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Value added product recovery and carbon dioxide sequestration from biogas using microbial electrosynthesis

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Issues like global warming and the associated climate change demand alternative green biofuel in place of the fossil fuel. Biogas, which mainly contains carbon dioxide and methane, is produced by anaerobic degradation of organic matter. Microbial electrosynthesis (MES), a novel type of bioelectrochemical systems (BES), can be used to improve the calorific value of biogas, and also to reduce the CO_2 content in the biogas, and thus increase the percentage of methane present in the biogas. In this study, MES has been used to sequester carbon dioxide present in biogas to produce electro-biocommodities like acetate, isobutyrate, etc. The biogas generated from an up-flow anaerobic sludge blanket (UASB) reactor treating sewage was fed into the cathodic chamber of MES cell, which consisted of carbon felt as a biocathode poised at –0.9 V *vs.* SHE. The abiotic anode was also made up of carbon felt and phosphate buffer solution was used as anolyte. The electrotrophic microbiome present on the cathode produced acetate (52.4 mM m^{-2} d^{-1}), isobutyrate (36.2 mM m^{-2} d^{-1}), propionate (41.6 mM m^{-2} d^{-1}), 2-piperidinone (26.7 mM m^{-2} d^{-1}) and traces of methyl derivatives of these compounds. Thus, it demonstrated successful CO_2 sequestration from the biogas and synthesized multi-carbon organic compounds and in turn produced biogas with higher methane content in it.

Keywords: Acetate production, Biocathode, Bioelectrochemical system, Isobutyrate, Methane, Microbiome, UASB reactor

Microbial electrosynthesis (MES) is a type of bioelectrochemical system (BES) that deals with the production of extracellular multi-carbon organic compounds from the reduction of carbon dioxide using the catalytic activity of biocatalyst under the influence of imposed potential[1]. The microbiome present in the cathodic chamber derives electrons from polarised cathodes and use CO_2 as an electron acceptor to synthesise various value added organic compounds. Generally, in MES biocathodes are used as working electrode with abiotic anode as the counter electrode to facilitate the generation of proton motive force and electrons, which are transferred to the cathodic chamber through the proton exchange membrane (PEM) and external circuit, respectively[2]. In MES cell comprising of abiotic anodes, water splitting takes place, which produces electrons and protons. An external voltage is applied to the electrochemical cell, which draws the electrons from the anodic chamber through the external circuit to the biocathode. If a renewable source of energy is used to drive the MES, then the process can be termed as artificial photosynthesis[3]. The use of bi-bioelectrodes in MES is also well documented in which anodic consortia oxidizes various inorganic species like sulphide to generate electrons[4]. The electrotrophic bacteria present in the cathodic chamber are mainly acetogenic in nature that utilises CO_2 to generate electrobiocommodities[5] by following Wood-Ljungdahl pathway[6]. The use of CO_2 as feedstock for bioelectrochemical production of commodity chemicals has 2-fold advantages, namely it helps in the sequestration of CO_2, which has major implications towards climate change due to its increased contribution towards global warming. Also, CO_2 is easily available in the atmosphere, thus it can be captured with relative ease in pure form and can be further used in MES for production of biofuels[7]. However on the downside, CO_2 is thermodynamically very stable thus requires an external energy source to reduce it to valuable by-products.

Till date, acetate has been most commonly reported to be produced by MES. However, various other organic compounds like ethanol[1], butyrate[8] and methane[9] have also been widely reported to be synthesised using mixed consortia in MES. Further reduction of acetate can lead to the production of biofuels in the form of ethanol and butanol. The

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problem associated with the intermittent nature of renewable sources of energy can be solved by storing the energy in carbon-carbon bonds of biofuels, which can be used at an appropriate time. The pH of catholyte greatly affects the performance of MES by governing the proton motive force, which is directly related to the yield of MES. Till now, researchers have found that acidic pH positively affects the yield of organic compounds from MES[10,11]. However, the performance of MES in slightly alkaline pH still requires further research effort to establish it. The possibility of synthesising various reduced products using MES cell at alkaline pH was looked upon in this research.

Biogas, which mainly contains carbon dioxide and methane, is produced by anaerobic degradation of organic matter. Anaerobic wastewater treatment technologies like up-flow anaerobic sludge blanket (UASB) reactor and anaerobic filters are popularly used for sewage treatment and biogas production at many places. The carbon dioxide fraction reduces the calorific value of biogas. Hence, to improve the calorific value of biogas, MES can be used to reduce the CO_2 content in the biogas to the value-added products, and thus increasing the percentage of methane present in the biogas. CO_2 reduction in MES can also produce CH_4 by the process of electromethanogenesis[12]. This would also enhance the quality of biogas by increasing the percentage of methane in it. On the other hand, electro-biocommodities synthesised by MES using biogas will also be of special interest as this process can cost-effectively produce them. Keeping this in mind, this study was focused on using biogas generated from an UASB reactor as feedstock for MES to synthesise multi-carbon organic compounds and also to increase the methane content of the biogas. Effect of different catholyte pH 4, 5, 6, 7 and 8 on the performance of MES was evaluated.

Materials and Methods

Bioelectrochemical reactor setup and operation

Total five identical MES cells were used during this study, each consisting of an anodic and a cathodic chamber separated by Nafion 117 membrane (DuPont, USA) with an area of 4.0 cm^2, which was used as a proton exchange membrane (PEM). The total effective volume of each cell was 50 mL, divided equally among the two chambers. The biotic cathode was made up of carbon felt, and stainless steel wires were used to connect the cathode with a potentiostat by which a potential of –0.9 V *vs*. SHE was imposed on it. The abiotic anode was also made up of carbon felt. Phosphate buffer solution of pH 7 was used as anolyte, and Lauryl tryptose (Himedia, India) broth was used as catholyte to provide sufficient nutrients for the growth of the microbiome. The cathodic pH of the cells was regularly monitored and maintained at a certain value. The cells were named as MES-1, MES-2, MES-3, MES-4, and MES-5 operated with catholyte pH of 4, 5, 6, 7 and 8, respectively. The cathodic chambers were hermetically sealed to maintain anaerobic condition. A sample of catholyte was collected daily, and it was analysed in GC to find the concentration of organic compounds formed. Biogas was collected from an UASB reactor located at IIT Kharagpur, which was used for the treatment of sewage, and this biogas was supplied continuously to the cathodic chamber of these five MES cells at the rate of 3.0 L d^{-1}. A potentiostat was used to impose a potential of –0.9 V *vs*. SHE on the cathode by using three electrode system consisting of calomel electrode (+0.241 V *vs*. SHE, Bioanalytical Systems Inc., USA) as a reference electrode, cathode as working electrode and anode as the counter electrode. All the electrode potentials reported in this article are *vs*. SHE unless stated otherwise. The pH of the catholyte of the MES cells was measured regularly, and it was maintained at a particular pH by applying 1 M NaOH or 1 M HCl solution depending on the cells designation as mentioned above. All the cells were operated till stable values of Coulombic efficiency (CE), current density and acetate production were obtained, which varied for different cells. The operating temperature of all the cells was maintained at 30±2°C.

Acclimatization of biocathode

Mixed anaerobic sewage sludge was used as inoculum for the biocathode of MES, which was collected from septic tank situated in IIT, Kharagpur, India. The sludge was kept in anaerobic conditions at a temperature of 30°C and was cultured for around two weeks using Lauryl tryptose broth, which is a suitable medium to be used for the culture of acetogens. After this, 10 mL of this sludge was transferred to the cathodic chamber of the MES cells. During the acclimatisation of the biocatalyst on the cathode surface, Lauryl tryptose broth was used but without lactose in it to switch the growth phase from

heterotrophic to autotrophic phase. Also during this phase biogas, which was collected from an UASB reactor was supplied continuously to the cathodic chamber at the rate of 2 mL min^{-1}. Such a lower rate of biogas purging was used to prevent the dislodging of newly formed biofilm on the cathode surface. Simultaneously, an imposed potential was also applied, which began from –0.1 V and went up to –0.8 V in steps. The cathodic potential was decreased daily by 0.1 V to make the electroactive microbes acclimated to electric current. During the growth phase, primarily CO_2 present in biogas was the only carbon source for the microbes. The microbes were allowed to grow by consuming biogas for a period of around one week, and after this period, steady production of acetate was observed. This proved that the biocatalysts were completely acclimatised to the biogas containing environment and the MES cells were ready for further experiments.

Polarization test

Theoretically, H_2 evolution at biocathode takes place at a potential of –0.409 V and at –0.290 V acetate is produced from CO_2 both at pH of 7.0[13]. However, due to various inherent overpotential losses present in the bio-electrochemical system, a more negative potential has to be imposed for the same purpose. As mentioned above, hydrogen-mediated electron transfer from the cathode to the microbes is seen to be dominating in MES rather than direct electron transfer. Hence, to determine the potential for H_2 evolution, which would facilitate electron transfer in between the cathode and biocatalyst, polarisation tests were carried out after the acclimatisation of the biocathode. During this test, the biocathodes were imposed with a potential ranging from –0.4 V to –1.0 V and correspondingly hydrogen production at different imposed potential was measured. The pH of the catholyte was maintained at 7.0 to minimise the effect of pH on this test. Biogas was purged continuously into the MES cells, and the catholyte was changed after each sweep. During this test, the current density at different applied potential was also measured, and when it stabilised, a sample of cathodic chamber off-gas was collected and was analysed to find hydrogen content. Two sweeps of the voltage were carried out, one starting from –0.4 V to –1.0 V and other from –1.0 V to –0.4 V. The average values of hydrogen evolution and current density obtained at a fixed reduction potential during both the sweeps were reported and used for calculation.

Chemical analysis

The concentration of VFA's in the catholyte was measured regularly using gas chromatography (GC). To quantify the concentration of VFA's, one mL filtrate sample was collected in a 1.5 mL GC vial and it was acidified with 100 µL of 3% H_3PO_4 before being analysed by a GC (Agilent Technologies GC-7890A, Penang, Malaysia) with flame ionization detector (FID) and DB-FFAP column (30 m × 0.25 mm × 0.25 mm). About 1.0 µL of the prepared sample was injected into the GC using nitrogen as the carrier gas with a flux of 30 mL min^{-1}. The injection port and the detector were maintained at 200 and 250°C, respectively. The GC oven was programmed to begin at 120°C for 2 min, next to increase at 13°C min^{-1} to 200°C, and then to hold at 200°C for an additional 2 min[14]. The measured VFAs constituent were expressed in mg L^{-1}.

Gas analysis

To find the amount of hydrogen gas produced during polarisation test cathodic chamber off-gas samples were collected during the test and were analysed using GC (Agilent Technologies GC-7890A, Penang, Malaysia). The sample was collected in 1.5 mL GC vial by water displacement method and analysed using TCD and molecular sieve column. One µL of the gas was injected into the system using GC syringe using nitrogen as the carrier gas with a flux of 31 cm sec^{-1} and at a flow rate of 6 mL min^{-1}. The injector and the TCD were both maintained at 130 °C initially. The initial column temperature was kept at 40°C, and the oven was programmed to hold this temperature for four minutes followed by a ramp of 10°C min^{-1} up to 70°C with a hold time of 5 min. The hydrogen content of the sample was estimated by comparing the areas obtained by analysing the samples and standard of hydrogen.

The biogas that was used as a feedstock was also analysed to find out its composition. The biogas was collected in a 1.5 mL GC vial by water displacement method, and it was examined using FID and molecular sieve column. One µL of the gas was injected into the GC (Agilent Technologies GC-7890A, Penang, Malaysia) using GC syringe. Nitrogen was used as the carrier gas with a flux of 35 cm s^{-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 min followed by a ramp of

20°C min^{-1} until 65°C with a hold time of 4 min. 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 cathodic chamber off gas collected from the MES cells.

Calculations

The production of various chemicals (in mol) at any time *t* was calculated as per Eq. (1).

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

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 $C_{pro,t0}$ 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).[2]

Coulombic efficiency, which is also known as cathodic electron efficiency or current efficiency, is the efficiency of capturing the electrons by the microorganisms from the electric currents to form products. The CE was calculated using Eq. (2)[2].

$$CE = \frac{n_{pro,t} \times f_{pro} \times F}{\int_{t_0}^{t} I \, dt} \times 100 \quad \ldots (2)$$

where, *CE* is the Coulombic efficiency (%), f_{pro} represents molar conversion factor (8 electron equivalent for acetate), *F* is Faraday's constant (96485 C mol^{-1}), *I* is the current supplied[2]. CE was calculated individually for all the products, and it was then added to get the total CE for the system.

Carbon fixing or carbon recovery efficiency (CRE) indicates the percentage of carbon used from biogas by the microbes to form products. Carbon recovery efficiency was calculated using the Eq. (3)[2].

$$CRE = \frac{n_{pro,t} \times f_{c,pro}}{n_{gas}} \times 100 \quad \ldots (3)$$

where, *CRE* is the carbon recovery efficiency (%), $f_{c,pro}$ 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[2]. CRE was also calculated for individual products, 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 an estimate of the amount of energy required to synthesise a unit of the product. It was calculated using Eq. (4).

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

where, $J_{pro,t}$ is the amount of electrical energy required to produce a unit mole of the product (kWh mol^{-1}), E_{cell} is the actual cell voltage during the operation of MES[2]. The total electrical energy required is reported as the sum of the energy required for each product as calculated from Eq. 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.

Hydrogen production yield (HPY) can be defined as the amount of hydrogen produced per unit of an applied potential. It was calculated using Eq. 5.

$$HPY = \frac{Hydrogen\ produced}{|Potential\ applied|} \quad \ldots (5)$$

where, *hydrogen produced* is in L d^{-1}, and *potential applied* is in Volts.

Results and Discussion

Polarization test of biocathode

Polarization tests were conducted to find the potential at which hydrogen evolution takes place in the MES cells because electron transfer from cathodes to microbes in MES generally mediated through hydrogen[15]. Theoretically, H_2 evolution using biocathode takes place at an imposed potential of –0.409 V. However, due to various overpotential losses, potential that is practically more negative has to be applied for the same. It is needless to mention that with more negative applied potential, hydrogen production also increases. But more negative imposed potential call for more operating costs. Also, with the increase in imposed potential various other parasitic reactions could start taking place, which may hinder the hydrogen evolution. These reactions would not only dampen hydrogen production but also disturb the purity of products. Thus, an optimum potential is to be determined that not only enhances hydrogen production but also keep the cost within limits. To find this potential, a ratio of hydrogen production to imposed potential was calculated for every potential. This was termed as hydrogen production per unit of imposed potential or hydrogen production yield (HPY) and the potential at which highest value of HPY achieved was considered as the optimum potential for hydrogen evolution. As mentioned earlier, during polarisation study two sweeps were

carried out one from –0.4 V to –1.0 V and the other from –1.0 V to –0.4 V. Fig. 1 represents current density acquired at different applied potentials. The average values during backward and forward sweep were considered while plotting this graph.

From the Fig. 1, it is evident that current density increases with the decrease in (more negative) applied potential. The highest current density that was delivered was ca. 65 A/m^2 at an imposed potential of –1.0 V. Comparable value of current density of ca. 30 A/m^2 was also obtained at a polarised potential of –0.4 V by Soussan *et al.*[16]. It is worth mentioning that current density mainly depends on the reactor based parameters like reactor design and configuration and also on the biocathode's properties.

Table 1 presents the HPY at various polarised potentials. The HPY values reported in Table 1 are conclusive enough to point out that highest HPY (20.56±0.39) was obtained at an applied potential of –0.9 V. During the test, hydrogen production was observed at all the potential, but it was very low at –0.4 V as the minimum theoretical potential for hydrogen evolution is very close to this value. Also during the test, the current density at more negative polarised potential stabilised quickly, when compared to higher values, indicating more stable performance at more negative imposed potential. Considering all these observations, –0.9 V was chosen as the applied potential for MES to synthesise organic compounds from biogas.

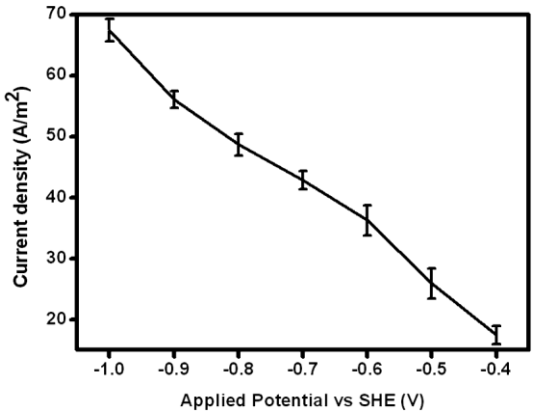

Fig. 1—Current density obtained at different applied potentials

Table 1—Hydrogen production yield at different imposed potentials

Imposed Potential (V)	Hydrogen production yield (HPY)
–0.4	6.56±0.44
–0.5	18.00±1.41
–0.6	18.33±0.59
–0.7	19.64±1.01
–0.8	20.47±0.22
–0.9	20.56±0.39
–1.0	20.13±0.18

Effect of pH on the performance of MES

Generally, acetogenic bacteria seem to dominate the MES for production of organic compounds from CO_2. Acetogens can grow and reproduce in pH ranging 5-8[6]. However in MES, CO_2 is bubbled into the catholyte making the pH acidic. Hence, the effect of catholyte pH ranging 4-8 on the performance of MES was evaluated. For the determination of performance at a particular pH, various parameters like CE, CRE, yield of acetate and maximum current density were observed for the different MES cells (Table 2).

It is evident from the Table 2 that, MES-2 operated with catholyte pH of 5.0 outperformed all the other MES setup. Acetate production normalised to cathode surface area for MES-2 reached an average value of 52.43±3.2 mM m^{-2} d^{-1}, highest among all the other MES. Not only acetate, but other multi-carbon organic compounds were also produced in sufficient quantity in MES-2. It has also been reported that a pH of 5.2 is best suited for electrosynthesis of organic compounds by CO_2 reduction[17]. Generally in MES, pH of the catholyte is maintained around 7 as it is well known that near neutral pH is best for the growth of microbes especially acetogens. But this does not always guarantee the best performance in terms of organic compound production. Various other factors govern the electrosynthesis of these compounds, and one major factor is the proton motive force generated by the transfer of protons from the anodic chamber to the cathodic chamber through the membrane.

Table 2—Performance comparison of the MES

MES used	Catholyte pH	CE (%)	CRE (%)	Acetate production (mM m^{-2} d^{-1})	Maximum Current density (A m^{-2})
MES-1	4	31.53 ± 4.2	16.16 ± 3.8	38.18 ± 2.1	42.23
MES-2	5	62.12 ± 3.6	21.95 ± 2.7	52.43 ± 3.2	65.65
MES-3	6	49.68 ± 3.3	20.65 ± 3.2	43.69 ± 3.1	44.72
MES-4	7	44.95 ± 3.1	18.39 ± 2.4	40.15 ± 2.6	36.67
MES-5	8	33.76 ± 2.6	17.65 ± 3.5	35.25 ± 4.6	31.72

Eq. 6 represents the basic electrochemical reaction for the formation of acetate from CO_2. It can be easily concluded from the equation that an acidic pH favours acetate production. However, very less acidic pH will render the biocatalyst inactive; hence, an optimum pH will not only increase the yield, but it will also keep the biocatalysts electroactive for a longer period. According to the results obtained from this study, it is clear that the optimum pH for the MES to synthesise organic compounds is 5.0.

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

Going into further details of the study, MES-2 stabilized to an optimum yield at a quite smaller time of about 21 days. Hence, it can also be hypothesized that MES with cathodic pH of 5 requires a less start-up time. In comparison, all the other MES took approximately 45 days to stabilize, more than twice as compared to MES-2. The highest acetate titer concentration that was obtained in MES-2 was ca. 11 g L^{-1}. Similar titer concentration of 10.5 g L^{-1} of acetate was also obtained by Marshall *et al.*[18] using enriched brewery wastewater sludge. The under-performance of MES-1 can be proved by the fact that acetogens generally do not tend to be active below the pH 5[6]. At a higher pH of 8, both the factors, namely proton motive force and activity of acetogens affected the performance of the setup negatively, which is easily visible by the least production rate of acetate.

Feed gas and cathodic chamber off gas analysis

As mentioned earlier biogas was collected from an UASB reactor and it was supplied continuously to the cathodic chamber of the cells at the rate of 3.0 L day^{-1}. Gas chromatographic analysis was carried out for both the inlet gas and the outlet gas to determine the composition of the same. The fed gas contained CH_4 (66±5%), CO_2 (25±2%), ammonia (4±1%) and few other gases like H_2S, N_2, etc. in negligible concentrations (<1%). The constituents of cathodic off gas with their concentration are presented in Table 3.

It is evident from data mentioned above that methanogenesis took place in all the 5 MES cells, with highest in MES-2 and the lowest in MES-1. Methanogenesis helped in increasing the methane content of the biogas, thus increasing its calorific value. But on the other hand, it also consumed electrons, which could have been used by the microbes to synthesize organic compounds. Thus, methanogenesis in a way decreases the productivity of MES. If the primary objective is the yield of organic compounds, then suppressor of methanogenesis should be targeted by using chemicals like sodium 2-bromoethanesulfonate[19]. Enhancing methane percentage in biogas and simultaneous organic compounds recovery can be achieved by using mixed culture containing both acetogenic and electro-methanogenic microbiome, which was the case in this study. Methanogens are ubiquitous in MES related studies and are frequently reported by various researchers[9]. Maximum methane production was observed in MES-2, where the pH was maintained at 5.0. In this cell, acetate production was highest, and thus methanogens received more substrate when compared to the other cells, and therefore higher methane production took place. Conversely, for MES-1, acetate production was the least and simultaneously methane produced was also the least. Thus, pH is a major factor which also affects methanogenesis in MES but indirectly.

Table 3—Cathodic chamber off gas composition of different MES

	Methane (%)	Carbon dioxide (%)	Ammonia (%)
MES-1	67.23±5.1	23.56±4.5	<1
MES-2	74.42±6.3	18.03±3.2	<1
MES-3	71.25±5.7	19.20±3.1	<1
MES-4	70.05±4.8	19.86±3.7	<1
MES-5	68.72±5.3	21.32±3.5	<1

Carbon dioxide was consumed the most by the biocathode of MES-2 and the least by MES-1. This can be elucidated by the fact that the biocathode of MES-2 was most active in terms of bioconversion of CO_2 to acetate and least for MES-1. Hence, the active biofilm consumed more CO_2 and converted it into multi-carbon organic compounds. Thus, a lesser concentration of CO_2 in cathodic chamber off-gas was found for MES-2 and on the contrary for MES-1. Ammonia was present in the inlet gas though in small quantity but it was used by the mixed consortia to produce 2-piperidinone. Thus, very less quantity of ammonia was found in the cathodic off-gas (<1%).

Production of electro-biocommodities

Along with the production of acetate, microbial electrosynthesis of other multi-carbon organic compounds like propionate, isobutyrate and 2-piperidinone also took place in the cells. As methane was present in the feed gas, methyl derivatives of these compounds like methyl propionate were also found in the catholyte but at very low concentration (<10 mg L^{-1}). The reactions for the formation of these compounds by CO_2 reduction are as given in Eq. 7 through 10.

$$2CO_2 + 8H^+ + 8e^- = CH_3COOH + 2H_2O \quad \ldots (7)$$

$$3CO_2 + 14H^+ + 14e^- = C_2H_5COOH + 4H_2O \quad \ldots (8)$$

$$4CO_2 + 20H^+ + 20e^- = C_3H_7COOH + 6H_2O \quad \ldots (9)$$

$$5CO_2 + 24H^+ + 24e^- + NH_3 = C_5H_9NO + 9H_2O \quad \ldots (10)$$

The bioproduction of butyrate through MES has also been reported previously[8]. The concentration of acetate in the catholyte was always found to be higher when compared to the other organic compounds. These can be explained by the fact that the production of these compounds namely propionate, isobutyrate and 2-piperidinone requires more electrons, 14, 20 and 24, respectively, when compared to acetate (8 electrons). They also require a greater proton motive force for their generation. These two are the major factors which dented the production of these compounds in a higher quantity through MES. The 2-piperidinone is a nitrogen-based compound and it was formed as ammonia was present in the feed biogas. Bioelectrochemical reduction of CO_2 in the presence of NH_3 leads to the formation of 2-piperidinone, which in turn assimilates 24 electrons into the product. The yields of different chemicals in the various cells are reported in Table 4.

During the analysis of the catholyte, trace concentration of ethanol (<4 mg L^{-1}) was also found but not daily. Ganigué *et al*.[8] also reported the production of ethanol through MES at a polarised potential of –0.8 V. Smaller organic molecules like formic acid has also been reported to be produced through MES using biocathodes[20]. The formations of these compounds are basically pH dependent. All these compounds require acidic pH, thus a higher proton motive force for their formation. This fact was also proven by the results obtained in this study. Also, the use of different ion selective membranes affects the yield of these chemicals and the stability of MES[21]. It is evident from Table 4 that, as pH of the catholyte increased, the production of these compounds decreased. Hence, the highest concentrations of these compounds were found in the catholyte of MES-1, where the pH was most acidic (pH 4) and least for MES-5 in which cathodic pH was most alkaline (pH 8).

Table 4—Yield of various organic compound in different set-ups

	Propionate (mM m^{-2} d^{-1})	Isobutyrate (mM m^{-2} d^{-1})	2-piperidinone (mM m^{-2} d^{-1})
MES-1	41.63±3.6	36.24±5.2	25.71±4.3
MES-2	35.36±4.6	33.15±4.6	21.02±5.4
MES-3	28.28±3.2	28.66±3.1	18.45±3.1
MES-4	20.57±5.4	23.25±4.2	12.73±4.3
MES-5	14.37±2.4	10.52±3.6	8.35±2.5

Efficiency of the MES

Coulombic efficiency can be defined as the efficiency of biocatalysts to accept electrons from the cathode and convert them into organic compounds. CE and CRE are directly linked with the yield of organic compounds, which are apparent from the Eq. 2 and Eq. 3. A higher yield basically results in higher CE and CRE. Accordingly, highest CE (62.12±3.6) and CRE (21.95±2.7) were also observed for MES-2. Hence, a higher CE indicates better acceptance of electrons by the biocathodes to reduce carbon dioxide and form valuable products. Using mixed wastewater treatment sludge, CE of 89.5% was reported at an imposed potential of –1.1 V *vs*. Ag/AgCl[22]. Such a high CE was obtained because of the application of more negative imposed potential, which in turn increased the yield of the MES. Marshall *et al*.[9] reported a similar CE of 67% using brewery wastewater sludge. Also, a lesser CE of ca. 50% at an imposed potential of –1.1 V *vs*. Ag/AgCl was also reported, which was due to the presence of methanogens in the mixed inoculum that was proved by the production of methane in the cathodic chamber[2]. Methanogens consumed electrons in the process of electromethanogenesis and thus CE was reduced. With the use of bipolar membrane to buffer pH change, CE up to 89% was achieved recently[23]. The use of reduced graphene oxide as cathode catalyst has also been reported to demonstrate a higher CE of 83% by enhancing the surface area of the electrode, thus improving the thickness of biofilm[24].

MES-2 reached a maximum current density of 65.65 A m^{-2}. Such a high maximum current density using mixed culture has not been reported to the best of our knowledge. Current density as high as 37 A m^{-2} was obtained using novel NanoWeb reticulated vitreous carbon cathode at an applied potential of –1.05 V *vs*. Ag/AgCl[25]. In their study, cathodic pH was maintained at 7.0 and that produced a current density of 37 A m^{-2} and in present study the MES-4, with cathodic pH of 7.0, also attained a similar current density of 36.67 A m^{-2}. Current density as low as 10 A m^{-2} was also reported when mixed wastewater sludge was used as inoculum[2].

Carbon recovery efficiency can be defined as the efficiency of biocatalysts to use carbons from carbon dioxide and simultaneously transfer them to multi-

carbon organic compounds. Again, the highest CRE of (21.95±2.7%) was obtained by MES-2, which basically states that ca. 22% of the carbon from CO_2 has been converted into organic compounds. Highest CRE of 26% was reported by Bajracharya *et al.*[2] where a batch mode of operation was used for MES. It is needless to mention that CRE would increase for batch processes as the feed gas is retained in the cell for a longer period. Therefore, in our case a lesser value of CRE was obtained as biogas was applied to the cathodic chamber in a continuous mode.

Future prospective of bio-production of organic chemicals through MES

The results obtained in this study shows that electro-biocommodities can be synthesised from biogas, but substantial interdisciplinary research has to be carried out before the field scale application of MES is possible. To compete with the conventional techniques used for the production of acetate, titer concentration in MES should be more than 20 g L^{-1} [26]. One of the greatest advantages of biofuel production through MES is the independence on the availability of arable land for the production of biomass. Also, the feedstock used for MES is available in sufficient quantity in the atmosphere. It also solves the problem of storing renewable electrical energy by storing them in carbon-carbon bonds, which can be easily transferred to required location and can be used at an appropriate time without significant losses.

The purity of the products of MES should be targeted as it decreases the separation cost in the downstream processes. However, in this study, purity of value-added product generation was not aimed and on the other hand, the possible bio-electrochemical routes for the production of different organic compounds were explored. These chemicals have several uses in the industries and if production yield of these compounds from CO_2 using MES can be increased by selecting appropriate catalyst then the cost associated with the production of various chemicals, where these chemicals are used as precursors, can be brought down drastically. For example, butyrate is extensively used in food, chemical and pharmaceutical industries as pure acid or in the form of esters as a food additive to increase food fragrance[27]. It also plays a vital role in plastic material and textile manufacturing industries. However, till date the economic aspect related to the MES cells has emerged as the main constraint in scaling up of this novel technology. Researchers need to find cost effective electrode materials, membranes, etc. that will not only cut down the cost but also would enhance the performance of the system. Also, the fixation of CO_2 into these organic compounds is providing the researchers with an added initiative to explore more in this direction.

Conclusion

The effect of cathodic pH on the performance of MES was evaluated and it was observed that pH of 5.0 is the optimal pH for simultaneous acetate production and increasing methane content in biogas through MES. Along with acetate, other organic compounds, such as isobutyrate, propionate and 2-piperidinone were also produced by the biocathodes. However, they were produced optimally at pH of 4.0. The optimal polarizing potential for these MES cells were also determined, and –0.9 V *vs.* SHE was found to be the optimal potential. The study also paves the way for various chemical recovery options through MES. Using this technology, simultaneous carbon sequestration and valuable product recovery can be achieved. It can also be used to increase the methane content of biogas, thus increasing its market value. Though MES is still in an embryonic stage, significant leading-edge research can take this technology to new heights in terms of sustainability and eco-friendliness.

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