathodes is however, limited by the poor stability of the enzymes in the system. In a fuel cell the lifetime of the enzymes typically vary from 7-10 days [11]. Rubenwolf et al, 2012 investigated the deactivation of laccase from *T. versicolor* in citrate buffer (pH 5) at room temperature. They observed that the enzyme remained stable for 2 days after which there was constant deactivation rate with a half-life of 7 days. A MFC operating with laccase air breathing cathode catalyst had a 4% drop in voltage after 4.75 days [12]. Laccase cost on an electrode in a microbial fuel cell was shown to retain its activity for a period of 30 days [7,13].

Laccases from fungi were shown to be inhibited by environmental factors such as pH [14]. The pH optima for fungal laccase with ABTS as substrate is in the range of 3-5. pH affects the total net charge of enzymes and the distribution of charges on their exterior surfaces and these changes affect the activity and structural stability of the enzyme.

In a MFC the oxidation of substrate by the microorganisms in the anode produces protons and electrons. The incomplete transfer of protons across the membrane results in an acidic environment at the anode and the movement of cations to the cathode increases both the

salinity and pH in the cathode chamber. pH gradients have adverse effects on the performance of MFCs by interfering with metabolic activity in the anode and increasing potential losses at the cathode. According to the Nernst equation these pH gradients cause high anodic equilibrium potential and/or low cathodic equilibrium potential that significantly lowers the cell voltage and causes a loss of ~60 mV per 1 pH change [1,15]. Fokina et al, 2015 have observed that increase in pH by one unit caused a decrease in oxygen reduction potential in the range of 30-80 mV in a biofuel cell using laccase as cathode catalyst [16]. Changes in charges with pH can also affect the activity, structural stability and solubility of enzymes. Salinity affects the movement of charged groups and the solubility (hence activity and stability) of enzymes.

In order to increase the stability of laccase in MFC cathodes and improve MFC performance various strategies (Section 2.1) to mitigate pH changes in the cathode chamber were explored in this study. The investigation was carried out in the context of treatment of azo dye containing wastewater.

2. Materials and Methods

2.1. Experimental design

The MFC used in the study was the 'H'-type reactor with a working volume of 200 ml in each chamber. The electrodes were constructed from carbon fibre (non-woven) with a surface area of 25 cm². Four systems were set up to mitigate pH changes in the cathode chamber. System 1 which will be referred to as 'Nafion' involved using Nafion 117 as the ion exchange membrane. System 2 referred to as 'pH control' involved automatically controlling the pH in the cathode through feedback control by addition of acid or base. System 3 referred to as 'buffer strength' involved using a buffer of higher strength 200 mM compared to other systems have used 100 mM acetate buffer (pH 4.5) as buffer. System 4 referred to as 'CEM' involved using CMI7000 membrane as the ion exchange membrane. These conditions are summarised in Table 1 including a rationale for each.

The composition in the anode was the same for all the reactors. The anolyte consisted of minimal salts medium containing (per litre): 0.46 g NH₄Cl, 0.22 g (NH)₂SO₄, 0.117 g MgSO₄, 7.7 g Na₂HPO₄·7H₂O, 2.87 g NaH₂PO₄ along with 1% (v/v) trace minerals as described by [18] and 1% (v/v) vitamin mix as described by [19]. The carbon source was

pyruvate at a concentration of 1 g L^{-1} and casein hydrolysate was added at 500 mg L^{-1} . The pH of the anolyte was initially adjusted to 7.

The catholyte for systems 1, 2 and 4 was 100 mM acetate buffer with laccase freely suspended at a pH of 4.5. System 3 consisted of 200 mM acetate buffer with laccase suspended at pH 4.5.

Nafion 117 membrane was pre-treated by heating in each of the following solutions at 60°C for 60 minutes: 0.1 M H_2SO_4 , 0.1M H_2O_2 and finally in deionised water. CMI7000 ion exchange membrane was soaked in 5% NaCl for 12 hours prior to use.

2.2 Operating conditions

The anode and cathode were connected to a resistor of $2 \text{ K}\Omega$. We have previously determined, for the MFC systems we use, the internal resistance to be $2 \text{ K}\Omega$ at which external resistance the power output would theoretically be expected to be maximum. The anode was inoculated with 10% v/v *S. oneidensis* MR-1 culture previously grown in Luria Bertani broth to an OD of 0.6 and the dye Acid Orange 7 was added at a concentration of 150 mg/l. The anode chamber was sparged for 10 minutes with nitrogen gas to remove any dissolved oxygen and maintain an anaerobic environment. No active stirring was involved in the anode during experiments.

The cathode chamber consisted of commercial laccase (Sigma–Aldrich) from *Trametes versicolor* (13.6 U mg^{-1}) in a buffer solution. In systems 1, 2 and 4 300 U L^{-1} of laccase was added to 100 mM acetate buffer with pH 4.5 and in system 3 300 U L^{-1} of laccase was added to 200 mM of acetate buffer at pH 4.5. The cathode chamber was maintained in aerobic conditions by supplying air through an air stone at a rate of 200 ml air per min. Experiments were conducted at a temperature of 30°C over a period of 10 days. Experiments were repeated two times.

2.3. Analytical Procedures

2.3.1 AO7 Decolourisation

The concentration of AO7 was measured at various time intervals using a UV-visible spectrophotometer at a wavelength of 484 nm which is the maximum absorption wavelength for the dye. The samples were first centrifuged at 8000 g for 10 minutes to remove the suspended biomass. The decolourisation efficiency was calculated by

$$DE (\%) = \frac{A_0 - A_t}{A_0} \times 100$$

A_0 and A_t are the absorbance units at the initial and each time point respectively. A time series is plotted for the absorbance values measured.

2.3.2 Electrochemical Analysis

The electric potential across the system was recorded every 10 minutes using a data acquisition system Picolog (Pico Technology, UK). The current through the unit was calculated using Ohm's Law:

$$Current (I) = \frac{Voltage (V)}{Resistance (\Omega)}$$

The power produced was calculated using the following formula:

$$P = I \times V$$

where P is power in Watts, I is current in amperes and V is the electric potential in volts.

The power and current per surface area of anode (25 cm²) was used to calculate the power and current density. To carry out polarisation tests, each MFC unit was connected to various external resistances ranging from 10 Ω to 1 M Ω and the potential measured using a multimeter.

2.3.3 Chemical Oxygen demand, pH and ionic strength measurement

The COD was measured by the standard closed reflux titrimetric method described by Environment Agency (UK), based on APHA method 5220D. COD reagent- Ficodox (Fisher Scientific) containing sulphuric acid, K₂Cr₂O₇, Ag₂SO₄ was added to appropriately diluted samples and digested at 150°C for two hours. The samples were then titrated against Ferrous Ammonium Sulphate (FAS, 0.025 M). The COD was calculated using the following formula:

$$COD \left(\frac{mg}{L} \right) = \frac{8000 * (V_b - V_s) * DF * M}{Sample\ volume}$$

where DF is the dilution factor, M is the molarity of FAS, V_b and V_s are the titrant volumes of FAS for blank and substrate respectively.

pH and ionic strength were measured using a calibrated benchtop combined pH and ionic strength meter (pH/CON 700 meter, Cole-Parmer, UK).

2.3.4 Coulombic Efficiency (CE)

The CE was calculated as follows [20]:

$$CE (\%) = \frac{M \int_0^t I dt}{b * F * V_{anode} * \Delta COD}$$

where M is the molecular weight of oxygen (32), I is current over a time period (A), b number of electrons exchanged per mole of oxygen, F is Faraday constant (96485 C mol⁻¹), V_{anode} is working volume of anode and Δ COD is change in COD over time (g L⁻¹).

2.3.5 Enzyme Activity

The activity of laccase was measured using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) as a substrate . A solution of 3 ml acetate buffer (100mM, pH 4.5), 0.1 ml ABTS (0.5 mM) and 0.1 ml of enzyme was used. The oxidation of ABTS by laccase was measured by a UV spectrophotometer at 420 nm [8]. The enzyme activity unit (U) was defined as the amount of enzyme required to oxidize 1.0μmol ABTS min⁻¹ at 25°C [21].

2.3.6 Detection of degradation metabolites by HPLC

The degradation products of AO7 were identified using high performance liquid chromatography. The HPLC system (DIONEX GS50) was equipped with a Phenomenex Gemini C18 reversed phase column (5 μm, 150 X 4.6 mm) and a Photodiode Array (PDA) detector (DIONEX PDA-100). Aromatic compounds were quantified using HPLC with known concentrations of reductive compounds of AO7, sulfanilic acid and 1-amino-2-naphthol. They were detected at wavelengths 248 nm and 284 nm respectively. The mobile phase consisted of 50% methanol and 50% 33 mM (pH 7) phosphate buffer. The presence of degradation products from the sample was confirmed by the retention times of the standards.

2.3.7 Toxicity Assay

The toxicity of the samples was measured by the Microtox toxicity assay using the bioluminescence bacteria *Vibrio fischeri* [22] . The samples were centrifuged at 4000g, the cells harvested, washed twice with sterile phosphate buffer and suspended in 2% NaCl. Luminescence was measured using a Fluostar Optima plate reader. The absolute light units were recorded and IC₅₀, a concentration which inhibits 50% of light was calculated for each sample. The IC₅₀ concentrations were expressed as the COD equivalent of the samples.

2.3.8. Statistical analysis of data

All experimental data indicated on the graphs are the means of duplicate experiments unless otherwise stated and the error bars in the graphs represent the standard deviation of the mean (SD). Statistical analysis of data was conducted by one way analysis of variance (ANOVA) using SPSS statistics package.

3. Results and Discussion

3.1 AO7 decolourisation

The decolourisation of Acid Orange 7 was measured at the maximum absorption wavelength for the dye (484 nm). The overall removal efficiency was 89% dye removal in the reactor containing Nafion compared to systems where pH was automatically controlled (82%), where buffer strength was increased (80%) and where a cation exchange membrane was used (78%). There was greater than 50% colour removal within 48 hours for Nafion-containing and automatically controlled pH systems (Figure 1).

At the end of the runs there was 78% COD reduction in the Nafion reactor followed by 76% in both pH control and buffer strength reactors. The CEM reactor showed a 74% COD reduction.

A combination of increased proton transport, mitigated pH changes in the cathode chamber (see section 3.2) and a high COD reduction are the likely reasons why Nafion performed better than other systems. Biologically, the azo bond is thought to be cleaved under anaerobic conditions in the anode chamber leading to decolourisation but the mechanism is not clear. Since azo dyes are large and usually charged, they are likely to be reduced extracellularly. It has been suggested that the decolourisation process is a fortuitous one where azo dyes might act as an electron acceptor supplied by carriers of the electron transport chain. There also suggestions that decolourisation is due to non-specific extracellular reactions occurring between reduced compounds of anaerobic metabolism e.g. sulphides and the azo dyes. Others suggest that anaerobic reductive cleavage of the azo bond is aided by azoreductases, the electron shuttling being aided by soluble redox mediators e.g. flavins [23,24].

3.2 pH and salinity changes in the cathode chamber

The initial pH was 4.5 in all the reactors and 2.5 mS cm⁻¹ ionic strength. The MFC with 200 mM buffer had an initial ionic strength of 5.32 mS cm⁻¹. From Figure 2 it can be observed that the nafion-MFC had a better catholyte pH control with pH changing from 4.5 to 5.5 as compared to CEM-MFC where the pH increased to 8.6. The increase in pH and the ionic strength in the cathode chamber of Nafion setup suggest the migration of cations to the cathode. Nafion is a sulfonated tetrafluorethylene copolymer that consists of a hydrophobic fluorocarbon backbone (–CF₂–CF₂–) to which hydrophilic sulfonate groups (SO₃⁻) are attached [25]. The negatively charged SO₃⁻ groups have high levels of proton conductivity that allows H⁺ ions to be transported to the cathode chamber of a MFC. Rozendal et al, 2006 suggested that although the diffusion coefficient of the metal ions are much smaller than the protons for Nafion membrane, the concentration of these ions is 10⁵ higher than the protons in a MFC [26]. Increase in both pH and ionic strength in CEM-MFC as compared to Nafion MFC may be explained in terms of higher cation transport as compared to proton transport. Nafion had a better control over the metal ion transport from anode to cathodic chamber as compared to the CEM. This is evident in this study where there is an increase in ionic strength from 2.5 mS to 6.25 mS cm⁻¹ in Nafion-MFC whereas CEM-MFC has an increase of 2.5 mS cm⁻¹ to 22.5 mS cm⁻¹. The difference between Nafion and CEM ionic strength was statistically significant (p <0.05) as observed by independent sample t-test. The increase in ionic strength in Nafion-MFC is due to cation transport from anode as Nafion possesses undesired affinity for cation species other than protons [26].

The increase in buffer strength from 100mM to 200mM maintained a similar pH range as the Nafion system suggesting that the increased salinity relative to the Nafion system may be the reason for its poorer dye decolourisation efficiency and lower maximum power density.

3.3 Current and Power generation

The maximum voltage recorded was 250 mV for the Nafion system. The other three systems recorded a much lower voltage with CEM showing the lowest voltage of 140 mV. Polarisation tests indicated a maximum power output of 16 mW m⁻² for Nafion, 13.3 mW m⁻² in pH controlled system, 11 mW m⁻² and 6.5 mW m⁻² for Buffer strength and CEM systems respectively (Figure 3). The internal resistances of the MFCs was calculated from the potential vs current density graph (Figure 4). Nafion produced the lowest internal resistance of 860 Ω followed by pH control 1001 Ω; buffer strength 1205 Ω and CEM 1302 Ω. The

power density and internal resistances followed the trend observed by Kim et al, 2007 with Nafion performing better than CEM [27]. The Coulombic efficiency for Nafion was 1.13%; for pH control 1.07%; for buffer strength 0.895% and for CEM 0.71%. The low coulombic efficiencies could be due to the azo dye out competing the anode for electrons generated via metabolism. Other anodic processes e.g. production of reduced end products such as acetate from pyruvate used as a cosubstrate could also account for the low CE's observed [28]. The improved power density in the Nafion system could be due to the reduction of metal ions migration and higher proton transport to the cathode minimising pH changes compared to other systems. This correlates with study by Scholz, 2008 which suggests that number of protons transported across Nafion is higher than CEM, thereby maintaining the pH equilibrium in Nafion system [17]. A similar system with 195 mg/L Acid Orange 7 (AO 7) as dye, Pt as catalyst and CMI 7000 as ion exchange membrane gave a Pmax value of 24 mW m⁻² [29].

Although the pH control MFC had better control over catholyte pH as compared to other systems, there was a significant increase in the ionic strength of the system. Based on salinity values the power density values for the pH control system would be expected to be intermediate between CEM and buffer strength systems. The fact that the power density of the pH control system was higher than that of the buffer strength CEM system suggests that maintaining optimal pH value in the cathode chamber to be more important to power generation than the differences in salinity in the systems tested.

3.4 Enzyme Activity

Figure 5 shows the trend for enzyme activity over time. Nafion 117 membrane was able to limit salinity and pH changes in the cathode chamber leading to prolonged enzyme activity in comparison to other systems ($p < 0.05$). Popat et al., 2012 argue that the ORR at pH 7 takes the form $O_2 + 2H_2O + 4e = 4OH^-$ and not $O_2 + 4H^+ + 4e = 4H_2O$ as is widely assumed. They suggest that the poor buffering of the catholyte and/or sluggish diffusion of hydroxide causes high cathodic overpotentials and is the main limiting factor of power production in MFCs [15]. The poor performance of the buffer and CEM systems with regard to enzyme activity is attributed to the production of OH^- ions which have been known to hamper electron transfer from T1 to T2/T3 Cu sites in laccase [30].

Increasing the buffer strength from 100mM to 200 mM improved enzyme activity (Fig. 5). This has also been observed in *Pleurotus ostreatus* laccase in which buffer strengths up to 100 mM increased the rate of enzyme activity [31]. In the case of CEM a large shift in pH as compared to the other systems, probably causes a big shift in the standard reduction potential of the ORR reaction which might lead to H₂O₂ formation (instead of water) causing negative feedback inhibition of laccase. The effect of H₂O₂ inhibition was observed by Milton et al, 2013 in a single chamber enzymatic fuel cell employing laccase for O₂ reduction.

The pH profile for *Trametes versicolor* laccase is a bell shaped curve with optimum activity at 4.5 and decreasing gradually as the pH increases [32]. This might explain the better performance of the pH control system over the buffer strength and CEM systems. pH affects the total net charge of enzymes and the distribution of charges on their exterior surfaces and these changes affect the activity and structural stability of the enzyme.

The laccase activity in this study in the nafion system remained stable for a period of 24 hours after that constant loss in activity each day was observed. Incubated laccase from *T. versicolor* in citrate buffer (pH 5) remained stable for 48 hours after which there was constant deactivation with a half-life of 7 days [33].

3.5 Detection of degradation products through HPLC

HPLC analysis revealed the presence of sulfanilic acid and 1- amino-2-naphthol, the aromatic products of AO7 degradation (Figure 6). The presence of these products from the analysis of the samples from day 3 to day 10 indicates their recalcitrant nature over time. The retention times were sulfanilic acid Rt= 1.7 mins and 1-amino-2-naphthol Rt= 2.7 mins respectively (Figure 6).

3.6 Toxicity Testing

The Microtox toxicity assay conducted at the end of the run using *Vibrio Fischeri* indicated that the degradation products were toxic. The half maximal inhibitory concentration (IC₅₀) for Nafion was at 200 mg COD L⁻¹ and around 150 mg COD L⁻¹ for all other samples (p < 0.05, Anova) (Figure 7).

The toxicity of the degradation products sulfanilic acid and 1- amino-2-naphthol is widely reported in various literature but an independent study done in our lab suggests that this can be reduced by using a second aerobic treatment step [23].

4. Conclusion

This study investigated various ways of mitigating pH changes in the cathode chambers of MFCs and their effect on laccase activity and decolourisation of a model azo dye Acid orange 7 in the anode chamber. The methods included using Nafion 117 and CMI7000 as membranes, automatic control of pH in the cathode chamber and using a high strength buffer.

Nafion 117 membrane was able to limit salinity and pH changes in the cathode chamber leading to prolonged enzyme activity and improved performance of the system in comparison to other MFCs. The pH optima for laccase is 4.5 and there is a gradual decrease in enzyme activity as the pH shifts from the optima. This is evident in MFC with CMI7000 membrane had the highest change in pH and ionic strength which contributed to low performance and decreased longevity of enzyme activity. The MFCs with pH control and increased buffer strength (200 mM) had higher power output due to better retention of enzyme activity than one with CMI7000 membrane. Although Nafion performed better than other MFCs, its cost may hinder its usage in wastewater treatments and scaled up reactors.

In the present study it is observed that pH control is essential for preserving laccase activity and increasing the performance of a MFC, but it does not guarantee sustained laccase activity that salinity increases also affects the activity and could be mitigated using a proton selective membrane. Therefore it is essential to decouple pH and salinity to develop efficient biocathodes for MFCs. Moreover, laccase has the versatility of being engineered to improve its efficiency. With the advent of protein engineering laccase holds potential to be an efficient catalyst for oxygen reduction reaction.

5. References

- [1] A. Rozendal, H.V.M. Hamelers, K. Rabaey, J. Keller, C.J.N. Buisman, Towards practical implementation of bioelectrochemical wastewater treatment, *Trends Biotechnol.* 26 (2008) 450–459. doi:10.1016/j.tibtech.2008.04.008.
- [2] X. Li, B. Hu, S. Suib, Y. Lei, B. Li, Manganese dioxide as a new cathode catalyst in microbial fuel cells, *J. Power Sources.* 195 (2010) 2586–2591. doi:10.1016/j.jpowsour.2009.10.084.
- [3] X.-B. Gong, S.-J. You, X.-H. Wang, J.-N. Zhang, Y. Gan, N.-Q. Ren, A novel stainless steel mesh/cobalt oxide hybrid electrode for efficient catalysis of oxygen reduction in a microbial fuel cell, 2014. doi:10.1016/j.bios.2013.12.015.
- [4] M.T. Noori, M.M. Ghangrekar, C.K. Mukherjee, V2O5 microflower decorated cathode for enhancing power generation in air-cathode microbial fuel cell treating fish market wastewater, *Int. J. Hydrogen Energy.* 41 (2016) 3638–3645. doi:10.1016/j.ijhydene.2015.12.163.
- [5] M. Horizons, Oxygen reduction reaction catalysts in microbial fuel cells for energy-efficient wastewater treatment : A review *Materials Horizons, Mater. Horizons.* 3 (2016) 382–401. doi:10.1039/C6MH00093B.
- [6] C. Santoro, S. Babanova, P. Atanassov, B. Li, High Power Generation by a Membraneless Single Chamber Microbial Fuel Cell (SCMFC) Using Enzymatic Bilirubin Oxidase (BOx) Air-Breathing Cathode, *J. Electrochem. Soc.* 160 (2013) 720–726. doi:10.1149/2.058310jes.
- [7] H. Luo, S. Jin, P.H. Fallgren, H.J. Park, P.A. Johnson, A novel laccase-catalyzed cathode for microbial fuel cells, *Chem. Eng. J.* 165 (2010) 524–528. doi:10.1016/j.cej.2010.09.061.
- [8] S. Bakhshian, H.-R. Kariminia, R. Roshandel, Bioelectricity generation enhancement in a dual chamber microbial fuel cell under cathodic enzyme catalyzed dye decolorization, *Bioresour. Technol.* 102 (2011) 6761–6765. doi:http://dx.doi.org/10.1016/j.biortech.2011.03.060.
- [9] C. Galhaup, D. Haltrich, Enhanced formation of laccase activity by the white-rot

- fungus *Trametes pubescens* in the presence of copper, *Appl. Microbiol. Biotechnol.* 56 (2001) 225–232. doi:10.1007/s002530100636.
- [10] O. Schaetzle, F. Barrière, U. Schröder, An improved microbial fuel cell with laccase as the oxygen reduction catalyst, *Energy Environ. Sci.* 2 (2009) 96–99. doi:10.1039/B815331K.
- [11] M.J. Cooney, V. Svoboda, C. Lau, G. Martin, S.D. Minteer, Enzyme catalysed biofuel cells, *Energy Environ. Sci.* 1 (2008) 320. doi:10.1039/b809009b.
- [12] S.R. Higgins, C. Lau, P. Atanassov, S.D. Minteer, M.J. Cooney, Hybrid biofuel cell: Microbial fuel cell with an enzymatic air-breathing cathode, *ACS Catal.* 1 (2011) 994–997. doi:10.1021/cs2003142.
- [13] I.S.P. Savizi, H.R. Kariminia, S. Bakhshian, Simultaneous decolorization and bioelectricity generation in a dual chamber microbial fuel cell using electropolymerized-enzymatic cathode, *Environ. Sci. Technol.* 46 (2012) 6584–6593. doi:10.1021/es300367h.
- [14] V. Madhavi, S.S. Lele, Laccase: Properties and applications, *BioResources.* 4 (2009) 1694–1717. doi:10.15376/biores.4.4.1694-1717.
- [15] S.C. Popat, D. Ki, B.E. Rittmann, C.I. Torres, Importance of OH⁻ transport from cathodes in microbial fuel cells, *ChemSusChem.* 5 (2012) 1071–1079. doi:10.1002/cssc.201100777.
- [16] O. Fokina, J. Eipper, L. Winandy, S. Kerzenmacher, R. Fischer, Improving the performance of a biofuel cell cathode with laccase-containing culture supernatant from *Pycnoporus sanguineus*, *Bioresour. Technol.* 175 (2015) 445–453. doi:10.1016/j.biortech.2014.10.127.
- [17] F. Scholz, The Suitability of Monopolar and Bipolar Ion Exchange Membranes as Separators for Biological Fuel Cells, *Energy Environ. Sci.* 42 (2008) 1740–1746.
- [18] E. Marsili, D.B. Baron, I.D. Shikhare, D. Coursolle, J.A. Gralnick, D.R. Bond, *Shewanella* secretes flavins that mediate extracellular electron transfer, *PNAS.* 105 (2008) 6–11.
- [19] E.A. Wolin, M.J. Wolin, R.S. Wolfe, Formation of Methane by Bacterial Extracts, *J.*

- Biol. Chem. 238 (1963) 2882–6. <http://www.ncbi.nlm.nih.gov/pubmed/14063318> (accessed July 5, 2016).
- [20] B.E. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: Methodology and technology, *Environ. Sci. Technol.* 40 (2006) 5181–5192. doi:10.1021/es0605016.
- [21] C. Eggert, U. Temp, K.L. Eriksson, A.P.P.L.E.N.M. Icrobiol, The Ligninolytic System of the White Rot Fungus *Pycnoporus cinnabarinus* : Purification and Characterization of the Laccase, *Appl. Environ. Microbiol.* 62 (1996) 1151–1158.
- [22] C. Hao, O.J., Chien-Jen, S., Chen-Fang, L., Fu-Tien, J., Zen-Chyuan, Use of microtox tests for screening industrial wastewater toxicity, *Water Sci. Technol.* 34 (1996) 43–50.
- [23] E. Fernando, Treatment of Azo Dyes in, (2014). <http://www.westminster.ac.uk/research/westminsterresearch>.
- [24] R.G. Saratale, G.D. Saratale, J.S. Chang, S.P. Govindwar, Bacterial decolorization and degradation of azo dyes: A review, *J. Taiwan Inst. Chem. Eng.* 42 (2011) 138–157. doi:10.1016/j.jtice.2010.06.006.
- [25] K.A. Mauritz, R.B. Moore, State of understanding of Nafion, *Chem. Rev.* 104 (2004) 4535–4585. doi:10.1021/cr0207123.
- [26] R. a. Rozendal, H.V.M. Hamelers, C.J.N. Buisman, Effects of Membrane Cation Transport on pH and Microbial Fuel, *Environ. Sci. Technol.* 40 (2006) 5206–5211. doi:10.1021/es060387r.
- [27] J.R. Kim, S. Cheng, S.-E. Oh, B.E. Logan, Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells., *Environ. Sci. Technol.* 41 (2007) 1004–9. doi:10.1021/es062202m.
- [28] G.J. Newton, S. Mori, R. Nakamura, K. Hashimoto, K. Watanabe, Analyses of current-generating mechanisms of *Shewanella loihica* PV-4 and *Shewanella oneidensis* MR-1 in microbial fuel cells, *Appl. Environ. Microbiol.* 75 (2009) 7674–7681. doi:10.1128/AEM.01142-09.
- [29] E. Fernando, T. Keshavarz, G. Kyazze, Enhanced bio-decolourisation of acid orange 7

- by *Shewanella oneidensis* through co-metabolism in a microbial fuel cell, *Int. Biodeterior. Biodegradation*. 72 (2012) 1–9. doi:10.1016/j.ibiod.2012.04.010.
- [30] F. Xu, Oxidation of phenols, anilines, and benzenethiols by fungal laccases: Correlation between activity and redox potentials as well as halide inhibition, *Biochemistry*. 35 (1996) 7608–7614. doi:10.1021/bi952971a.
- [31] M.M. Hassan, A.M. Elshafei, B.M. Haroun, M.A. Elsayed, A.M. Othman, Biochemical Characterization of an Extracellular Laccase From *Pleurotus ostreatus* ARC280, *J. Appl. Sci. Res.* 8 (2012).
- [32] I. Stoilova, A. Krastanov, V. Stanchev, Properties of crude laccase from *Trametes versicolor* produced by solid-substrate fermentation, *Adv. Biosci. Biotechnol.* 1 (2010) 208–215. doi:10.4236/abb.2010.13029.
- [33] S. Rubenwolf, S. Sané, L. Hussein, J. Kestel, Prolongation of electrode lifetime in biofuel cells by periodic enzyme renewal Theoretical background, *Bioenergy and Biofuels*. 96 (2012) 841–849. doi:10.1007/s00253-012-4374-8.

6. Appendix

Figure 1: Comparison of decolourisation rates of AO7 in the various MFC systems.

Figure 2: Initial (time 0) and final (10 days) pH and ionic strength in each setup.

Figure 3: Power density curve with Pmax for each system.

Figure 4: Current density vs Voltage plot (Slope= Internal Resistance).

Figure 5: Laccase Activity: Change in absorbance (Δ ABS)/Change in time (Δ Time) Vs time

Figure 6: HPLC analysis of degradation products.

Figure 7: Relative luminescence inhibition in relationship to COD. There was no statistically significant difference in the levels of toxicity in the systems tested after a run time of 10 days.

Figure 1

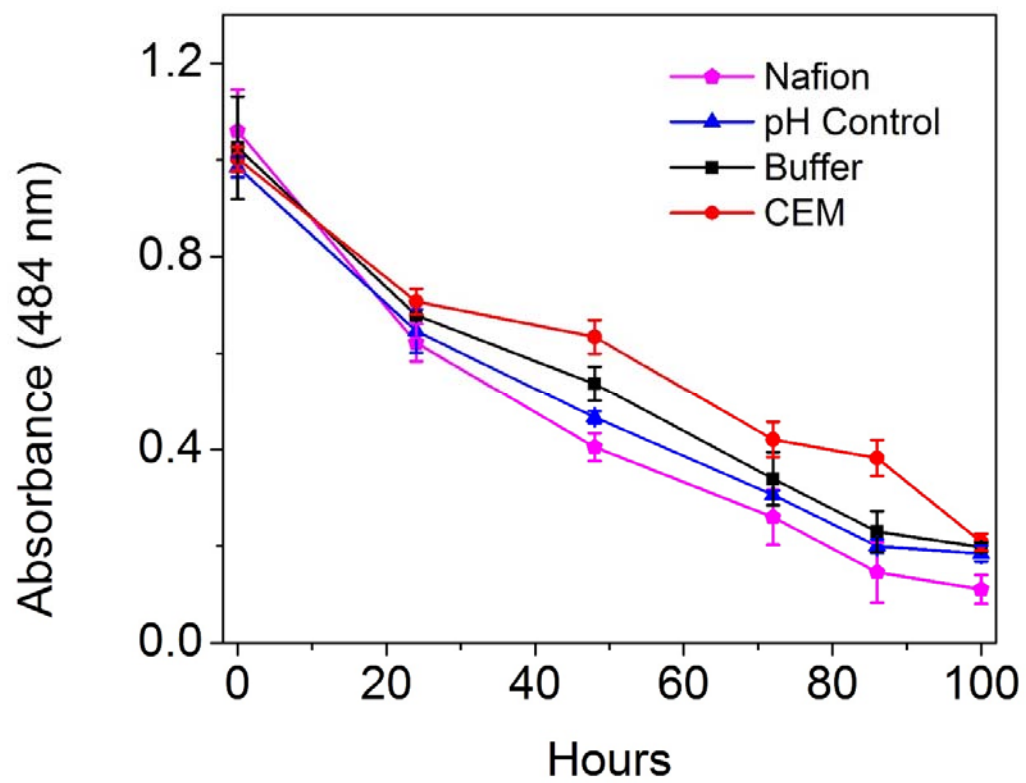


Figure 2

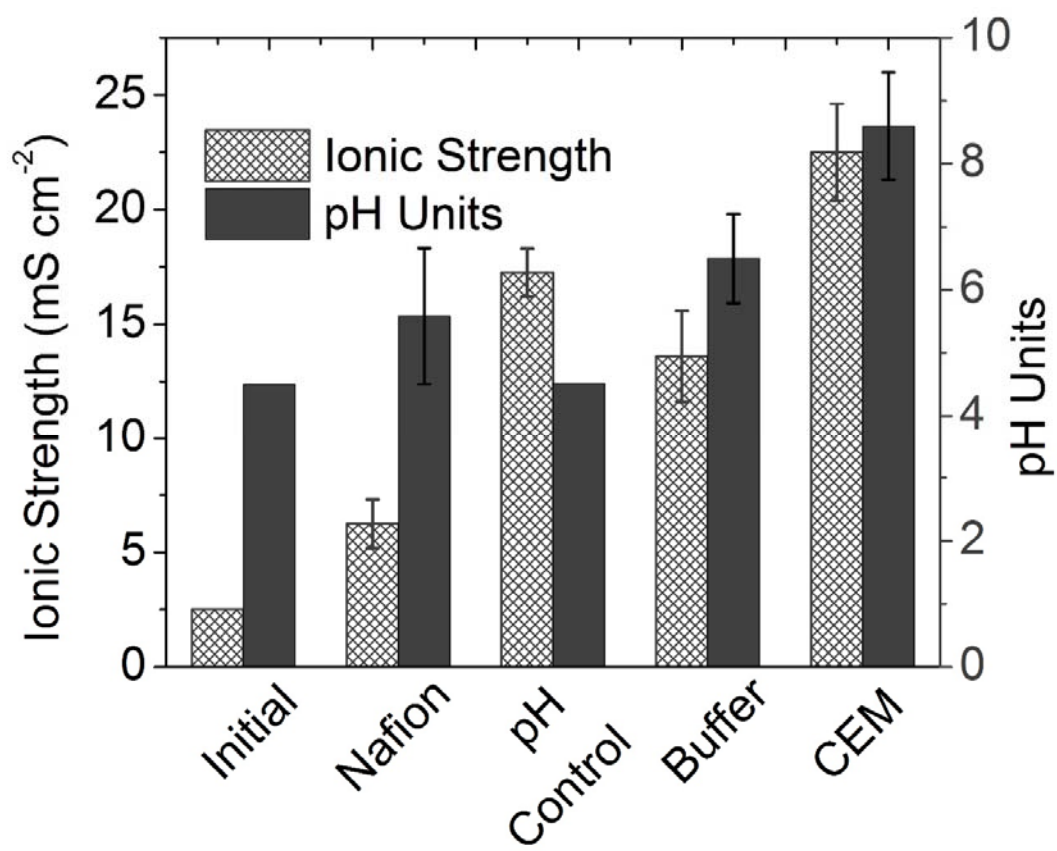


Figure 3

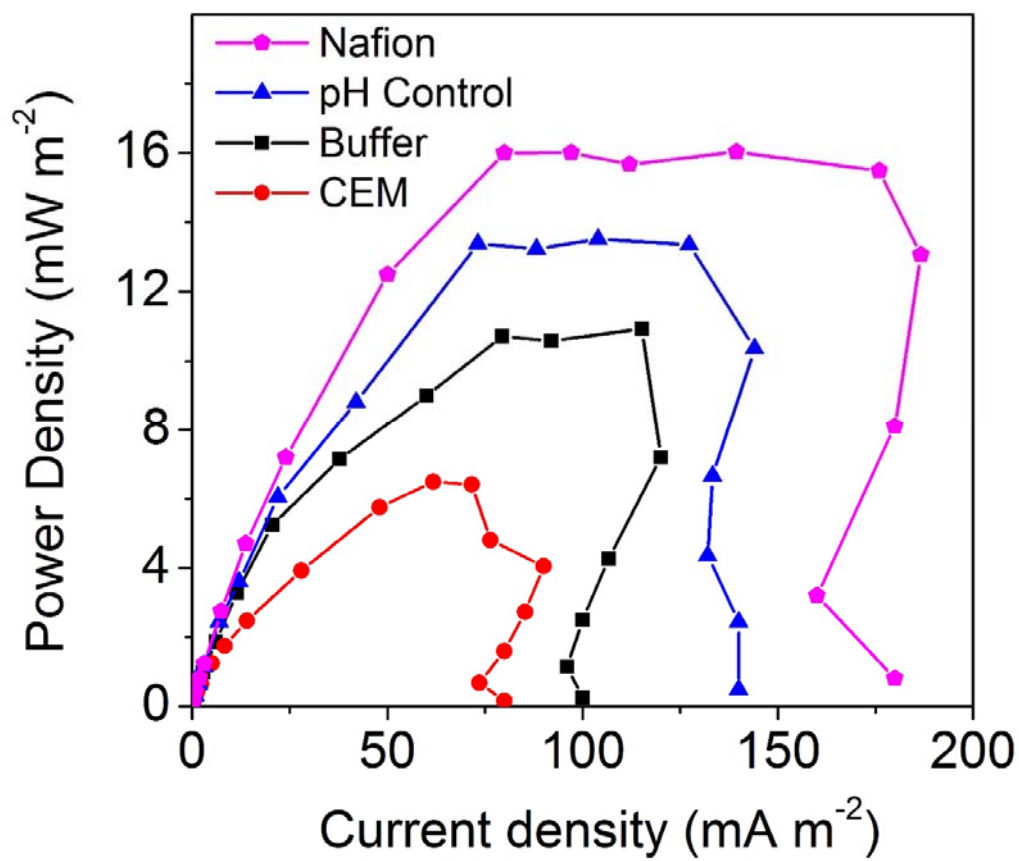


Figure 4

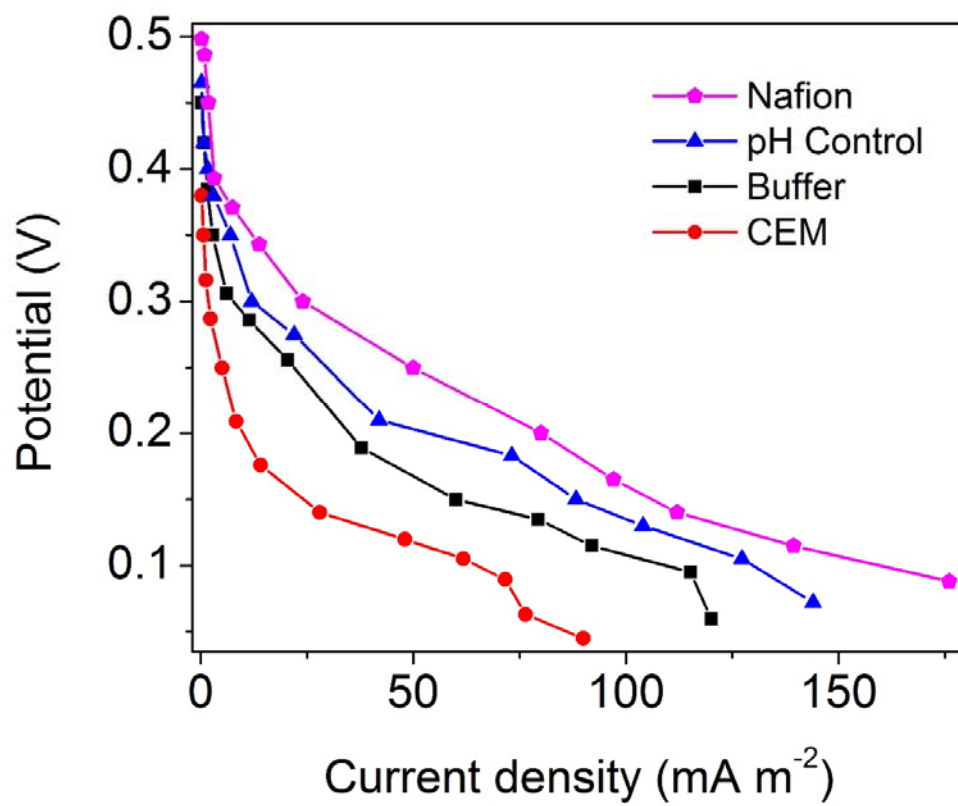


Figure 5

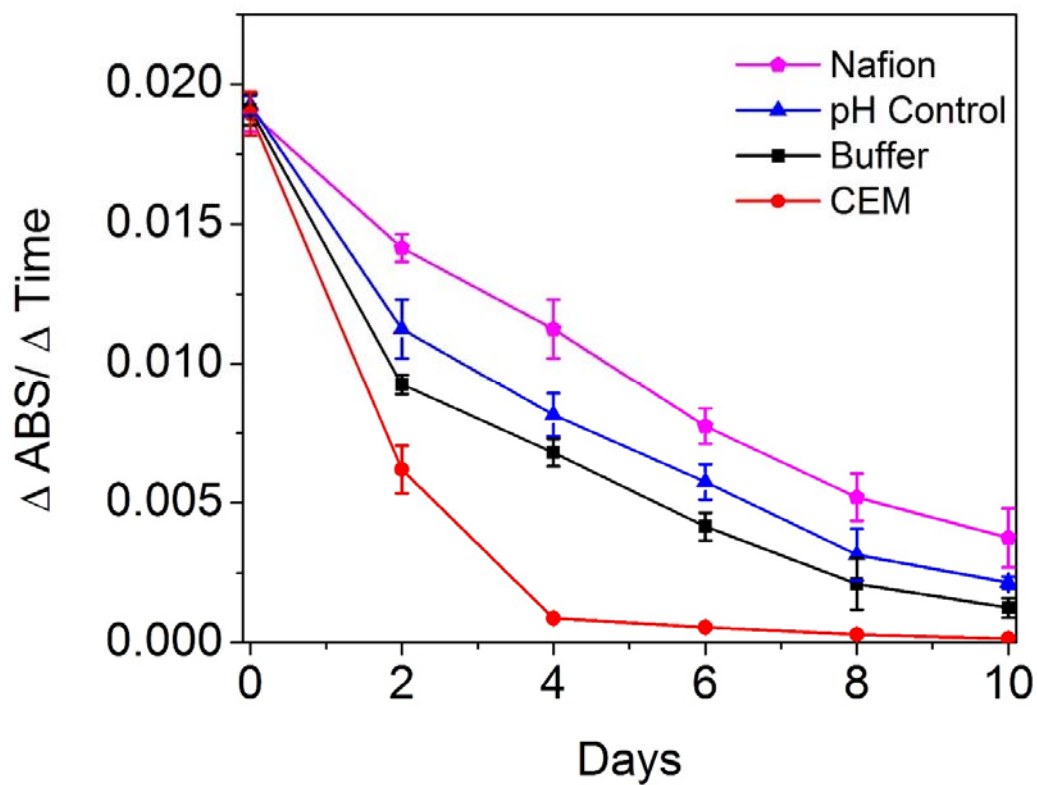


Figure 6

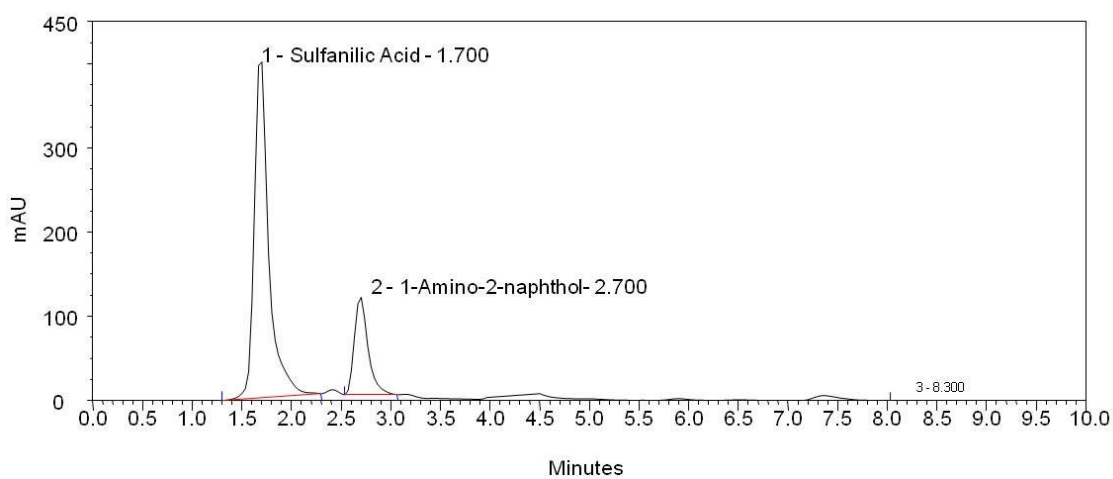


Figure 7

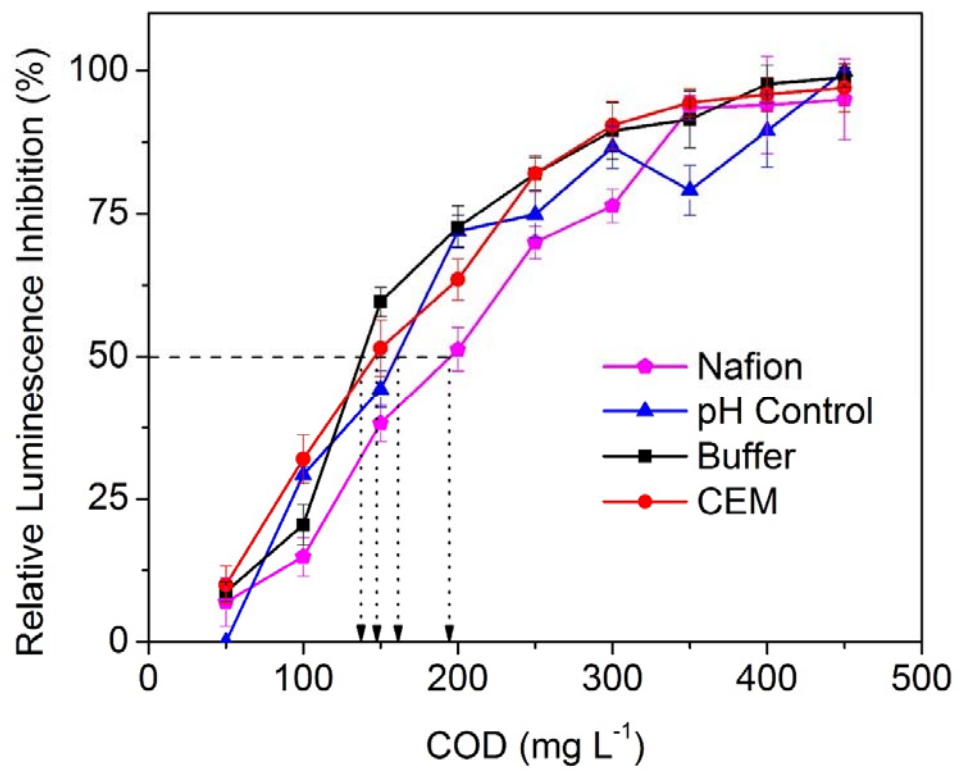


Table 1. Summary of variables employed in this study

| MFC System | pH mitigation measure | Other conditions | Rationale |
|------------------------|--|---|---|
| [1] Nafion | Nafion 117 membrane | Buffer in cathode chamber 100 mM acetate pH 4.5 | High proton affinity and transport [17]. |
| [2] pH control | Automatic pH control by acid (HCl) or base (NaOH) addition | Buffer in cathode chamber 100 mM acetate pH 4.5 to begin with; CMI7000 membrane | Tight control of pH. |
| [3] Buffer strength | Buffer strength increase | Buffer in cathode chamber 200 mM acetate pH 4.5; CMI7000 membrane. | Decouples effects due to pH changes from those due to salinity changes. |
| [4] CEM | CMI7000 membrane | Buffer in cathode chamber 100 mM acetate pH 4.5 | Cheap and commonly used membrane. |