Bioresource Technology 238 (2017) 313–324

Contents lists available at ScienceDirect

Bioresource Technology

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

Bioanode as a limiting factor to biocathode performance in microbial electrolysis cells

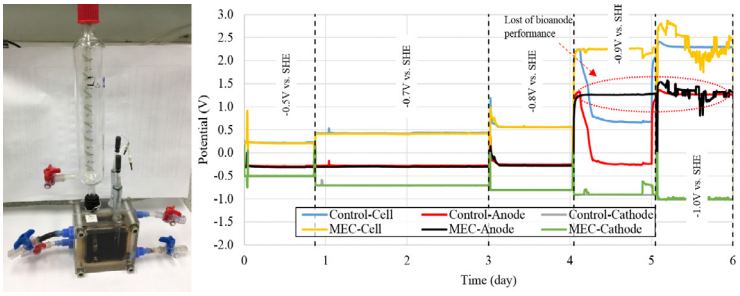
Swee Su Lim [a,b], Eileen Hao Yu [a,*], Wan Ramli Wan Daud [b], Byung Hong Kim [b,c], Keith Scott [a]

[a] School of Chemical Engineering and Advanced Materials, Newcastle University, Newcastle upon Tyne NE1 7RU, United Kingdom
[b] Fuel Cell Institute, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Malaysia
[c] Bioelectrochemistry Laboratory, Water Environment and Remediation Research Centre, Korea Institute of Science and Technology, Republic of Korea

HIGHLIGHTS

- In MECs hydrogen production from biocathode may be limited by bioanode.
- Electrogens enriched bioanode can maintain active at high applied potential up to 1.0 V.
- High demands of electrons on hydrogen production at cathode could exhaust bioanode.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 December 2016
Received in revised form 18 March 2017
Accepted 22 March 2017
Available online 24 March 2017

Keywords:
Bioanode
Biocathode
Biological microbial electrolysis cell
Limiting factor
Applied potential

ABSTRACT

The bioanode is important for a microbial electrolysis cell (MEC) and its robustness to maintain its catalytic activity affects the performance of the whole system. Bioanodes enriched at a potential of +0.2 V (vs. standard hydrogen electrode) were able to sustain their oxidation activity when the anode potential was varied from −0.3 up to +1.0 V. Chronoamperometric test revealed that the bioanode produced peak current density of 0.36 A/m^2 and 0.37 A/m^2 at applied potential 0 and +0.6 V, respectively. Meanwhile hydrogen production at the biocathode was proportional to the applied potential, in the range from −0.5 to −1.0 V. The highest production rate was 7.4 L H_2/(m^2 cathode area)/day at −1.0 V cathode potential. A limited current output at the bioanode could halt the biocathode capability to generate hydrogen. Therefore maximum applied potential that can be applied to the biocathode was calculated as −0.84 V without overloading the bioanode.

1. Introduction

Bioelectrochemical systems (BESs) appear to be an interesting research focused on the study of converting waste to energy or value added chemical compounds (Liu et al., 2015; Luo et al., 2014). Intensive contribution to the knowledge has increased by folds since the last decade (Escapa et al., 2016; Kumar et al., 2017). BECs are devices that can perform oxidation and reduction by either producing or consuming current (Ketep et al., 2013; Rivera et al., 2017). The devices manipulate the uses of biocatalysts such as living microorganism as whole cell catalysts and specific enzymes as non-viral organic catalysts in their system. The systems are typically named according to their purpose and the use of these biocatalysts, for examples, microbial fuel cell (MFC) and microbial electrolysis cell (MEC) both based on their use of microorganisms as catalysts and its production of electrical current and biohydrogen, respectively (Kadier et al., 2016).

The ability of MEC to produce hydrogen and treat wastewaters simultaneously is potentially very useful. Earlier laboratory exper-

* Corresponding author.
E-mail address: eileen.yu@newcastle.ac.uk (E.H. Yu).

http://dx.doi.org/10.1016/j.biortech.2017.03.127
0960-8524/

iments on hydrogen-producing MECs were conducted by placing cation exchange membrane (CEM) or anion exchange membrane (AEM) to isolate both anode and cathode into two separated reaction chambers (Liu et al., 2005; Rozendal et al., 2006). As early cathode mainly containing metal-based catalysts for hydrogen evolution, the purpose was to optimise the condition without affecting the microbial community in the anode while clean hydrogen can be obtained in cathode. Even though the advantage of getting highly pure hydrogen was attractive, membrane separators did caused serious drawback during the operation. As membrane separating both anolyte and catholyte but allowing selective ions to pass through, it could increase the accumulation of specific ions and cause imbalance to electrical charges in both chambers (Kumar et al., 2017; Rozendal et al., 2007). Then after, single-chamber membraneless MECs were introduced to eliminate the impact of electrical charges barrier and internal resistance caused by membrane separators (Call and Logan, 2008). Despite of better performance in energy usage and higher hydrogen production rate during the initial working stage, single-chamber membrane-less MECs were suffer from performance dropped after long time operation. This is because hydrogen produced from cathode may undergo diverse pathways and converted into low value products which is detrimental to the overall MEC performance. The ability of the anode to re-oxidise hydrogen in the same electrolyte directly increases the electrical current and reduces efficiency caused by reluctant hydrogen cycling phenomena (Lee and Rittmann, 2010). In additional to the artificial phenomena, proliferation of homoacetogenic or/and methanogenic microorganisms could have reduced hydrogen production and accumulation in the system (Ruiz et al., 2013). It is either been converted into acetate and utilised by the biofilm on the anode or transformed to methane and reducing the purity of the offgas product. Despite the fact that extensive studies have been carried out to solve the mass transport limitations on MECs from double-chamber using separators to membrane-less MECs, none of these studies were focused on the usage of biocatalysts in both anode and cathode.

Rozendal et al. (2008) began a comprehensive biocatalysts study of a MEC by deploiting three step start-up procedure and polarity reversal method in accordance to turn the electrochemically activated-bioanode into biocathode for hydrogen production. Years after, with the same setup, Jeremiasse et al. (2010) studied the first full biological MECs by combining both bioanode and biocathode in which both oxidation and reduction processes was performed by electrochemically active microorgansms. The same study was also performed by Liang et al. (2014) to test the effect of bicarbonate and cathode potential on the three step start-up biocathode. In their results, the study was focused on the hydrogen-producing biocathode and its performance based on a range of applied potentials providing little information on the bioanodes. It was assumed that the bioanode could supply sufficient current required for biocathodes to generate hydrogen. Lately, simpler start-up procedure was adapted for enriching autotrophic hydrogen-producing biofilm which making the utilisation of both bioanode and biocathode in a same system more reliable and easier (Batlle-Vilanova et al., 2014; Jourdin et al., 2015; Zaybak et al., 2013). But once again, the studies were half-cell experiments only focused on biocathode and not information was reported on the anode. Other advantages of using biocathode MECs were also demonstrated in wastewater treatment to remove inorganic substance such as sulphate, nitrate and heavy metals by supplying electrons from an external power supply. However, those studies only involved inorganic reduction reactions without generating any hydrogen (Cheng et al., 2012; Coma et al., 2013; Luo et al., 2014). Although information was included on how bioanode react during the polarisation test on one of the studies, the biocathode was meant for sulphate reduction instead of hydrogen production (Coma et al., 2013).

It is believed that in hydrogen-producing biocathode, microbial community was dominated by sulphate-reducing bacteria called *Desulfovibrio* sp. (Croese et al., 2014). The species possesses specific outer membrane enzymes called hydrogenases and c-type cytochromes facilitated hydrogen evolution and electron transport from cathode as electron donor (Aulenta et al., 2012). These electrochemically active proteins is postulated responsible for hydrogen evolution in the biocathode as almost similar to the hydrogen cycling mechanisms but with slightly diverge pathway (Kim et al., 2015; Rosenbaum et al., 2011). Typically, biocathode worked perfectly under moderate conditions eg. neutral pH and ambient temperature, low ionic concentration with the presence of certain organic and inorganic matters (Jeremiasse et al., 2010; Jourdin et al., 2015; Rozendal et al., 2008). In contrary, the operational condition for abiotic catalysts required much nerd conditions for hydrogen evolution and this seems turn the disadvantage of biotic cathode into opportunity to replace high-cost alternative abiotic cathode (Escapa et al., 2016). Some studies also reported that biocathode could outcompete abiotic cathode in milder operational conditions in term of hydrogen production, energy usage, self-regenerate and stability where making the scale-up application possible (Batlle-Vilanova et al., 2014; Jourdin et al., 2015; Liang et al., 2014). Yet, the controversial of biocathode outperform abiotic cathode still subject to debate and apparently further studies should be carried on to draw concrete evidences whether biocathode is suitable for MEC application (Jafary et al., 2015; Jeremiasse et al., 2010). In order to use biocatalysts in both anode and cathode of MEC, one has to consider the limitation of both biocatalyst in the MEC system in term of standard reduction potential and current supply. Firstly, standard reduction potential is important for prediction of minimum potential in order to initial redox reactions between the electrodes in MEC (Kumar et al., 2017; Rosenbaum et al., 2011). Theoretically, a bioanode which uses acetate as its main carbon source could oxidise electron donors to form proton and electron as described in (Eq. (1)). The electrons contribute energy to power the system or to lower the total energy need into the MEC system. At the cathode, protons react with the electrons to form hydrogen (Eq. (2)).

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3 + 9H^+ + 8e^- \quad E'_o = -0.28\ \text{V (vs. SHE)} \tag{1}$$

$$2H^+ + 2e^- \rightarrow H_2 \quad E'_o = -0.41\ \text{V (vs. SHE)} \tag{2}$$

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3 + H^+ + 4H_2 \quad E'_o = +0.13\ \text{V (vs. SHE)} \tag{3}$$

The minimal electrical potential that is required to drive the reaction is 0.13 V. However, more energy is required (>0.13 V) due to overpotentials to overcome energy barriers in the system (Rozendal et al., 2006). Thermodynamically, this voltage is relatively smaller required to derive hydrogen from water electrolysis compared to 1.21 V at neutral pH. Meanwhile, it could go up to 1.8–2.0 V for water electrolysis under alkaline condition due to overpotential at the electrodes (Liu et al., 2005). Secondly, the robustness of anode should be considered for better MEC performance as it could limit the current supply to cathode (Kumar et al., 2017; Rago et al., 2016; Wang et al., 2010). Weak anode with more positive open-circuit potential tends to perform poorly in supporting cathode reduction reaction when a fixed voltage was applied between the electrodes (Wang et al., 2010). As a result of weak anode, more current was required from external power to drive the reduction reaction in cathode resulting higher energy

consumption. However, this phenomena was mainly found in conventional MECs with abiotic cathode and the question whether the bioanode coupled with biocathode would react the same way still remains concealed.

To make the MEC feasible, at least same amount of energy needs to be supplied by the anode to margin the energy invested in the cathode. The first working MEC was published under (Liu et al., 2005) showing that the principle of hydrogen production from biocatalyst electrodes was possible. However, the system was not optimised and the hydrogen production rate was low whilst higher potentials were applied due to high overpotentials in the system. Jeremiasse et al. (2010) reported an MEC system that can reach a maximum current density of 1.4 A/m^2 at an applied voltage of 0.5 V or 3.3 A/m^2 at an optimum cathode potential of −0.7 V with a biocathode. Their work mostly focused on the MEC system and how the biocathode performed with different applied potentials from a power supply. Most studies only focused on the biocathode itself in a half-cell experiments without much information about bioanode (Aulenta et al., 2012; Batlle-Vilanova et al., 2014; Jeremiasse et al., 2010; Jeremiasse et al., 2012; Jourdin et al., 2015; Rozendal et al., 2008).

There is limited information on the function of bioanode as the supporting electrode to a biocathode in MEC systems. Some questions are still unanswered such as how the bioanode responds when the applied potential on the biocathode is changed, what is the limiting potential a bioanode can handle before it loses its ability to produce electrons and will it have the same performance when the set potential on the anode is high? In this study, the main objective was to enrich the bioanode, test it at higher applied potential −1.0 V and in MEC to assess its robustness. The anode should be able to supply the electrons to the cathode of MEC, therefore reduces the total electric energy required from hydrogen production. We believe that sufficient electron supply from substrate oxidation by bioanode activity is vital to support the hydrogen evolution in a biocathode and therefore maintaining the energy demand from external power supply as low as possible. In order to have an optimum hydrogen production rate from the biocathode, the anode plays an important role as a support to the biological MEC system. It may lower external energy supply to the system and increase energy recovery in term of hydrogen evolution on the one hand and it could be a limitation factor to the whole system together with other problems like substrate crossover and precipitation of mineral on the electrodes on the other (Jeremiasse et al., 2010). Due to the fact that bio-catalysts will be used in both anode and cathode, double-chamber membrane-based MEC will be used for better environmental control in both chambers. Moreover, special designed electrolytes to accommodate different reactions and end products are vital for the grown and re-generation of independent microbial dominated species in both separated chambers (Escapa et al., 2016; Jafary et al., 2015; Kadier et al., 2016). The information is useful to provide parameters for actual operating condition and to assess the effectiveness and feasible of the system in practical applications.

2. Materials and methods

2.1. Electrochemical cells and experimental setup

Double-chamber electrochemical cells of 25 mL volume were used. Each chamber was constructed from polyacrylate, with external dimensions of 7 × 7 × 2 cm and with internal dimensions of 5 × 5 cm cross section and 1 cm thickness in the direction of current flow for the fluid space. Two identical chambers were assembled together as described as Fig. S1. A cation exchange membrane (CMI-7000, Membrane International Inc., USA) was place between the two chambers. Graphite felt (RVG-2000, Mersen, USA) was used as electrodes with geometric size of 5 × 5 (cross-section) × 0.5 cm thickness.

For bioanode enrichment, platinum coated graphite felt with a platinum loading 0.5 mg/cm^2 was used as the cathode. A silver/silver chloride reference electrode (RE-5B, BASi, USA) was inserted into the anode chamber for monitoring potentials. Anolyte flow through the cell was via two pipe connections at opposite side of the chamber. The cathodic chamber, incorporated a hole for collecting gas products. A 80 mL glass tube, with a septa on the top was fixed into the holes and filled with cathodic medium. The produced gas was collected and measured by the means of water displacement method. Prior to start, both anode and cathode chambers were filled with deionised water and the electrodes were soaked overnight prior to use.

2.2. Enrichment of bioanodes and biocathodes

Bioanode was first enriched by coupled with Pt-coated cathode. Once the reactor produced a stable current, the Pt-coated cathode was replaced with a new plain graphite felt to start the enrichment of biocathode. The strategy was performed for obtaining bioanode first and then biocathode in order to obtain both bioelectrochemically active electrodes in microbial electrolysis cells (MECs). Inoculums were obtained from an anode in a microbial fuel cell and a anode control (cultivated without connecting an external circuit to cathode) which has been operated over a year (Spurr, 2016). Those electrodes had been identified as being colonised by dominating microorganism *Geobacter* sp. and *Desulfovibrio* sp., respectively. A four-channel potentiostat (Quad Potentiostat, Whistonbrook Technologies, UK) was used in both enrichment processes. A fixed potential of +0.2 V vs. standard hydrogen electrode (SHE) was first applied on anode during bioanode enrichment before changing the fixed potential to −0.7 V vs. SHE on biocathode while biocathode enrichment took place. At the initial stage of biocathode enrichment, the applied potential +0.2 V vs. SHE was still fixed on the bioanode in order to protect the bioanode from losing its ability to produce a stable current. Once the Pt-coated cathodes were changed with the plain graphite felts, the cathodic chambers were injected with 25 mL inocula 1:1 in ratio as mentioned above. Hydrogen grade 99.99% was fed into cathode chamber once a day and recycled via a glass tube's headspace to encourage the growth of hydrogen-oxidising microorganisms for at least a week before switching the fixed potential from anode operation to cathodic operation (Rozendal et al., 2008). A 40-channel data logger (NI USB-6225, National Instruments, UK) was also used in the experiments to record electrodes and cell potentials. Both anode and cathode media were fed continuously through their respective chambers at flow rates of 10 mL/h using peristaltic pumps (120S, Watson-Marlow, UK). The anode medium was as follows: (g/L): $NaCH_3COO$ 0.41, NH_4Cl 0.27, KCl 0.11, $NaH_2PO_4{\cdot}2H_2O$ 0.66, $Na_2HPO_4{\cdot}2H_2O$ 1.03, Wolfe's vitamin solution 10 mL/L and modified Wolfe's mineral solution 10 mL/L (Lim et al., 2012). The carbon source ($NaCH_3COO$) in the medium was 10 mM unless stated otherwise. The cathodic solution contained (g/L): $NaH_2PO_4{\cdot}2H_2O$ 0.66 and $Na_2HPO_4{\cdot}2H_2O$ 1.03 during the bioanode enrichment process while the biocathode medium was prepared as previous study for biocathode enrichment (Rozendal et al., 2008). Control MFCs and MECs were setup in conjunction with the enrichment process of bioanode and biocathode. The same condition and media were used without added any inocula into the reactors.

2.3. Polarisation test and cyclic voltammetry of MEC

After stable currents were obtained with applied potentials of +0.2 V, the bioanodes were subjected to a range of chronoampero-

metric test at −0.3, −0.2, 0, +0.2, +0.4, +0.6, +0.8 and 1.0 V. However, the range of the analysis on biocathode was −0.5, −0.7, −0.8, −0.9 and −1.0 V. The biocathodes were analysed under polarisation test after a stable current was observed under applied potential −0.9 V. Cyclic voltammetry were performed either with (PGSTAT128N, Metrohm, Netherland) equipped with FRA32M module or Quad potentiostat (with available CV function). All potentials are reported with reference to the standard hydrogen electrode (SHE).

2.4. Analytical methods

The pH and conductivity were measured before the liquids were filtered through 0.2 μm syringe filters. The samples were kept in refrigerator under 4 °C prior analysis. Gas volume produced at the biocathode was captured through a glass tube using water replacement method and the actual gas volume was recorded every 24 h. Then the samples were collected through a septa on the top of the glass tube by using a syringe and analysed using a gas chromatography (GC-8A, Shimadzu, UK). Two columns molecular sieve 5A (mesh range 40–60) and Chromosorb 101 (mesh range 80–100) were used and operated at 40 °C. The carrier gas was research grade 99.99% N_2 at a pressure of 100 kPa. A thermal conductivity detector was used to detect the gas based on their retention times.

2.5. Kinetic analysis and calculations

Energy consumed and recovered from both bioanode and biocathode were calculated to summarise the overall efficiency of the system used in this study. Firstly, in the cathode, actual hydrogen volume was calculated as

$$V_{H2} = V_h \cdot X_{H2} \quad (4)$$

where V_{H2} (L) is pure hydrogen volume, V_h (L) is the headspace volume of the gas captured in the glass tube, X_{H2} is fraction of hydrogen in the gas samples. The pure hydrogen volume was then used to compute hydrogen production rate as

$$Q_{H2} = V_{H2}/(A_{cat} \cdot t) \quad (5)$$

where Q_{H2} (L H_2/m^2 cathode/day) is hydrogen production rate, A_{cat} (m^2) is cathode surface area and t (day) is production time.

The efficiency of the hydrogen recovery from cathode was determined based on Faraday's law of electrolysis process as

$$r_{cat}\ (\%) = Q_{recovery}/Q_{supply} \quad (6)$$

where $Q_{recovery}$ (C) = η·F·z is charge use to reduce proton to hydroge η is hydrogen recovery in mole, F is faraday constant (96,485 C/mol), z is the valency number of proton which is 1. Meanwhile, Q_{supply} (C) = ∫I(t)dt is total charge supplied from the power supply within the specific time of recovery.

Secondly, the anodic columbic efficiency was obtained according to (Logan et al., 2006)

$$r_{CE}\ (\%) = Q_{produce}/Q_{oxidise} \times 100 \quad (7)$$

where $Q_{produce}$ (C) = ∫I(t)dt, $Q_{oxidise}$ (C) = S·b·F·V_r, S is substrate consumed in term of COD (mg O_2/L), b is stoichiometric number of electron produced per mol of oxygen reduced (4 mol/e^-), F is Faraday constant and V_r is anodic reactor volume.

Besides, modified Monod-type equation was used to estimate the anode current density related to substrate concentration as follows (Foad Marashi and Kariminia, 2015)

$$I = I_{max} \cdot S/(K_s + S) \quad (8)$$

where I (A/m^2) is the current density generated from anode, I_{max} is maximum current density, S is substrate concentration and K_s is half-saturated substrate concentration.

The overall energy efficiency is calculated based on (Call and Logan, 2008)

$$\eta_{e+s}\ (\%) = W_h/(W_e + W_s) \times 100 \quad (9)$$

where the energy yield relative to the electrical input is

$$\eta_e\ (\%) = W_h/W_e \times 100 \quad (10)$$

and the total amount of energy produced from the substrate oxidation according to

$$\eta_s\ (\%) = W_h/W_s \times 100 \quad (11)$$

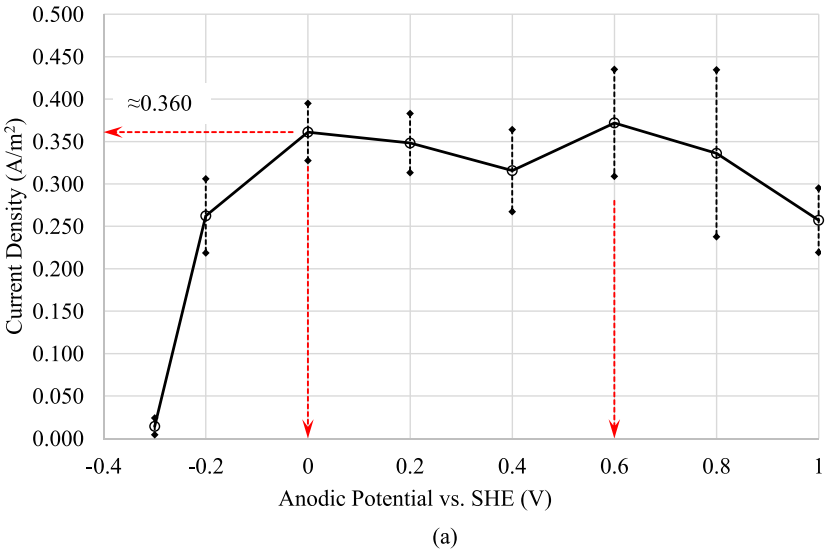
where W_h, W_e, and W_s (J) is the energy content of H_2, supplied electrical energy and energy released from substrate oxidation.

3. Results and discussion

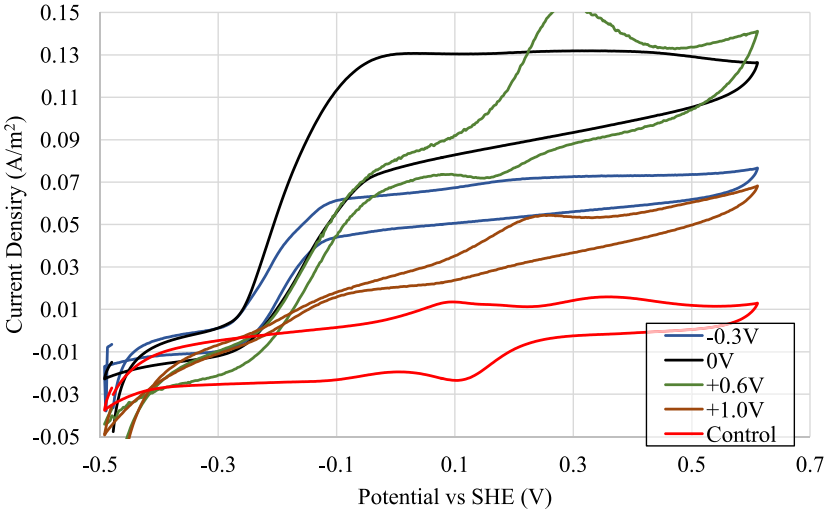
3.1. Effect of applied potential activity on bioanode

Four bioelectrochemical cells were setup in MFC mode, including two controls. All the operating condition for the controls were the same with experimental bioelectrochemical cells without adding any sources of inoculum. First, the anode of these cells were inoculated and a stable current were produced after a week of culturing under a fixed potential +0.2 V. Next, the bioanodes were subjected to chronoamperometry for at least a day before cyclic voltammetry analysis. The current density produced based on different applied potentials are shown in Fig. 1(a) as computed from the chronoamperometric results. There are two maximum current densities, 0.361 ± 0.034 A/m^2 and 0.372 ± 0.063 A/m^2, observed at 0 and +0.6 V, respectively, through a range of applied potential from −0.30 to +1.00 V. The first maximum current at 0 V was due to the contribution of electrogenic bacteria *Geobacter* sp. based on the inoculums added into the bioelectrochemical cells had been determined dominated by the species (Spurr, 2016). It is postulated that lower enrichment potential (−0.2 to +0.4 V) was the most suitable potential for the growth of dominating electrogenic species such as *Geobacter* sp. (Aelterman et al., 2008; Busalmen et al., 2008; Ketep et al., 2013; Torres et al., 2009; Zhu et al., 2013). Meanwhile second higher current occurred at +0.60 V was suspected to either inducing dominating-electrogenic or – non-electrogenic bacteria or both on the anode surface. New redox couples was detected which explained that new electron transfer mechanism might be used at this potential (Busalmen et al., 2008). Intensive works have been done by to study the effect of fixed potential used to enriched bioanode-respiring bacteria community (Aelterman et al., 2008; Torres et al., 2009; Wei et al., 2010; Zhu et al., 2013). The enriched bioanode posed different electrochemical behaviour and biofilm characteristic when different potential was applied because of the divergence of bacteria community. The lower the applied potential closed to the bioanode midpoint potential tended to suppress non-electrogenic microbes on the anode whilst favouring the electrogenic species and increasing the growth and portion of the electrogen such as *Geobacter* sp. in the bioanode community (Ketep et al., 2013; Torres et al., 2009). Other way of obtaining the highly pure community is performing secondary enrichment using the culture from primary bioanode effluent (Ketep et al., 2013; Liu et al., 2008). Table 1 summarised the enrichment potentials which have been used in previous studies.

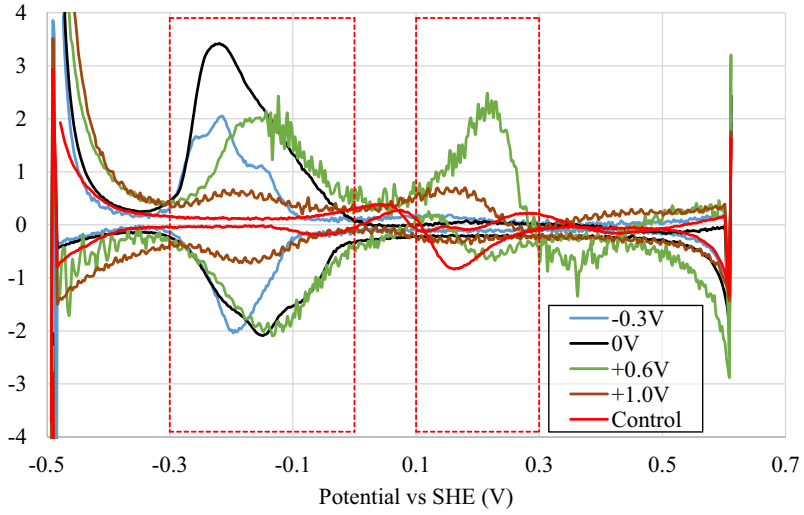
Chronoamperometric analysis revealed that the enriched bioanode could provide almost similar current density at the anode potential over 0 V (Fig. 1(a)). Cyclic voltammogram (Fig. 1(b)) indicated that enriched bioanode from +0.2 V can survive at higher poised potential up to 1.0 V. The bioanode enriched at +0.2 V produced two half wave with the midpoint potentials at −0.20 and



(a)



(b)



(c)

Fig. 1. (a) Current density produced during bioanode chronoamperometry test at different applied potentials; (b) Response of the bioanode cyclic voltammogram fixed at selected applied potentials; and (c) First derivative of the cyclic voltammograms showing the bioanode active midpoint occurred at −0.2 V and +0.2 V. The midpoint +0.2 V was showed to be active in both oxidation and reduction reactions. In contrary, oxidation reaction was more favoured at the midpoint −0.2 V as stronger oxidation wave was observed.

Table 1
Summary of enrichment parameter applied in chronoamperometry mode to enrich electrogenic consortia at anode. Current density can only be compared within the same study due to variety system configurations and substrates were used. The community of microbes diverges as enrichment potential changed from one condition to another.

Enrichment potential V (vs. SHE)	Current density A/m^2	Midpoint potential V (vs. SHE)	Main substrate mM[3]	Microbial community/significant observation	Reference
+0.37	0.600	+0.15	15 (NaAc);	16% *Geobacter* sp.	Torres et al. (2009)
+0.02	2.000	+0.14	100 (PBS)	90% *Geobacter* sp.	
−0.09	6.000	−0.16		92% *Geobacter* sp.	
−0.15	10.300	−0.16		99% *Geobacter* sp.	
+0.70	0.046	−0.10	12 (NaAc); 50 (PBS)	Higher enrichment potential favoured bioanode electroactivity as electron transfer components increased	Zhu et al. (2013)
+0.20	0.047	−0.10		Power overshoot when higher potential was introduced due to the lack of sufficient electron transfer components to shuttle electrons	
−0.04	0.035	−0.10			
−0.26	0.005	−0.10			
+0.40	2.500	−0.10	10 (NaAc);	Dominated *Geobacter* sp.	Liu et al. (2008)
+0.40	5.000	−0.10	50 (PBS)	More dominated *Geobacter* sp. achieved through secondary enrichment	
+0.20	0.636	−0.20	18 (NaAc);	Same start-up time; lower respiration rate and highest biomass production at lower enrichment potential	Aelterman et al. (2008)
0.00	0.927	−0.20	64 (PBS)		
−0.20	0.817	−0.20			
0.00	0.600	N/A	10 (Glucose);	Lower charge transfer resistance; higher substrate driving force; accelerated start-up time	Wang et al. (2009)
1000 Ω[1]	0.086	N/A	50 (PBS)	Higher charge transfer resistance; lower substrate driving force; slower start-up time	
+0.04	5.500	−0.16	5 (NaAc); 5 (PBS)	Primary enrichment; *Geobacter* sp. and *Desulfuromonas* sp. were dominating species on bioanodes	Ketep et al. (2013)
−0.16	6.000	−0.16		Secondary enrichments produced almost the same current as primary enrichment but can survive at lower enrichment potential; *Geobacter* sp. almost disappear	
−0.16	5.650	−0.16		*Desulfuromonas* sp. was the only dominating species after tertiary enrichment; Midpoint potential −0.16 V almost disappears after tertiary enrichment.	
−0.16	< 0.03	N/A		Primary enrichment produced no current due to low enrichment potential	
+0.40	1.035	−0.11	20 (NaAc); 47 (PBS)	Small amount of biomass was gained while highest enrichment potential was used and substrate oxidation reduced significantly	Wei et al. (2010)
0.00	1.025	−0.11		Biomass was gained and power density was increased; Significant substrate oxidation; current generation was proportionate to biomass for all condition; single culture *Geobacter sulfurreducens* was used in the study	
−0.16	0.660	−0.11			
500 Ω[1]	0.470	−0.11			
+0.40	1.143	−0.23	5 (NaAc); 5 (PBS)	Pure culture *Geobacter sulfurreducens* was used	Bond and Lovley (2003)
500 Ω[1]	0.065	N/A			
+0.8	2.400[2]	+0.70	5.5 (NaAc); 0.43 (PBS)	Pure culture *Geobacter sulfurreducens* was used; new redox coulples were detected indicated new electron transfer mechanism was performed at higher enrichment potential	Busalmen et al. (2008)
+0.3	1.500[2]	+0.03			

[1] Potentiostat was replaced by a resistance and the enrichment potential was depended on cathode performance.
[2] Normalised current density (ratio value without unit).
[3] NaAc-Sodium acetate, PBS-Phosphate buffer solution.

+0.20 V as shown in Fig. 1(c) and probably resulted from different electron transfer mechanisms. A more positive applied potential may also have resulted in a larger current output, especially when the potential was increased more than +0.4 V. New redox couples at the potential may indicate that new electron transfer mechanism could exist with more positive anode potential (Busalmen et al., 2008). First derivative (Fig. 1(c)) analysis showed the first midpoint potential occurred at −0.20 V with both observable active oxidation and reduction activity, however, the second midpoint potential occurred at +0.20 V showed the catalytic activity was more weak compared to the first potential and favours oxidation rather than reduction activity. The −0.20 V mid-point potential was mainly reported in literature and confirmed that it was the activity of electrogenic microbes such as *Geobacter* sp. and *Shewanella* sp. (Liu et al., 2008; Marsili et al., 2008; Torres et al., 2009). This could be either due to the multiple redox centre exposed on the surface of the microbes cells or redox–active mediators secreted by specific microbes which having the potential of −0.2 V (Carmona-Martínez et al., 2013; Jain et al., 2012; Marsili et al., 2008). Dark colour biofilm was found on the surface of the bioanode enriched at +0.20 V. The colour changes has been observed by other researchers as a change of biofilm community on the anode, for example the colour of the biofilm changed from orange-brown to thinner and darker colour when the potential increase from −0.15 V to +0.37 V (Torres et al., 2009). Based on this report, we suggest that a mixed community dominated by electrogens was grown simultaneously with non-electrogens at +0.20 V. Therefore the community can survive at higher potential and posing the second catalytic activity on +0.20 V when bioanode potential was fixed >+0.40 V. Nonetheless, the bioanode behaviour fixed at potential more than +0.40 V only showed favourable oxidation activity compared to reduction. Free flavins were normally secreted by the electrogen to facilitate the mediated electron transfer between outer membrane cytochromes and electrode (Carmona-Martínez et al., 2013; Jain et al., 2012). Once the flavins had been excreted from the electrogens, they start to accept electrons from cytochromes located at the outer membrane of electrogen and transfer electron to electrode in a reducing form. The reduced flavins were oxidised on the anode surface and probably been wash out from the continuously-fed bioanode before they could actually recycled back to the electrogens again to transfer electrons.

Fig. 2(a) and (b) show the maximum/minimum point of catalytic waves in the Fig. 1(c) versus a range of applied potentials. Fig. 2(a) revealed that the first electron transfer mechanism (deducted from the catalytic wave occurred at −0.20 V midpoint potential) was still active but exhibit low activity even when the poised potential was set near to the −0.2 V midpoint potential, eg. −0.3 V. The catalytic wave was intensified while the poised potential was set more positive than −0.3 V. Therefore, more substrate could be converted to energy and more electron can be transferred to the electrode (LaBelle and Bond, 2009). Electrode

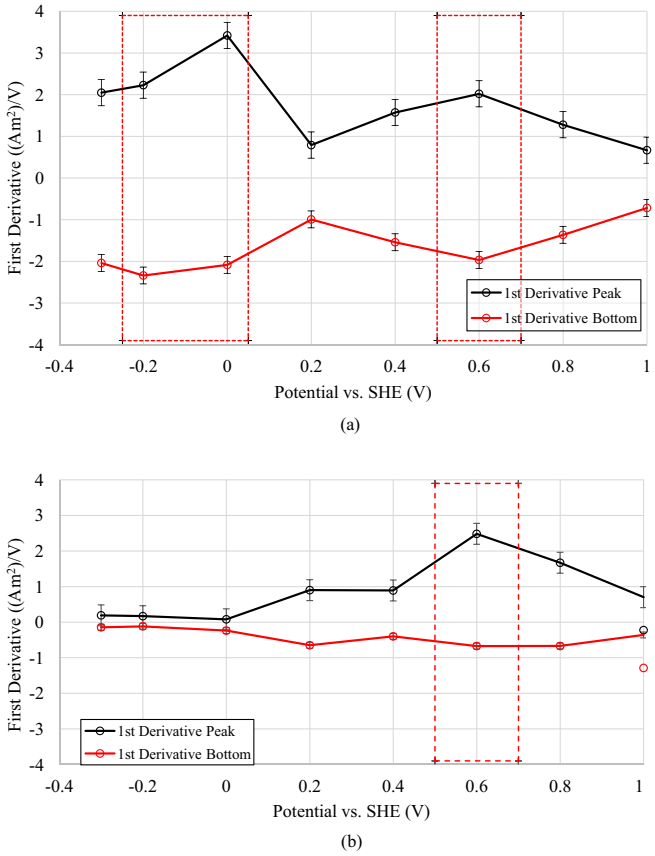


Fig. 2. The response of peak and bottom values of the catalytic waves at (a) −0.2 V and (b) +0.2 V to different poised potentials derived from the first derivative (Fig. 1 (c)). The red dash line emphasises significant catalytic waves in the range of applied potentials. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with more positive poised potential was favourable for the electrogenic bacteria to discharge their used electron and conserve energy via direct electron transfer (DET) or mediated electron transfer (MET). The catalytic wave started to decrease after the poised potential was set more positive than 0 V. As observed from the first derivative in Fig. 1(c), a second catalytic wave started to appear at +0.2 V midpoint indicating that the bioanode could use another pathways to transfer the electron to the anode. Electrogenic bacteria were able to diverge its metabolic pathway to accommodate the changes of conditions for growth and survival, especially when poised potential was changed from its original condition (Aelterman et al., 2008; Busalmen et al., 2008; Ketep et al., 2013; Wang et al., 2010). In additional to the divergent pathways, the changes of microbial community that favour particular microbes but suppress the primary electrogenic microbes might be possible as the species can easily adapt to the changes of potential than the primary species in the community (Torres et al., 2009). As a results, the second electron transfer mechanism (catalytic wave occurred at +0.20 V) started to appear when the poised potential was set more positive than +0.20 V. Fig. 2(b) shows the second peak/bottom points at +0.20 V midpoint, the catalytic activity and was at its best when the potential was set more than +0.60 V. There are two possible explanation on the second midpoint activity, either non-electrogen grew together side-by-side with the electrogen to create a robust biofilm that can use a wide range of high potential anode as electron acceptor or the electrogenic microbes had few electron transfer pathways that could be switched among them when the surrounding environment changes, eg. from +0.20 V to

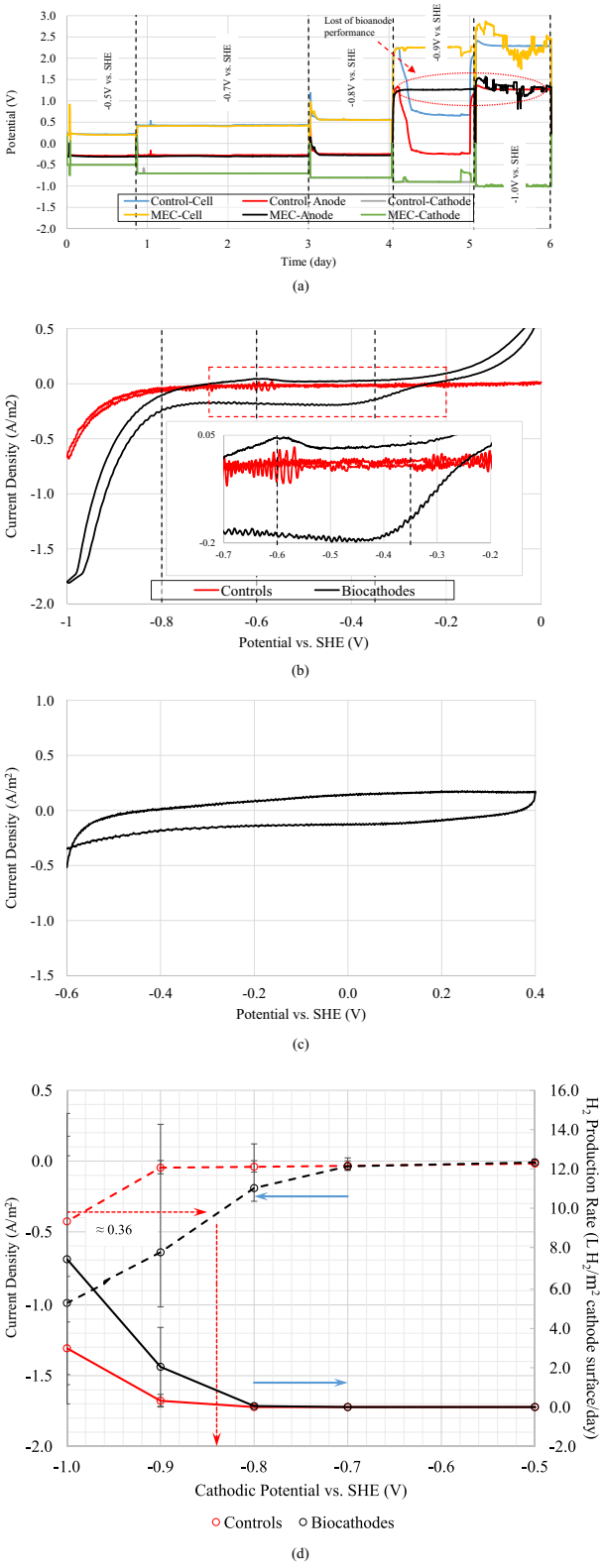


Fig. 3. (a) Cell and half-cell potentials of control cells and full biological MECs. Small legend in each region indicated the potential applied at cathode; (b) cyclic voltammogram of biocathodes after chronoamperometric tests. A magnified graph is inserted showing a small active midpoint potential at −0.6 V where hydrogen were oxidised; (c) cyclic voltammogram of bioanodes after biocathode chronoamperometric tests; (d) Current and hydrogen production across a range of applied potential. Noted that the red dash arrow line was used to determine the upper limit potential that could be applied on the cathode. Assume maximum current was produced at bioanode and the current was supplied to the biocathode for hydrogen evolution.

Table 2
Overview of the use of bioelectrodes reported in the literature.

Cathodic potential V (vs. SHE)	Current density A/m^2	Hydrogen production rate $m^3 H_2/m^3$ reactor/day	Hydrogen recovery %	Vital ingredient in catholyte	Biocathode catalyst	Vital ingredient in anolyte	Bioanode catalyst	Mode of operation	Reference
Double-chamber MEC with both electrochemically active bioanode and biocathode									
−0.70	3.30	0.04	21	CO_2	Enriched electrochemically active culture from MEC	Acetate	Enriched electrochemically active culture from MEC	Continuous	Jeremiasse et al. (2010)
−0.75	4.40	0.01	–	CO_2	Hydrogenophilic dechlorinating culture	CO_2	Hydrogenophilic dechlorinating bacteria	Batch	Villano et al. (2011)
−1.00	0.99	0.17	96	CO_2	Enriched electrochemically active culture from MFC	Acetate	Enriched electrochemically active culture from MFC	Continuous	This study
Double-chamber Half-cell MEC focused on biocathode performance									
−0.70	1.20	0.63	49	CO_2	Effluent from an active bioelectrochemical cell	Ferricyanide/ferrocyanide	–	Continuous	Rozendal et al. (2008)
−0.70	0.60	2.20	–	Acetate then CO_2	Inoculum from UASB and enriched over 5 years in MECs	Ferrocyanide	–	Continuous	Jeremiasse et al. (2012)
−0.75	1.88	9.2 L H_2/m^2/day	–	CO_2	Mixed microbial consortia from pond sediments and WWTP anaerobic digester	Phospate buffer	–	Batch	Jourdin et al. (2015)
−1.00	47 A/m^3	0.89	175	CO_2	Inoculum from urban WWTP and MFC treating WW	Same as catholyte	–	Batch	Batlle-Vilanova et al. (2014)
−0.90	3.00	8 mM/day	100	Lactate + SO_4^{2-}	*Desulfovibrio paquesii*	Same as catholyte without Lactate + SO_4^{2-}	–	Batch	Aulenta et al. (2012)
MEC with abiotic cathode									
0.8[1]	11.00	1.54	54	Same as anolyte	Platinum-coated cathode	Acetate	Inoculum from previous working MFC	Single-chamber MEC; batch	Rago et al. (2016)
0.8[1]	1.27	0.22	73	Same as anolyte	Type 304 Stainless steel mesh 60	Acetate	Pre-colonised bioanode in two-chamber MFC	Single-chamber MEC; batch	Rivera et al. (2017)
1.0[1]	2.30	0.3	23	Gas collection chamber without solution	Platinum-plating cathode	Acetate	Effluent from an active bioelectrochemical cell	Double-chamber MEC; continuous	Rozendal et al. (2007)
3.0[1]	7.50	0.38	49.5	Same as anolyte	Ti/RuO mesh cathode	Liquid fraction of pressed municipal solid waste (LPW) pH 5.5	MEC fed with grounded submerged aquatic plants	Single-chamber MEC; batch	Zhen et al. (2016)
0[2]	–	–	–	Bicarbonate buffer	Platinum-coated cathode	Propionate	Camel manure and anaerobic digested sludge	Double-chamber MEC; batch	Hari et al. (2016)
−0.55[3]	2.67	H_2 started to produced when anodic potential <−0.15	–	Phosphate buffer solution	Platinum-coated cathode	Acetate	Sewage sluge from municipal WWTP	Double-chamber MEC; batch	Wang et al. (2010)
−1.059	9.63	0.51	19.84	Same as anolyte	Activated sludge	Acetate	Activated sludge	Single-chamber MEC; batch	Liang et al. (2014)
MEC where the biocathode is not for hydrogen-producing purpose									
−0.2	+75 mA	Alkalinity produced from cathodic denitrification partially (19%) neutralised the acidity of the anodic reaction	85.3[4]	Acetate	Activated sludge from municipal WWTP	Same with catholyte without Ac or NO_3^-	–	Half-cell double-chamber MEC; continuous	Cheng et al. (2012)
	−40 mA		87.3[5]	NO_3^-					

Table 2 (*continued*)

Cathodic potential V (vs. SHE)	Current density A/m^2	Hydrogen production rate m^3 H$_2$/m^3 reactor/day	Hydrogen recovery %	Vital ingredient in catholyte	Biocathode catalyst	Vital ingredient in anolyte	Bioanode catalyst	Mode of operation	Reference
−0.4	0.03	1.9 g/L acetate; 2.09 g/L propionate; 2.25 g/L butyrate; 26.82 mg/L butanol; 16.04 mg/L ethanol; 0.16 mmol H2 (after 70 days operation)	–	CO_2	Pre-enriched culture from bog sediment	Same as catholyte	Pre-enriched culture from bog sediment	Double-chamber MEC; batch	Zaybak et al. (2013)
0.8[1]	–	0.49 mg/day SO_4^{2-} removal	5.9[6]	SO_4^{2-}	Pre-enriched domestic WW using 0.1 g/L SO_4^{2-}	Acetate	Enriched electrochemically active culture from previous MFC treating phenol	Double-chamber MEC; fed-batch	Luo et al. (2014)
	–	5.81 mg/day SO_4^{2-} removal	47.7[6]					Double-chamber MEC; continuous	
−0.9	–	39% SO_4^{2-} removal	27[6]					Double-chamber MEC; fed-batch	

[1] Applied voltage between anode and cathode.
[2] Anodic potential was controlled, no cathodic potential was recorded.
[3] determined from graph at 0.6 V applied voltage.
[4] Coulombic efficiency for substrate oxidation.
[5] Cathodic denitrification.
[6] calculated based on electron recovery.

+0.60 V. Although the bioanode could survive in higher potential, toxic compounds and mineral deposition on the surface of the anode could cause the obstruction to the microbes to transfer electrons to anode surface (Ketep et al., 2013; Torres et al., 2009). Besides, the energy force that drives abiotic reaction, eg water electrolysis, was higher compared to biotic reaction when the potential was set more positive (>+0.60 V).

3.2. Biocathode performance and bioanode limitation

All enriched bioanodes from previous experiment were further deployed in dual-chamber MECs for examining biocathode performance. Fig. 3(a) shows the cell and electrode potentials of the control cathodes (without inoculum) and biological MECs recorded under chronoamperometric tests. Interestingly, bioanode as a biocatalyst maintained its potential in between −0.30 ± 0.02 V when −0.50 to −0.80 V potentials were applied on the cathode. Even though the bioanode could maintain its potential when cathode was set as low as −0.8 V, it started to lose its performance when more current was required to draw from the anode to support cathode at higher working potential more than −0.9 V. On the other hand, the control anode could maintain its potential until −0.9 V was applied to the cathode.

Cyclic voltammetry was performed on both bioanode and biocathode after each chronoamperometric test. Fig. 3(b) and (c) shows the voltammograms of the biocathode and bioanode, respectively. On the other hand the relationship between hydrogen production and current density with cathodic potentials is shown in Fig. 3(d). By analysing the biocathode voltammogram, the first catalytic activity occurred at −0.35 V which is suspected to be the non-hydrogen-producing activity whilst the second catalytic activity started to occur at −0.8 V and below. A small hydrogen oxidation peak happened at −0.6 V proved the biocathode reversible catalysis activity accelerated by a specific enzyme called hydrogenase (Aulenta et al., 2012; Batlle-Vilanova et al., 2014). Meanwhile, based on the Fig. 3(c), bioanodes which worked as counter electrode lost their ability to catalyse oxidation reaction after chronoamperometric test. As per hypothesis mentioned in the introduction, the amount of electron consumed in cathode should be, at least, fulfilled by the electron produced by anode by substrate oxidation to balance and/or reduce energy demand from external power supply, the bioanode no longer retain its biocatalytic activity at the end. For instance, at cathodic potential −1.0 V, the current density was recorded as 0.99 A/m^2 but the maximum current density that the bioanode could produce was 0.36 A/m^2. The bioanode, at least, need to provide an extra 0.63 A/m^2 to close this energy gap. As a result of they could not produce enough current to support the biocathodes, power supply

Fig. 4. The effect of acetate concentration to current density and Coulombic efficiency (CE) of bioanode fixed at +0.2 V vs. SHE.

forced anode potential to increase sharply (−0.28 – +1.26 V) to induce abiotic reaction eg. water electrolysis or produce peroxides with the present of oxygen. The growth of the bioanode were totally halted and probably killed by toxic products produced abiotically through a high potential. Moreover, oxygen may be produced from water electrolysis due to the more positive potential was applied on the anode after the biofilm could not keep up its oxidation activity to produce more electron. Additional oxygen contamination in the system would subsequently trigger the formation of peroxides and other inorganic anions which are toxic to the bioanode (Milner, 2015). The abiotic reactions were dominated in the anode as power supply had to withdraw high current from anode to support the current consumed in cathode. There was no considerable current flow or hydrogen production activity when applied potential was set from −0.5 V to −0.7 V as shown in Fig. 3 (d). Although substantial current started flowing into the biocathode at −0.8 V, the current yet favoured any hydrogen production in the biocathode unless more negative potentials (−0.9 to 1.0 V) were used. Cathodic overpotential could be the main reason why potentials lower than −0.8 V was required (Jeremiasse et al., 2010; Rozendal et al., 2008). Theoretically, hydrogen evolution potential is −0.42 V (Nernst equation, pH 7.0). That means at least −0.38 V was lost in term of overpotential in this setup. The outcome is accordant to the previous study on a hydrogen-producing microorganism, *Desulfovibrio* sp., that equal or less reducing potential than −0.9 V is needed due to insufficient electron transfer above −0.8 V (Aulenta et al., 2012). In contrary, mediators was used to reduce the overpotential between cathode and cell surface and facilitate electron transfer. Villano et al. (2011) tested methyl viologen in their study and proved that the mediator could effectively reduce the overpotential up to 0.3 V and brought the potential closed to −0.45 V, which is slightly lower than standard hydrogen reduction potential −0.41 V. However, the latter solution appears not suitable in practical application as mediator will be required most of the time.

Abiotic current flow became significant with an applied potential more negative than −0.90 V. However, the biocathode only consumed significant amount of energy starting from −0.70 V and below as moderate current flow was observed at this point. Therefore, the working potential of biocathode in this system should be between −0.70 and −0.90 V. In order to protect the bioanode from losing its performance as biocatalytic electrode, maximum current that can be withdraw from the bioanode is determined as 0.36 A/m^2 from Fig. 1(a). If same amount of energy was required to support the biocathode then the maximum working potential that can be applied is about 0.84 V which is determined from Fig. 3(d) assuming that the same amount of current produced in anode was supplied to the cathode. This information is important to determine the optimum condition for the system to promote biohydrogen production and not water electrolysis. Significant amount of hydrogen was produced at potential more negative than −0.80 V even a reductive current was significant observed before this potential. It seems that a minimum energy is required to overcome the activation energy, which leads to overpotentials and activate microorganism's hydrogenase to produce hydrogen. A strategy to applied lower potentials in

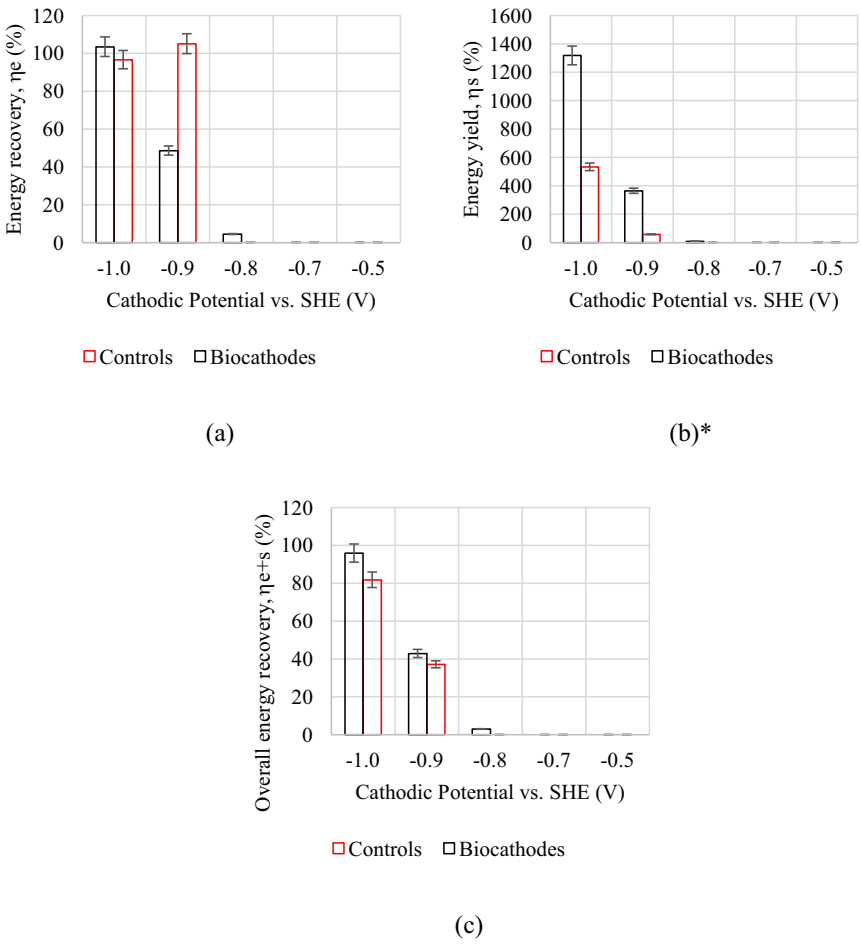


Fig. 5. Energy recovery (a), energy yield (b) and overall energy recovery (c) from MECs at different applied cathodic potentials. *Calculated based on the maximum oxidation activity of bioanode at 0 V.

chronoamperometry form were used in few studied to examine hydrogen production until a significant hydrogen was detected (Aulenta et al., 2012; Batlle-Vilanova et al., 2014). The reason why higher potential was required is to compensate for the hydrogen lost by diffusion and overpotentials such as higher pH electrolyte. Another strategy to promote hydrogen production is to keep hydrogen partial pressure as low as possible by continuously removing it from the system and maintain the pH of electrolyte at least around 7.0 (Rozendal et al., 2008). The pH of electrolyte is normally maintained between 6.5 and 7.5. If the value is lower than 6.0 or under acidic condition, less energy will be consumed and higher applied potential (>−0.7 V) could be used as higher concentration of proton is available in bulk solution (Batlle-Vilanova et al., 2014; Kumar et al., 2017). The latter strategy did increased the hydrogen yield, however, it also could increase the cost of investment and operation because of the complexity of the system configuration and controlled devices that had been used. Furthermore, a portion of hydrogen lost through membrane depends on operating temperature. Higher temperature tends to increase the diffusion coefficient as reported in Rozendal et al. (2008). Besides, it also depends on the natural of the MEC either to produce hydrogen or clean inorganic matters. For instance, standard reduction potential of sulphate (SO_4^{2-}/HS^- −0.213 V; SO_4^{2-}/S^0 −0.191 V) is much lower than proton production (H^+/H_2 −0.414 V) (Coma et al., 2013; Luo et al., 2014). If the MEC system was used to clean sulphate contaminates instead of hydrogen production, then slightly higher potential could be applied. Table 2 presents an overview of the usage of biocathode in hydrogen production and non-hydrogen producing purposes.

3.3. Energy recovery and overall performance

Once the bioanodes were enriched with stable current output, they were tested in different substrate concentrations to observe the effect of the concentration in term of current density and Coulombic efficiency. Fig. 4 shows the current density and CE plot pertaining to acetate concentration up to 20 mM. Modified Monod equation was used to determine the Monod coefficient I_{max} and K_s as mentioned in Eq. (8) (Foad Marashi and Kariminia, 2015). Based on the equation, I_{max} and K_s were determined as 0.5138 A/m^2 and 1.5163 mM. In this study, 10 mM acetate concentration was used because it is the most applicable concentration which could sustained about 86.8% of I_{max} and 45% Coulombic efficiency. Even higher acetate concentration (>10 mM) could bring up the current density (93.0% of I_{max}), the CE dropped significantly to 15% at 20 mM acetate concentration. Meanwhile, lower acetate concentration (<10 mM) generated lower current which may jeopardised the whole MEC system in term of energy recovery. As a result there would be not enough electrons to be supplied to cathode for hydrogen evolution.

Fig. 5 summarised the overall energy recovery in term of electrical power, substrate oxidation and hydrogen produced. From the graphs, it seems that external power supply play an important role in driving hydrogen production in cathode rather than electron-producing anode. For instances, at cathodic potential −1.0V, η_s biocathode was significantly high about 1317% and it means larger portion (1317 − 100 = 1217%) of the hydrogen recovery was not contributed by substrate oxidation in bioanode. However, it is quite opposite for η_e biocathode where the efficiency is 103% where the excessive 3% was not provided by the electrical energy (Call and Logan, 2008; Logan et al., 2008). Biocathode energy recovery was first observed starting from −0.8 V cathodic potential compared to the control where still remains zero. A remarkable overall recovery nearly 100% was recorded at cathodic potential −1.0 V.

4. Conclusions

This study demonstrated that the performance of bioanode can be a factor that can limit the biocathode in a MEC system. The bioanode enriched at −0.2 V vs. SHE can survive at higher applied potential up to 1.0 V and posted two significant catalytic activities at midpoint potentials −0.2 V and +0.2 V. The catalytic waves could be shifted between each other depend on the potentials fixed on the anode. This may due to community shifted or the changes of metabolic pathways of dominating microbes. Meanwhile, biocathode could produce hydrogen with applied potential lower than −0.8 V, said −0.9 V. However, the applied potential −0.9 V on biocathode killed the bioanode as it was not able to generate enough current to support the need of the biocathode. In the operation of a biocathode, the potential vs. current density behaviour for effective operation during hydrogen evolution may not be compatible with the effective operation of the bio-anode. The obtained current density may result in less than ideal anode potentials for effective anode biofilm operation at a given cathode potentials. Applied potential of 0.84 V was determined as maximum value that can be applied to biocathode without overloading the bioanode. The capability and robustness of bioanode are important to ameliorate the limitation to biocathode and whole system.

Acknowledgements

This research was financially supported by EPSRC project EP/N009746/1. Swee Su Lim was sponsored by Skim Latihan Akedemik IPTA (SLAI) under the Malaysian Ministry of Education. Data supporting this publication is openly available under an 'Open Data Commons Open Database License'. Additional metadata are available at: http://dx.doi.org/10.17634/150659-1. Please contact Newcastle Research Data Service at rdm@ncl.ac.uk for access instructions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.03.127.

References

Aelterman, P., Freguia, S., Keller, J., Verstraete, W., Rabaey, K., 2008. The anode potential regulates bacterial activity in microbial fuel cells. Appl. Microbiol. Biotechnol. 78 (3), 409–418.
Aulenta, F., Catapano, L., Snip, L., Villano, M., Majone, M., 2012. Linking bacterial metabolism to graphite cathodes: electrochemical insights into the H2-producing capability of Desulfovibrio sp. ChemSusChem 5 (6), 1080–1085.
Batlle-Vilanova, P., Puig, S., Gonzalez-Olmos, R., Vilajeliu-Pons, A., Bañeras, L., Balaguer, M.D., Colprim, J., 2014. Assessment of biotic and abiotic graphite cathodes for hydrogen production in microbial electrolysis cells. Int. J. Hydrogen Energy 39 (3), 1297–1305.
Bond, D.R., Lovley, D.R., 2003. Electricity production by Geobacter sulfurreducens attached to electrodes. Appl. Environ. Microbiol. 69 (3), 1548–1555.
Busalmen, J.P., Esteve-Nuñez, A., Feliu, J.M., 2008. Whole cell electrochemistry of electricity-producing microorganisms evidence an adaptation for optimal exocellular electron transport. Environ. Sci. Technol. 42 (7), 2445–2450.
Call, D., Logan, B.E., 2008. Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane. Environ. Sci. Technol. 42 (9), 3401–3406.
Carmona-Martínez, A.A., Harnisch, F., Kuhlicke, U., Neu, T.R., Schröder, U., 2013. Electron transfer and biofilm formation of Shewanella putrefaciens as function of anode potential. Bioelectrochemistry 93, 23–29.
Cheng, K.Y., Ginige, M.P., Kaksonen, A.H., 2012. Ano-cathodophilic biofilm catalyzes both anodic carbon oxidation and cathodic denitrification. Environ. Sci. Technol. 46 (18), 10372–10378.
Coma, M., Puig, S., Pous, N., Balaguer, M.D., Colprim, J., 2013. Biocatalysed sulphate removal in a BES cathode. Bioresour. Technol. 130, 218–223.
Croese, E., Jeremiasse, A.W., Marshall, I.P.G., Spormann, A.M., Euverink, G.-J.W., Geelhoed, J.S., Stams, A.J.M., Plugge, C.M., 2014. Influence of setup and carbon source on the bacterial community of biocathodes in microbial electrolysis cells. Enzyme Microb. Technol. 61–62, 67–75.

Escapa, A., Mateos, R., Martínez, E.J., Blanes, J., 2016. Microbial electrolysis cells: an emerging technology for wastewater treatment and energy recovery. From laboratory to pilot plant and beyond. Renew. Sustain. Energy Rev. 55, 942–956.
Foad Marashi, S.K., Kariminia, H.-R., 2015. Performance of a single chamber microbial fuel cell at different organic loads and pH values using purified terephthalic acid wastewater. J. Environ. Health Sci. Eng. 13, 27.
Hari, A.R., Katuri, K.P., Logan, B.E., Saikaly, P.E., 2016. Set anode potentials affect the electron fluxes and microbial community structure in propionate-fed microbial electrolysis cells. Sci. Rep. 6, 38690.
Jafary, T., Daud, W.R.W., Ghasemi, M., Kim, B.H., Md Jahim, J., Ismail, M., Lim, S.S., 2015. Biocathode in microbial electrolysis cell; present status and future prospects. Renew. Sustain. Energy Rev. 47, 23–33.
Jain, A., Zhang, X., Pastorella, G., Connolly, J.O., Barry, N., Woolley, R., Krishnamurthy, S., Marsili, E., 2012. Electron transfer mechanism in Shewanella loihica PV-4 biofilms formed at graphite electrode. Bioelectrochemistry 87, 28–32.
Jeremiasse, A.W., Hamelers, H.V.M., Buisman, C.J.N., 2010. Microbial electrolysis cell with a microbial biocathode. Bioelectrochemistry 78 (1), 39–43.
Jeremiasse, A.W., Hamelers, H.V.M., Croese, E., Buisman, C.J.N., 2012. Acetate enhances startup of a H2-producing microbial biocathode. Biotechnol. Bioeng. 109 (3), 657–664.
Jourdin, L., Freguia, S., Donose, B.C., Keller, J., 2015. Autotrophic hydrogen-producing biofilm growth sustained by a cathode as the sole electron and energy source. Bioelectrochemistry 102, 56–63.
Kadier, A., Simayi, Y., Abdeshahian, P., Azman, N.F., Chandrasekhar, K., Kalil, M.S., 2016. A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alexandria Eng. J. 55 (1), 427–443.
Ketep, S.F., Bergel, A., Bertrand, M., Achouak, W., Fourest, E., 2013. Lowering the applied potential during successive scratching/re-inoculation improves the performance of microbial anodes for microbial fuel cells. Bioresour. Technol. 127, 448–455.
Kim, B.H., Lim, S.S., Daud, W.R.W., Gadd, G.M., Chang, I.S., 2015. The biocathode of microbial electrochemical systems and microbially-influenced corrosion. Bioresour. Technol. 190, 395–401.
Kumar, G., Bakonyi, P., Zhen, G., Sivagurunathan, P., Koók, L., Kim, S.-H., Tóth, G., Nemestóthy, N., Bélafi-Bakó, K., 2017. Microbial electrochemical systems for sustainable biohydrogen production: surveying the experiences from a start-up viewpoint. Renew. Sustain. Energy Rev. 70, 589–597.
LaBelle, E., Bond, D.R., 2009. Cyclic voltammetry of electrode-attached bacteria. Bioelectrochemical Systems: From Extracellular Electron Transfer to Biotechnological Application. Wageningen University, The Netherlands.
Lee, H.-S., Rittmann, B.E., 2010. Significance of biological hydrogen oxidation in a continuous single-chamber microbial electrolysis cell. Environ. Sci. Technol. 44 (3), 948–954.
Liang, D., Liu, Y., Peng, S., Lan, F., Lu, S., Xiang, Y., 2014. Effects of bicarbonate and cathode potential on hydrogen production in a biocathode electrolysis cell. Front. Environ. Sci. Eng. 8 (4), 624–630.
Lim, S.S., Daud, W.R.W., Md Jahim, J., Ghasemi, M., Chong, P.S., Ismail, M., 2012. Sulfonated poly(ether ether ketone)/poly(ether sulfone) composite membranes as an alternative proton exchange membrane in microbial fuel cells. Int. J. Hydrogen Energy 37 (15), 11409–11424.
Liu, H., Grot, S., Logan, B.E., 2005. Electrochemically assisted microbial production of hydrogen from Acetate. Environ. Sci. Technol. 39 (11), 4317–4320.
Liu, Y., Harnisch, F., Fricke, K., Sietmann, R., Schröder, U., 2008. Improvement of the anodic bioelectrocatalytic activity of mixed culture biofilms by a simple consecutive electrochemical selection procedure. Biosens. Bioelectron. 24 (4), 1006–1011.
Liu, G., Zhou, Y., Luo, H., Cheng, X., Zhang, R., Teng, W., 2015. A comparative evaluation of different types of microbial electrolysis desalination cells for malic acid production. Bioresour. Technol. 198, 87–93.
Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and technology. Environ. Sci. Technol. 40 (17), 5181–5192.
Logan, B.E., Call, D., Cheng, S., Hamelers, H.V.M., Sleutels, T.H.J.A., Jeremiasse, A.W., Rozendal, R.A., 2008. Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environ. Sci. Technol. 42 (23), 8630–8640.
Luo, H., Fu, S., Liu, G., Zhang, R., Bai, Y., Luo, X., 2014. Autotrophic biocathode for high efficient sulfate reduction in microbial electrolysis cells. Bioresour. Technol. 167, 462–468.
Marsili, E., Rollefson, J.B., Baron, D.B., Hozalski, R.M., Bond, D.R., 2008. Microbial biofilm voltammetry: direct electrochemical characterization of catalytic electrode-attached biofilms. Appl. Environ. Microbiol. 74 (23), 7329–7337.
Milner, E.M., 2015. Development of an aerobic biocathode for microbial fuel cells. In: School of Chemical Engineering and Advanced Materials, vol. PhD, Newcastle University.
Rago, L., Monpart, N., Cortes, P., Baeza, J.A., Guisasola, A., 2016. Performance of microbial electrolysis cells with bioanodes grown at different external resistances. Water Sci. Technol. 73 (5), 1129–1135.
Rivera, I., Bakonyi, P., Buitrón, G., 2017. H_2 production in membraneless bioelectrochemical cells with optimized architecture: the effect of cathode surface area and electrode distance. Chemosphere 171, 379–385.
Rosenbaum, M., Aulenta, F., Villano, M., Angenent, L.T., 2011. Cathodes as electron donors for microbial metabolism: which extracellular electron transfer mechanisms are involved? Bioresour. Technol. 102 (1), 324–333.
Rozendal, R.A., Hamelers, H.V.M., Euverink, G.J.W., Metz, S.J., Buisman, C.J.N., 2006. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int. J. Hydrogen Energy 31 (12), 1632–1640.
Rozendal, R.A., Hamelers, H.V.M., Molenkamp, R.J., Buisman, C.J.N., 2007. Performance of single chamber biocatalyzed electrolysis with different types of ion exchange membranes. Water Res. 41 (9), 1984–1994.
Rozendal, R.A., Jeremiasse, A.W., Hamelers, H.V.M., Buisman, C.J.N., 2008. Hydrogen production with a microbial biocathode. Environ. Sci. Technol. 42 (2), 629–634.
Ruiz, Y., Baeza, J.A., Guisasola, A., 2013. Revealing the proliferation of hydrogen scavengers in a single-chamber microbial electrolysis cell using electron balances. Int. J. Hydrogen Energy 38 (36), 15917–15927.
Spurr, M.W.A., 2016. Microbial Fuel Cell-based Biosensors for Estimation of Biochemical Oxygen Demand and Detection of Toxicity, Unpublished manuscript.
Torres, C.I., Krajmalnik-Brown, R., Parameswaran, P., Marcus, A.K., Wanger, G., Gorby, Y.A., Rittmann, B.E., 2009. Selecting anode-respiring bacteria based on anode potential: phylogenetic, electrochemical, and microscopic characterization. Environ. Sci. Technol. 43 (24), 9519–9524.
Villano, M., De Bonis, L., Rossetti, S., Aulenta, F., Majone, M., 2011. Bioelectrochemical hydrogen production with hydrogenophilic dechlorinating bacteria as electrocatalytic agents. Bioresour. Technol. 102 (3), 3193–3199.
Wang, X., Feng, Y., Ren, N., Wang, H., Lee, H., Li, N., Zhao, Q., 2009. Accelerated start-up of two-chambered microbial fuel cells: effect of anodic positive poised potential. Electrochim. Acta 54 (3), 1109–1114.
Wang, A., Liu, W., Ren, N., Zhou, J., Cheng, S., 2010. Key factors affecting microbial anode potential in a microbial electrolysis cell for H_2 production. Int. J. Hydrogen Energy 35 (24), 13481–13487.
Wei, J., Liang, P., Cao, X., Huang, X., 2010. A new insight into potential regulation on growth and power generation of Geobacter sulfurreducens in microbial fuel cells based on energy viewpoint. Environ. Sci. Technol. 44 (8), 3187–3191.
Zaybak, Z., Pisciotta, J.M., Tokash, J.C., Logan, B.E., 2013. Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems. J. Biotechnol. 168 (4), 478–485.
Zhen, G., Kobayashi, T., Lu, X., Kumar, G., Hu, Y., Bakonyi, P., Rózsenberszki, T., Koók, L., Nemestóthy, N., Bélafi-Bakó, K., Xu, K., 2016. Recovery of biohydrogen in a single-chamber microbial electrohydrogenesis cell using liquid fraction of pressed municipal solid waste (LPW) as substrate. Int. J. Hydrogen Energy 41 (40), 17896–17906.
Zhu, X., Tokash, J.C., Hong, Y., Logan, B.E., 2013. Controlling the occurrence of power overshoot by adapting microbial fuel cells to high anode potentials. Bioelectrochemistry 90, 30–35.