Increasing methane content in biogas and simultaneous value added product recovery using microbial electrosynthesis

Sovik Das, Pritha Chatterjee and M. M. Ghangrekar

ABSTRACT

Electrosynthesis of multi-carbon compounds from the carbon dioxide present in biogas is a nascent approach towards purification of biogas. Microbial electrosynthesis (MES) cells, fabricated using different electrode materials, were operated using different electrolytes and mixed anaerobic culture as biocatalysts in the cathodic chamber under an applied cathode potential of -0.7 V vs SHE. The rate of production of acetate, isobutyrate, propionate and 2-piperidinone from reduction of CO_2 in the cathodic chamber of the MES was 0.81 mM/day, 0.63 mM/day, 0.44 mM/day and 0.53 mM/day, respectively. As methane was also present in the biogas, methyl derivatives of these acids were also found in traces in catholyte. It was observed that the use of nickel foam as an anode, 1 M $NiSO_4$ solution as anolyte, graphite felt as a cathode, phosphate buffer solution as catholyte at a pH of 5.2 proved to be the best possible combination for MES for this study to get enhanced product yield at higher energy efficiency.

Key words | biocathode, bioelectrochemical system, biogas, carbon dioxide reduction, electroreduction, microbial electrosynthesis cell

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INTRODUCTION

Microbial electrosynthesis (MES) is a novel application of bioelectrochemical systems (BESs) to achieve electrosynthesis by external application of electricity and by using microorganisms as biocatalysts (Rabaey & Rozendal 2010). These groups of bacteria, which are able to accept electrons directly from the electrode surface or through mediators like hydrogen, are known as electrotrophs (Lovley 2011). A typical MES has two chambers, anodic and cathodic chambers separated by a proton exchange membrane (Figure 1). Either MES can start from CO_2 or from other organics; however, electrosynthesis by reduction of CO_2 is most widely reported. In the cathodic chamber, electrons are supplied using external power source, which leads to the reduction of CO_2 to various organic compounds. On the other hand in the anodic chamber splitting of water occurs, when the anode is abiotic, which generates protons and oxygen (Rabaey & Rozendal 2010). The protons are transferred through the PEM from the anodic chamber to the cathodic chamber, where it reacts with carbon dioxide in the presence of biocatalyst to generate organic compounds.

The first proof of such electrosynthesis via fermentation of CO_2 in a MES was documented by Nevin *et al.* (2010), in which CO_2 was reduced by *Sporomusa ovata* producing acetate and 2-oxobutyrate at an imposed cathodic potential of -0.6 V vs. Ag/AgCl. Other pure acetogenic cultures like *Acetobacterium*, *Clostridium* etc. could also convert CO_2 to acetate, formate, butyrate, butanol and ethanol (Marshall *et al.* 2012; Ganigué *et al.* 2015). Multispecies inoculum have given similar or better performance, but it is difficult to compare as various studies reported earlier were carried out under different conditions and at different applied potentials (Blanchet *et al.* 2015). Mixed culture of microbes was used by Bajracharya *et al.* (2015) and Jourdin *et al.* (2014) producing acetate and traces of methane from CO_2 in the cathode chamber. Polarized stainless steel cathode, in pure cultures of *Geobacter sulfurreducens*, was reported to generate reduction currents of up to 30 A/m^2 while reducing CO_2 producing glycerol (Soussan *et al.* 2013).

Volumetric production rates and yields reported in all the above studies using mixed or pure culture inoculum were low. Acetate production was reported to be increased
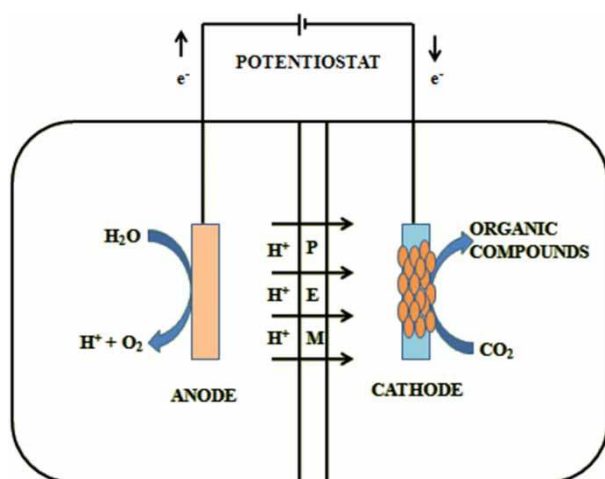
Figure 1 | Schematic diagram of MES cell.

by 2.3 times using Ni nanowire coating on graphite stick at imposed potential of -0.6 V vs. Ag/AgCl as compared with untreated graphite cathode (Nie *et al.* 2013). Jourdin *et al.* (2014) developed a novel biocompatible, highly conductive three-dimensional cathode manufactured by direct growth of flexible multiwalled carbon nanotubes on reticulated vitreous carbon for the improvement of MES, which allowed an enhanced bacterial attachment and biofilm development within its hierarchical porous structure, thus 1.7 and 2.6 fold higher current consumption and acetate production rate was obtained as compared to a carbon plate control for the microbial reduction of carbon dioxide by mixed cultures.

Hydrogen produced on the cathode by water electrolysis has been proved to be an essential mediator in the microbial electrochemical reduction of CO_2 (Blanchet *et al.* 2015). Bajracharya *et al.* (2015) used a graphite-felt electrode with stainless steel current collector to produce acetate at the maximum rate of 1.3 mM·d^{-1}, along with methane and hydrogen at -1.1 V vs. Ag/AgCl. Stainless steel worked not only as a current collector but also as a hydrogen-evolving cathode in that study. Acetate has been reported to be the major product of CO_2 reduction in a MES until this date. However, the autotrophic production of acetate is not very attractive from the economic standpoint due to its low market price. A study by Ganigué *et al.* (2015) demonstrated for the first time the bioelectrochemical CO_2 transformation to butyrate, which was proved hydrogen driven.

BES has many advantages over conventional techniques and one of them is environmental sustainability, as in BES simultaneously valuable products are recovered with pollution abatement. MES has the potential of mitigating the

greenhouse effect of CO_2 by using it as a feedstock for the synthesis of various chemicals (Rabaey *et al.* 2011). Thus, MES provides a unique opportunity of purifying the biogas generated from small scale up-flow anaerobic sludge blanket (UASB) reactors or utilizing anodic off gas containing CO_2 generated in anodic chamber of microbial fuel cell in cathodic chamber to convert this greenhouse gas in to basic chemicals like acetate, butyrate, formate, etc. using microorganisms as biocatalysts. These intermediate chemicals produced will further be used for production of other valuable chemicals (Nevin *et al.* 2010).

Based on earlier research it was hypothesized that micro-organisms in a MES can use the carbon dioxide present in the biogas, from an anaerobic wastewater treatment plant, to synthesize value added organic chemicals. In this study the possibility of purification of biogas generated by an UASB reactor for value added product recovery in a MES cell was explored. Further research is needed toward optimization of operating conditions like type of electrolyte and electrode by determining their individual and interactive effects on product recovery from carbon dioxide. Anode is an important part of the MES and its performance greatly affects the overall performance of the system. In addition, the effect of anode material on the performance of MES has not been looked into earlier by researchers. Therefore, in this study, the effect of different anode and cathode materials on the performance of the MES was evaluated. The possibility of using different anolytes like nickel sulphate and phosphate buffer, to enhance the performance of MES, was also explored.

MATERIALS & METHODS

Reactor setup

Four MES cells were fabricated using acrylic sheet. Anodic and cathodic chamber had an equal volume of 25 mL each. Nickel foam and graphite felt was used as anode and cathode material in different combinations (Table 1). Different

Table 1 | Configuration of MES used in the study

	Anode material	Cathode material	Anolyte	Catholyte
MES-1	Graphite felt	Graphite felt	PBS	PBS
MES-2	Nickel foam	Graphite felt	PBS	PBS
MES-3	Nickel foam	Nickel foam	PBS	PBS
MES-4	Nickel foam	Graphite felt	1 M NiSO$_4$	PBS

Uncorrected Proof

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anolytes were also used to determine the optimal configuration for MES (Table 1). Phosphate buffer solution of pH 5.2 was primarily used as both anolyte and catholyte. It was reported by Jourdin *et al.* (2016) that pH of 5.2 helps in suppressing methanogenesis without using any additional chemical suppressors accordingly the same pH was used as catholyte, unless stated otherwise. Nafion 117 was used as the proton exchange membrane separating the anodic and cathodic chambers. All the MESs were operated until a stable performance was noticed, which varied depending upon the setup and anodic/cathodic materials.

The cathodic chamber of the MES cell was inoculated using 5 mL of mixed anaerobic sludge, which was collected from a septic tank in IIT Kharagpur, India. Anaerobic condition was maintained in the cathodic chamber by hermetically sealing it. Biogas generated from UASB reactor treating sewage in IIT Kharagpur campus was used as cathodic feedstock for the MES. The biogas was fed continuously into the MES cells at a rate of 3 L d^{-1} by water displacement method. The flow rate of the input biogas was intentionally kept very less so that it does not disturb the biofilm formed on the cathode. The MES cells were operated at a temperature of 27 ± 5 °C. The cathodes of the MES cells were poised at -0.7 V vs SHE using a locally made potentiostat (Huang *et al.* 2009) and calomel reference electrode ($+0.238$ V vs. SHE, Bioanalytical Systems Inc., USA), which was also placed in the cathodic chamber. The catholyte was replaced regularly with fresh phosphate buffer solution to prevent the saturation of products formed.

Chemical analysis

Catholyte and anolyte samples were collected every day to measure pH and VFA. To find the concentration of VFAs, 1 mL filtrate sample was collected in a 1.5 mL gas chromatograph (GC) vial and it was acidified with 100-μL of 3% H_3PO_4 (Chen *et al.* 2007) before being analyzed on GC (Agilent Technologies GC-7890A, Penang, Malaysia) with flame ionization detector (FID) and DB-FFAP column (30 m × 0.25 mm × 0.25 mm). The sample injection volume was 1.0 μL. Nitrogen was used as the carrier gas with a flux of 30 mL min^{-1}. The injection port and the detector were maintained at 200 °C and 250 °C, respectively. The GC oven was programmed to begin at 120 °C for 2 min and then to increase at the rate of 13 °C min^{-1} to 200 °C, and then to hold at 200 °C for an additional 2 min. The measured VFA constituents were expressed in mg L^{-1} d^{-1}. The pH was measured using electrode (Thermo, Massachusetts, USA).

Biogas analysis

The biogas collected from the UASB reactor, was quantified to find the composition using GC system (Agilent Technologies GC-7890A, Penang, Malaysia). The biogas was collected in a 1.5 mL GC vial by water displacement method and was analysed using FID and molecular sieve column. One μL of the gas was injected into the system using GC syringe. Nitrogen was used as the carrier gas with a flux of 35 cm sec^{-1} at a flow rate of 8 mL min^{-1}. The injector and the FID were both maintained at 150 °C initially. The column temperature was initially set at 35 °C and the oven was programmed to hold the initial temperature of 35 °C for 5 minutes followed by a ramp of 20 °C min^{-1} until 65 °C with a hold time of 4 minutes. The percentage of carbon dioxide, methane and ammonia present in the sample was calculated by comparing the areas obtained by analysing the sample and that of the standard. The same method was adopted to find the composition of the outlet gas collected from the MES cells.

Nickel quantification

The amount of nickel leaching from nickel foam anode in MES-3 and the quantity of nickel passing through Nafion 117 from the anolyte to the catholyte was quantified using ultraviolet spectrophotometer (Scoggins 1970). Ammonical KCN solution containing 2 gm of KCN and 10 mL of ammonium hydroxide per 100 ml of the solution was used as the reagent. Samples of volume 5 mL were collected from the MES cells and 10 mL of the reagent was added to it. The absorbance of the resulting solution was measured at 550 nm using Spectrophotometer (PG Instruments, China). Using the same method the standard samples were run to get the calibration curve for this method. Accordingly, the absorbance values of the samples were converted into the concentration values by using calibration curve. These concentration values in mg/L were further reported in the text.

Calculations

Production of various chemicals (in mol) at any time t was calculated as per Equation (1)."

$$n_{pro,t} = \frac{V_{cat} \times (C_{pro,t} - C_{pro,t_0})}{M_{pro}} \tag{1}$$

where, $n_{pro,t}$ is the moles of the product formed in the time interval t, V_{cat} is effective volume of the cathodic chamber

(L), $C_{pro,t}$ and C_{pro,t_0} are the final and initial concentration of the product of interest, respectively (mg L^{-1}) and M_{pro} is the molar mass of the product (mg) (Bajracharya *et al.* 2015).

Current efficiency, which is also known as cathodic electron efficiency (*CEE*), is the efficiency of capturing the electrons by the microorganisms from the electric currents to form products. *CEE* was calculated using Equation (2) (Bajracharya *et al.* 2015).

$$CEE = \frac{n_{pro,t} \times f_{pro} \times F}{\int_{t_0}^{t} I\, dt} \times 100 \tag{2}$$

where, *CEE* is the CEE (in %), f_{pro} represents molar conversion factor (e.g. 8 electron equivalent for acetate), F is Faraday's constant (96,485 C mol^{-1}), I is the current supplied (Bajracharya *et al.* 2015). CEE was calculated individually for all the products and it was then added to get the total CEE for the system.

Carbon fixing or carbon recovery efficiency (CRE, η_c) indicates the percentage of carbon used from biogas by the microbes to form products. CRE was calculated using the Equation (3) (Bajracharya *et al.* 2015).

$$\eta_c = \frac{n_{pro,t} \times f_{c,pro}}{n_{gas}} \times 100 \tag{3}$$

where, η_c is the CRE (in %), $f_{c,pro}$ is the number of moles of carbon in a mole of the product (e.g. 2 moles of carbon in one mole of acetate), n_{gas} is the moles of CO$_2$ in the gas (Bajracharya *et al.* 2015). CRE was also calculated for individual product and the summation of the CRE of all the individual products is reported as total CRE of the system.

The energy efficiency of the system is calculated as the amount of energy required in kWh per unit of the product using Equation (4).

$$J_{pro,t} = \frac{E_{cell} \times \int_{t_0}^{t} I\, dt}{n_{pro,t}} \tag{4}$$

where $J_{pro,t}$ is the amount of electrical energy required to produce unit mole of the product (in kWh mol^{-1}), E_{cell} is the actual cell voltage during the operation of MES (Bajracharya *et al.* 2015). Total electrical energy required is reported as sum of the energy required for each individual product as calculated from Equation (4). To calculate these parameters for evaluating the performance of MES continuous monitoring of voltage and current was done using data acquisition/switch unit (Agilent Technologies, Penang, Malaysia) connected with a computer system.

Electrochemical analysis

Cyclic voltammetry (CV) was performed in non-turnover conditions to understand the action of biofilm and different electrolytes on the electrode material. The analyses were carried out after the MESs reached stable performance. The measurements were performed using three electrode systems consisting of cathode as the working electrode, an Ag/AgCl reference electrode saturated with 3 M KCl and platinum rod as counter electrode (+205 mV vs SHE). The working electrode and the reference electrode were placed in the same chamber and counter electrode in the other chamber. Electrochemical workstation (Autolab PGSTAT 302N potentiostat, Metrohm, Utrecht, The Netherland) was used for the analysis. A scan rate of 1 mV/s and a potential range of −1 V to +1 V was used during CV analysis. The current response against different applied voltages for CV was recorded with NOVA 1.9 software.

RESULTS & DISCUSSIONS

Electroreduction of CO$_2$

The efficiency of the MES actually depends on the enriched microbial culture. The microbial consortia and the interaction between the microbes and the electrodes in turn govern the rate of product formation, which is a key indicator of the efficiency of the MES. Parameters like CEE and CRE that are measured for MES are directly related to the rate of product formation. For this study the rate of formation of liquid organic chemicals were measured and reported. The rate of production of acetate was found to be highest in all the MES cells than the rate of production of other compounds. This is because the production of acetate requires the least number of electrons, compared to other products formed in the system. Among the MESs used, acetate production rate was highest for MES-2 (0.81 ± 0.07 mM d^{-1}, Table 2), where nickel foam was used as anode and graphite felt as cathode. During the operation of MES-1, with graphite felt as both anode and cathode, it was observed that the pH of the anolyte became highly alkaline (10.12 ± 0.2) with duration of operation. Due to the migration of H$^+$ ions from the anolyte to

Table 2 | Performance of the MESs used for the study and comparison with few other selected studies

Reactor	Biocatalyst	Anode	Cathode	Anolyte	Catholyte	Applied potential vs Ag/AgCl (V)	Liquid product(s)	Rate of production (mM d^{-1}/g m^{-2} d^{-1})	CEE (%)	Reference
MES-1	Mixed culture	Graphite felt	Graphite felt	PBS	PBS with CO_2 as carbon source	−0.9	Acetate Isobutyrate Propionate 2-piperidinone	0.65 ± 0.02 /17.33 ± 0.53 0.42 ± 0.03/16.43 ± 1.17 0.30 ± 0.02/9.87 ± 0.66 0.35 ± 0.03/15.40 ± 1.32	51.55 ± 4.5	This study
MES-2	Mixed culture	Nickel foam	Graphite felt	PBS	PBS with CO_2 as carbon source	−0.9	Acetate Isobutyrate Propionate 2-piperidinone	0.81 ± 0.07/21.60 ± 1.87 0.63 ± 0.027/24.64 ± 0.78 0.44 ± 0.03/14.47 ± 0.99 0.53 ± 0.03/23.32 ± 1.32	68.81 ± 3.3	
MES-3	Mixed culture	Nickel foam	Nickel foam	PBS	PBS with CO_2 as carbon source	−0.9	Acetate Isobutyrate Propionate 2-piperidinone	0.22 ± 0.0177/5.87 ± 0.27 0.12 ± 0.01/4.69 ± 0.39 0.09 ± 0.01/2.96 ± 0.33 0.1 ± 0.02/4.40 ± 0.88	34.42 ± 5.4	
MES-4	Mixed culture	Nickel foam	Graphite felt	1 M $NiSO_4$ soln.	PBS with CO_2 as carbon source	−0.9	Acetate Isobutyrate Propionate 2-piperidinone	0.67 ± 0.03/17.87 ± 0.80 0.51 ± 0.1/19.95 ± 3.91 0.36 ± 0.077/11.84 ± 2.30 0.43 ± 0.01/18.92 ± 0.44	54.31 ± 4.1	
–	Mixed culture	Graphite felt	Stainless steel and graphite felt assembly	PBS of pH 3–4	PBS with $NaHCO_3$	−1.1	Acetate	1.3	40–50	Bajracharya *et al.* (2015)
–	*Sporomusa ovata*	Unpolished graphite sticks	Unpolished graphite sticks	Growth medium	Growth medium	−0.6	Acetate & oxybutyrate	0.17	86 ± 21	Nevin *et al.* (2010)
–	Enriched culture from bog sediment	Carbon fiber rods	Carbon fiber rods	Basal medium	Basal medium	−0.6	Acetate	0.05	35.2 ± 4.4	Zaybak *et al.* (2013)
–	Pond sediments and WWTP sludge	Platinum wire	NanoWeb reticulated vitreous carbon	Na_2HPO_4, KH_2PO_4 soln.	NH_4Cl, $MgCl_26H_2O$, $CaCl_2$, Na_2HPO_4, KH_2PO_4 & trace elements soln.	−1.05	Acetate	0.47 ± 0.07	78.5	Jourdin *et al.* (2014)

*WWTP – wastewater treatment plant.

the catholyte, the pH of anolyte increased from the starting pH of 5.2 to 10.12 ± 0.2.

Graphite felt does not have any catalytic effect on the splitting of water owing to its inert and non-reactive nature. Therefore, the aim was to find an alternative anode material that could enhance the performance of the anode. It was reported by Han *et al.* (2015) and Masa *et al.* (2016) that nickel foam is a suitable material for enhancing water splitting reaction. Hence, graphite felt was replaced by nickel foam as anode material in MES-2. On the other hand, graphite felt was used as biocathode as it encourages bacterial attachment owing to its higher surface area. Graphite felt is a porous material with cross-linking fibres that increase the specific surface area of the material. It is also biocompatible; hence, these properties make it a suitable material to be used as a biocathode material. The lack of catalytic activity of graphite felt was evident from the results with lower production rate of acetate (0.65 ± 0.02 mM d^{-1}) in MES-1, where graphite felt was used as the electrode material for both anode and cathode.

In MES-3, nickel foam was used as cathode material but it didn't produce good enough yield, which might be due to the fact that nickel is a known toxic to microorganisms (Macomber & Hausinger 2011) and thus hindered the attachment of microbes on the nickel foam surface. The pH of the catholyte was 5.10 ± 0.2, due to the formation of organic acids in the cathodic chamber. The leaching of nickel from nickel foam under acidic pH is a well-known phenomenon (Xian *et al.* 2017) and thus nickel could have leached from nickel foam in the cathodic chamber. To ascertain the phenomenon of leaching of the nickel from nickel foam in the catholyte of MES-3, the concentration of nickel in the catholyte was measured. During the first few days after the start-up of the MES cell, the concentration of nickel in the catholyte of MES-3 was found less than 1 mg L^{-1}. After that, the concentration of nickel raised to 18 ± 3 mg L^{-1} for the remaining days of operation. Such a high concentration of nickel has a toxic effect on the growth of microbes. All these factors would have suppressed the growth of microbes on nickel foam electrode and thus dampening the performance of MES-3.

Use of nickel foam as anode enhanced the production of organic compound on one hand but on the other hand, the longevity of the anode was sacrificed. Nickel foam anode might have reacted with phosphate ions present in phosphate buffer solution to form nickel phosphate thus corroding the anode. After the completion of the experiment, it was found that the nickel foam was reduced in its size and became brittle thus indicating corrosion. In this

way, the longevity of the anode was hampered. To counteract this phenomenon PBS was replaced with 1 M NiSO$_4$ solution as anolyte in MES-4 with the aim of increasing the durability of the operation. While operating MES-3 using PBS as anolyte the anode remained stable for around 25 days during the operation of the MES. However, while using nickel sulphate solution as anolyte the durability of the nickel foam anode increased and steady performance of MES-4 was observed for around 60 days of operation of the MES. However, the average production of organic compounds using nickel sulphate as anolyte was found to be lower when compared to the MES cells where PBS was used as anolyte.

Current densities consumed by the four MESs used in the study are shown in Figure 2. A maximum current density of 31.1 A m^{-2} was consumed in MES-2, followed by MES-4 (26.12 A m^{-2}). The least value of current density of 10.04 A m^{-2} was demonstrated by MES-3. These results are in line with the other performance parameters that were observed for all the four MESs. As it is evident from Figure 2, current density of MES-2 kept on increasing up to 50 days of operation and stabilized after that. MES-4 took more time to stabilize, but a lesser value of current density was achieved by it when compared with MES-2. During the operation of MES-4, change in the colour of the catholyte from colourless to green was noted, which was due to the transfer of Ni^{2+} ions through Nafion 117 from the anolyte to the catholyte. The nickel ions further reacted with phosphate ions present in the PBS, which was used as catholyte in MES-4 and formed nickel phosphate (Ni$_3$(PO$_4$)$_2$) (Equatopn (5)). The green colour of the catholyte can be attributed to the formation of this
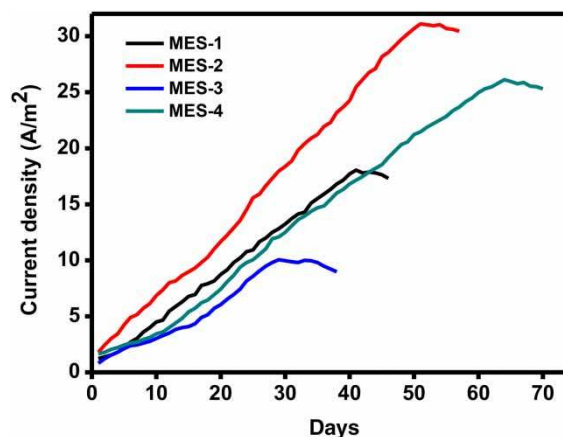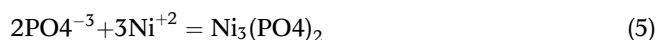

Figure 2 | Current density of the four MESs during the period of operation.

compound.

$$2PO4^{-3} + 3Ni^{+2} = Ni_3(PO4)_2 \qquad (5)$$

The concentration of nickel transferred through the membrane was measured regularly and a concentration of 23 ± 4 mg L^{-1} of nickel was found in the catholyte of MES-4 throughout the duration of operation. Izquierdo-Gil et al. (2012) have also previously reported migration of cations through Nafion 117 used as separator. Thus, the presence on nickel ions, which is toxic to the microbes in the catholyte, might have suppressed the activity of bacteria, thus adversely affecting the performance of the MES. The solution to this problem lies in replacing Nafion 117 membrane with a more selectively permeable membrane that would prevent the migration of nickel ions from the anodic chamber to the cathodic chamber. Another solution could be the use of another suitable anolyte that will not only enhance the performance of MES cell but also would be non-toxic to the microbes if it migrates to the cathodic chamber. However, use of nickel sulphate solution prevented the corrosion of nickel foam anode, thus increasing the longevity of the anode. It is one of the major findings of this study. Hence, it can be suggested that for longer periods of operation, nickel foam anode can be used with 1 M NiSO$_4$ to counter parasitic reactions, but if higher yield is required nickel foam should be replaced with PBS; however in this case the longevity of the anode would have to be compromised.

Cathodic electron efficiency

CEE is the ratio of the amount of products formed to the number of electrons used by the biocatalyst for the formation of these products. Going in line with the other efficiencies used in the scientific field, a higher CEE denotes a better performance of the MES, which emphasizes on the fact that electrotrophs were better able to accept electrons from the cathode and thus increasing the productivity of the system. CEE is directly linked to the rate of synthesis of organic compounds in the cell; hence a higher productivity certainly results in higher CEE. The highest rate of production of acetate was observed for MES-2 (0.81 ± 0.07 mM d^{-1}) and correspondingly the highest CEE for acetate was also observed for MES-2 ($68.81 \pm 3.3\%$) (Table 2). Bajracharya et al. (2015) also obtained similar CEE (40–50%) while using mixed culture and PBS as electrolyte with NaHCO$_3$ as carbon source. Nevin et al. (2010) reported

a higher CEE of $86 \pm 21\%$ using pure culture of *Sporomusa ovata*. Zaybak et al. (2013) reported a lower CEE of $35.2 \pm 4.4\%$, which could be due to a lesser production rate obtained in their study (0.05 mM d^{-1}) probably due to a lower imposed potential of -0.6 V vs Ag/AgCl electrode, which is quite low when compared to the other reported values. Jourdin et al. (2014) also reported a higher CEE using mixed culture from pond sediment and sludge. They used a higher negative imposed potential of -1.05 V vs SHE that could be the underlying fact for such a higher CEE when compared to this study.

CRE and production of additional compounds

CRE is the measure of the ratio of the moles of carbon generated in the products to the moles of carbon used by the biocatalyst from the feed biogas. Bacteria in MES could only use CO$_2$ from the biogas containing a mixture of other gases to reduce it to other organic compounds. They could also use carbon dioxide to generate methane by the process of methanogenesis, which would, in one way decrease the yield of organic compounds but on other hand would enhance the methane content of the biogas. As the aim of the study was to increase the yield of organic compounds so methanogenesis suppression was intended by maintaining a pH of 5.2 in the cathodic chamber. The inlet biogas, which was collected from a UASB reactor, was consisting of methane ($51 \pm 4\%$), carbon dioxide ($36 \pm 2\%$), ammonia ($4 \pm 1\%$) and a mixture of other gases like nitrogen, hydrogen, hydrogen sulphide etc. As explained earlier CRE is also directly affected by the rate of production of the multi-carbon organic compounds. Hence, a higher rate of production definitely yields higher CRE when all the other parameters remain constant. The composition of cathode off gas was also determined and it is presented in Table 3. The CRE of the MES used in this study are also reported in Table 3 and from the same it is evident that
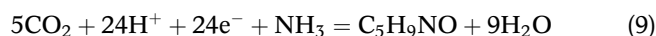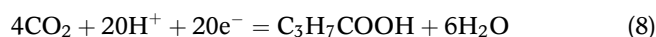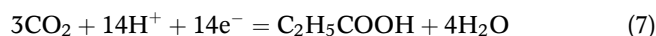
Table 3 | Cathode off gas composition and CRE of the MES cells

	Methane (%)	Carbon dioxide (%)	Ammonia (%)	CRE (%)	Electrical energy required (kWh mol^{-1})
MES-1	53 ± 5	23 ± 3	<1	13.41 ± 3.9	4.14
MES-2	56 ± 3	20 ± 2	<1	17.03 ± 2.4	3.24
MES-3	52 ± 4	32 ± 4	<1	4.21 ± 1.4	13.07
MES-4	53 ± 4	22 ± 3	<1	14.19 ± 2.0	3.88

Note – Rest of the cathode off gas consisted of nitrogen, hydrogen, hydrogen sulphide etc.

MES-2 has the highest CRE, which also corresponds to the fact that its rate of production of organic compounds was also the highest.

As mentioned earlier acetate was the most widely reported product of MES using CO_2 as feed. With acetate, isobutyrate, propionate and 2-piperidinone was also obtained in the catholyte but their yields were less as compared to acetate. Traces of methyl derivatives of these volatile fatty acids were also found in the catholyte, which was formed due to the presence of methane in the feed biogas. 2-piperidinone found in the catholyte is aliphatic heteromonocyclic organic compound that is used as building block and chemical reagent for the synthesis of various pharmaceuticals products. The biogas was collected from a UASB reactor and it contained ammonia. Ammonia can react with carbon dioxide in the presence of biocatalysts to form 2-piperidinone following Equation (9). From the reactions (6) to (9) shown below it is evident that acetate requires the least number of electrons (8) to be produced from CO_2 and 2-piperidinone requires the maximum (24). Thus, the rate of production of acetate was also the highest when compared to the other products.

$$2CO_2 + 8H^+ + 8e^- = CH_3COOH + 2H_2O \qquad (6)$$

$$3CO_2 + 14H^+ + 14e^- = C_2H_5COOH + 4H_2O \qquad (7)$$

$$4CO_2 + 20H^+ + 20e^- = C_3H_7COOH + 6H_2O \qquad (8)$$

$$5CO_2 + 24H^+ + 24e^- + NH_3 = C_5H_9NO + 9H_2O \qquad (9)$$

Electrical energy required

It is very important to find out the economic feasibility of the products formed to compete with the other already established process of production of the same. As electrical energy drives the process of MES, so the estimation of electrical energy required per unit production of these chemicals is a key parameter that gives an idea about the economic feasibility of the process. The rate of production again governs this parameter, as it was the case for CEE and CRE. However, in this case, rate of production affects the electrical energy required indirectly i.e. higher the productivity, lesser is the electrical energy required. From Table 2, it is obvious that MES-3 showed the least rate of production of all the compounds thus increasing the electrical energy required for the production ($13.07 \, kWh \, mol^{-1}$) of these compounds, which was calculated using Equation (4). On the other hand, MES-2 produced the highest rate of production of all the compounds, thus leading to a lesser electrical energy required for the production of unit moles of these organic compounds.

Cyclic voltammetry

CV of abiotic graphite felt did not show any prominent reduction and oxidation peaks but on the other hand, biotic graphite felt shows a steep oxidation peak at a potential of around 0.2 V vs Ag/AgCl (Figure 3). This peak could be the oxidation peak of the previously synthesized acetate, which was consumed by the microbes during the analysis. Marshall *et al.* (2012) also reported similar voltammograms with abiotic and biotic cathodes, where biocathode had a
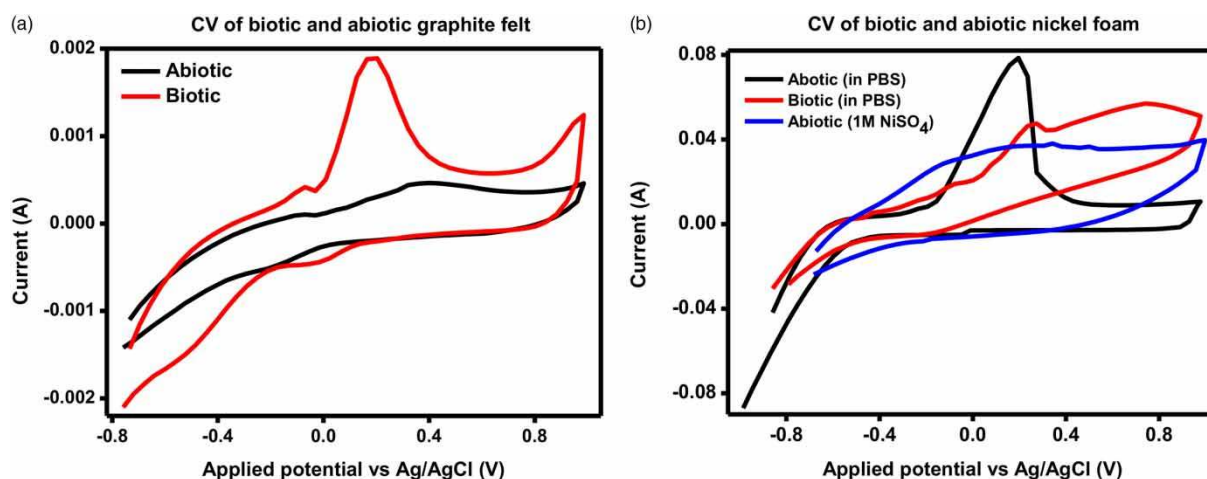

Figure 3 | Cyclic voltammograms of (a) graphite felt and (b) nickel foam under different conditions.

reduction peak at −0.5 V and no noticeable peak was observed for abiotic cathode. Two, not so prominent, reduction peaks were observed at potential of +0.1 V and −0.5 V vs Ag/AgCl, respectively. The second reduction peak may correspond to the reduction of CO_2 by the microbes leading to the production of multi-carbon organic compounds. Abiotic nickel foam in phosphate buffer solution showed a steep peak at a potential of around +0.2 V vs Ag/AgCl electrode, which signifies the reaction of phosphate with nickel forming nickel phosphate. As mentioned above, nickel foam did not support the growth of biofilm on it so distinct oxidation and reduction peaks were not visible on the CV of biotic nickel foam. Abiotic nickel foam in 1 M $NiSO_4$ solution also did not show any differentiable peaks, thus emphasizing on the fact that nickel foam can be used as anode with 1 M $NiSO_4$ solution as anolyte without corroding it. Nickel foam is suitable to be used as anode material in MES but the use of PBS as anolyte along with nickel foam is not advisable as electrode as it can be corroded, thus affecting the longevity of the performance of MES.

Way forward to the synthesis of acetate and isobutyrate in MES

Currently abiotic processes like carbonylation of methanol synthesize 75% of acetate used by industries and the rest is synthesized using biological route like fermentation of ethanol (Cheung *et al.* 2011). These synthetic processes involve the use of costly catalysts, which increases the cost of acetate. Biological processes are relatively cheaper but the rate of production for these processes is very low, which limits the application of biotic processes for the production of acetate. On the other hand, isobutyrate is mainly produced by the fermentation of starch in the presence of calcium carbonate, which helps in neutralizing the acids formed during the reaction. During biological production of butyrate many by-products are formed which degrades the quality of final product. Purification of butyrate involves huge capital investments, which further increases the cost of the purified product. 2-piperidinone, which was also synthesized from biogas, can be economically produced using MES thus decreasing the cost of production of various pharmaceutical products, where the same is used as precursor. Propionic acid is widely used in food industries as antifungal agent and it is generally prepared using various petrochemical routes and fermentation processes. However, the yield of these processes is very less, which increases the per unit production cost of the product. Thus, synthesis of propionate using MES can solve this problem and can make the process economically viable.

In the near future if MES is looked as an option to replace these traditional technologies for the production of organic compounds, then the production rates of MES need considerable improvements. In addition, the cost of separation of various products formed is a major concern related to the field scale application of MES. Noble technique of implementing MES in three-chambered reactor for simultaneous production and separation of the products was demonstrated by Gildemyn *et al.* (2015). The raw material used in the case of MES is apparently cheaper thus minimizing the bulk of the production cost. MES does not involve the use of any additional chemicals for the production of organic compounds; but on the other hand, reactor fabrication and the cost of materials used during its fabrication add up to the final cost of products formed. Therefore, the production rate of MES should be raised high enough to reduce per unit cost of the final products.

CONCLUSIONS

Electrotrophic bacteria were able to utilize CO_2 present in biogas to produce organic acids in a MES. This way by using MES, CO_2 concentration in the biogas could be reduced. Hence, nickel foam as an anode, 1 M $NiSO_4$ solution as anolyte, graphite felt as cathode, phosphate buffer solution as catholyte was observed to be the best possible configuration for MES. A maximum of 44% reduction in percentage of carbon dioxide in biogas was obtained with the yield of organic compounds like acetate (0.81 ± 0.07 mM d^{-1}), isobutyrate (0.63 ± 0.02 mM d^{-1}), propionate (0.44 ± 0.03 mM d^{-1}), 2-piperidinone (0.53 ± 0.03 mM d^{-1}). Use of MES for reduction of CO_2 content in biogas generated in small-scale wastewater treatment plant in this way will pave a new dimension and encourage users to prefer anaerobic treatment over aerobic treatment by providing way out for reuse of CO_2 present in biogas.

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Uncorrected Proof

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