Biochemical Engineering Journal 194 (2023) 108896

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Electrochemical synthesis of propionic acid from reduction of ethanol and carbon dioxide at various applied potentials

Narnepati Krishna Chaitanya [a], Akanksha Rajpurohit [a], Pavithra S. Nair [a], Pritha Chatterjee [a,b,*]

[a] Department of Civil Engineering, Indian Institute of Technology Hyderabad, Kandi, Sangareddy, Telangana 502285, India
[b] Department of Climate Change, Indian Institute of Technology Hyderabad, Kandi, Sangareddy, Telangana 502285, India

ARTICLE INFO

Keywords:
Microbial electrosynthesis
Ethanol
Propionic acid
Acetic acid
CO_2 reduction
Imposed potential

ABSTRACT

The synthesis of value-added compounds other than acetate at high rates while utilizing cheap electrode materials is one of the current difficulties faced in microbial electrosynthesis. The present study investigated the production of higher chain volatile fatty acids from the reduction of ethanol and CO_2 using unmodified carbon felt at different negative cathode potentials viz. −0.8, −1.0 and −1.2 V vs Ag/AgCl. Propionic acid was produced as secondary main product after acetic acid. The applied voltage of −1.2 V reported the highest amount of acetic acid with production rates of 949 mg L^{-1} d^{-1} (15.81 mM d^{-1}). The increased cathode potential from −0.8 to −1.2 V enhanced the maximum acetic acid production. The maximum acetic acid production achieved at a voltage of −1.2 V was 1.72 and 1.17 folds higher than MES operation at −0.8 and −1.0 V, respectively. Highest amount of propionic acid with the production rates of 322 mg L^{-1} d^{-1} (4.34 mM d^{-1}) was obtained at −1.0 V, which was 1.20 and 2.12 times higher than the MES operation at −0.8 and −1.2 V, respectively. Scanning electron microscopy revealed dense biofilm and strong attachment of diverse microorganisms on the biocathode.

1. Introduction

The concentration of carbon dioxide (CO_2) in the atmosphere has been steadily rising over the past decades, and it is a major contributor to global warming [1]. Capture of carbon and production of fuels or chemicals from CO_2 is a key strategy to achieve a carbon-neutral economy. One of the most promising processes for reducing CO_2 into methane, volatile fatty acids and alcohols is microbial electrosynthesis (MES) [2,3]. Bio-cathodic microorganisms of electrochemical cells aid in the transfer of electrons during the MES process, which in turn reduces CO_2 into fuels and chemicals [4–6]. Generally, in MES, abiotic generation of oxygen (oxidation) occurs at the anode (Eq. (1)), and reduction of CO_2 or any other compound occurs at the cathode (Eqs. (2)–(5)) [3]. This process requires an external power supply to drive the chemical synthesis.

At anode

$$H2O \rightarrow 2e- + 2H+ + O2; E_0' = -0.82 \text{ V vs SHE} \quad (1)$$

At cathode

$$CO2 + 7H+ + 8e- \rightarrow CH3COO- + 2H2O; E_0' = -0.28 \text{ V vs SHE} \quad (2)$$

$$2H+ + 2e- \rightarrow H2; E_0' = -0.41 \text{ V vs SHE} \quad (3)$$

$$CO_2 + 4\ H_2 \rightarrow CH_3COO^- + H^+ + 2\ H_2O \quad (4)$$

$$CO2 + 8H+ + 8e- \rightarrow CH4 + 2H2O; E_0' = -0.24 \text{ V vs SHE} \quad (5)$$

Nevin et al. [7] investigated the proof of principle of MES from CO_2 using pure culture (*Sporomusa ovata*) as biocatalyst. Acetate and 2-oxobutyrate were produced using a negatively poised graphite cathode; at a potential of - 0.4 V vs SHE. After this study, researchers employed mixed cultures from different anaerobic sources for electroreduction of CO_2 to methane and value-added chemicals at the biocathode [8,9]. Further improvements in production rates of VFAs were carried out by using electrodes of different materials including chemically modified carbon-based electrodes, like carbon rod or stick, carbon felt, carbon cloth, reticulated vitreous carbon (RVC), etc. [9–13].

Even after numerous research on MES only the primary organic compound acetate has been observed as a major product of CO_2 reduction [14]. Since, acetate (C2) has a low market value compared to the medium chain carboxylic acids (C4-C6) [15,16]; researchers should now shift their focus on expanding the product spectrum.

First instance of chain elongation from CO_2 was demonstrated by

* Corresponding author at: Department of Civil Engineering, Indian Institute of Technology Hyderabad, Kandi, Sangareddy, Telangana 502285, India.
E-mail address: Pritha@ce.iith.ac.in (P. Chatterjee).

https://doi.org/10.1016/j.bej.2023.108896
Received 23 December 2022; Received in revised form 19 January 2023; Accepted 13 March 2023
Available online 15 March 2023

Ganigué et al. [17] in which butyrate was obtained using mixed culture in MES, at a rate of 0.04 g L^{-1} d^{-1}. Following this, another study attempted to achieve butyrate in a tubular MES configuration [14]. They reported that butyrate was the major product, and the production rate was 0.16 g L^{-1} d^{-1}, when MES was operated at an initial pH close to 5.0 and hydrogen partial pressure greater than 1 atm. Furthermore, Vassilev et al. [18] reported the production of medium chain carboxylic acids; 0.042 g L^{-1} d^{-1} of isobutyrate, 0.072 g L^{-1} d^{-1} of nC4 and 0.038 g L^{-1} d^{-1} of nC6 and corresponding alcohols such as isobutanol, n-butanol, and n-hexanol which were also produced in trace quantities. However, acetate was still the predominant product synthesized at a rate of 0.141 g L^{-1} d^{-1} [18]. Although medium chain fatty acids are obtained in MES from CO_2, the low product titre and production rates are a hindrance to achieve sustainability.

On the other hand, some researchers have used additional electron donor such as ethanol, acetate etc. [19,20]. In this context, a long-term continuous bioelectrochemical chain elongation from CO_2 and acetate utilising a mixed microbial culture was reported by Raes et al. [20]. In this study, the maximum production rate of butyrate was around 0.54 g L^{-1} d^{-1} at an applied cathodic current of 9.3 A m^{-2} [20]. Additionally, it was reported that caproate production from acetate and CO_2 was observed but at very low rates (0.07–0.18 g L^{-1} d^{-1}). Moreover, usage of ethanol alone as electron donor in cathode chamber of MES is not sufficient to produce MCCAs, because the chain elongating bacteria like *Clostridium kluyveri*, require inorganic carbon or CO_2 and organic carbon for their cell synthesis [21,22]. Recently, Jiang et al. [19] demonstrated caproate synthesis from ethanol and CO_2 in MES. They reported the final concentration of caproate as 7.66 g L^{-1} and a maximum production rate of 2.41 g L^{-1} d^{-1}.

Furthermore, by increasing the negative potential, more electrons could be provided to the bacteria which in turn can increase the production of organic molecules [23]. However, as there is a cost associated with each unit of electrical energy supplied to MES, the operating cost rises with the increase in applied potential. This highlights the need for electrode potential tuning to effectively produce organic compounds using MES. The productivity of the process can be significantly increased by optimising this parameter, which controls the bioelectrochemical processes occurring in the cathodic chamber of a MES [24].

From this perspective, our current study is aimed to understand the influence of applied potential in MES under optimal conditions obtained from previous experiments. In our previous mixed culture study, design of experiments was carried out in serum bottles for optimizing the operating conditions such as pH, ethanol concentration, and hydrogen partial pressure (data not shown). The optimal values viz. pH 7.15, ethanol concentration 2318.7 mg L^{-1} obtained in the serum bottles experiments were chosen for the present batch MES study. The hypothesis of this study is that ethanol addition along with CO_2 in the presence of electroactive-bacteria under different operating voltages can enhance the production rates of VFA and chain elongation above acetate [25].

2. Materials and methods

2.1. MES reactor setup

A double chambered microbial electrosynthesis cell (MES) was fabricated using acrylic sheets (Fig. S1). Both anode and cathode chamber had a working/total volume of 280/400 mL and was separated by a proton exchange membrane (Nafion117, Vinpro Technologies, Hyderabad). Before use, the Nafion117 sheet was pretreated by boiling (at 70 °C) sequentially in 30% H_2O_2, deionized water (pH 7.2), 0.5 M H_2SO_4, and again deionized water each for a duration of 1 h to increase the porosity [27,28]. Pt coated Ti wire of 1.6 mm diameter and 50 mm length was used as the anode material (ipgi Instruments, Chennai). Pt coated Ti wire weaved through carbon felt (Vinpro Technologies, Hyderabad) with the dimensions of 3.5 cm × 5.7 cm (approximate projected surface area of 20 cm^2) was used as the cathode material. The porosity and conductivity of the carbon felt used were 96% and 2.85 S cm^{-1}, respectively.

Prior to use, the carbon felt and Pt-coated Ti wire was pretreated using 1 M HCl for 24 h and followed by 1 M NaOH for further 24 h to remove any impurities and to open the active reactive sites on the electrode surface [28]. Pt-coated Ti wires were used to establish the connecting electrodes as current collectors. The electrodes were dipped completely in anolyte and catholyte respectively and catholyte mixing was done with the help of magnetic stirrer. Composition of the catholyte used in this study: was 0.2 g L^{-1} of NH_4Cl, 0.04 g L^{-1} of $MgCl_2.6\ H_2O$, 0.015 g L^{-1} of $CaCl_2$,6 g L^{-1} of Na_2HPO_4, 3 g L^{-1} of KH_2PO_4 and the anolyte contained 44 mg L^{-1} of Na_2HPO_4 and 25 mg L^{-1} of KH_2PO_4[11]. Trace elements of concentration 1 mL L^{-1} (DSMZ 320) and vitamins 2.5 mL L^{-1} (DSMZ 141) were added to the catholyte to supply adequate nutrients required for the growth of microbes. Butyl rubber septa (Vohra rubber hoses, Chandigarh) was employed to avoid air entrance and ensure an anaerobic environment at both the chambers.

2.2. Culture enrichment

Anaerobic sludge collected from an anaerobic wastewater treatment plant was subjected to heat treatment at 85 °C for two hours to suppress the thermal-intolerant methanogens [29]. After heat treatment, 5 mL of sludge was used as inoculum to enrich homoacetogens in serum bottles by growing them at optimum conditions pH 7.15 and ethanol concentration 2318.7 mg L^{-1}.

2.3. MES reactor operation

The work was divided into two stages (1) startup phase and (2) influence of applied potential on MES reactor under optimal operating conditions. The startup phase began with a decrease in step-wise applied potential to acclimate electroactive bacteria to the current [30]. Enriched culture with a 25% of working volume was used as inoculum in the cathodic chamber of the MES setup.

Pure CO_2 gas was purged into the cathodic chamber of MES at the rate of 10 mL min^{-1} for 60 min, once in every three days, as the carbon source. The flow rate of CO_2 gas was selectively kept low to avoid disrupting the biofilm that had grown on the cathode surface. The anolyte was flushed with N_2 gas continuously to avoid oxygen bubbles forming at the surface of the anode and dissolved oxygen crossover to the cathode chamber [31]. A gas bag (0.5 L) filled with N_2 was connected to the cathodic chamber outlet, to ensure anaerobic condition and for gaseous sample collection. In the second stage, the reactor was operated at different applied potentials viz. − 0.8, − 1.0 and − 1.2 V vs Ag/AgCl, at optimal operating conditions such as pH of 7.15 and ethanol concentration of 2318.7 mg L^{-1}. Also, pure CO_2 gas was sparged for 60 min, once every four days. The CO_2 feeding was considered as a cycle and the reactor was operated at each potential till the VFA production was stable. The MES reactor was operated at a room temperature of 27 ± 5 °C. The experiments were carried out under dark conditions to avoid phototrophic activity [11].

2.4. Electrochemical analysis

Because of the consistent formation of a biofilm with more electrochemically active microorganisms on the cathode, the bioelectrochemical activity of the biocathode improves over time. By using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) at various points in time, these specific processes can be accurately explored in MES systems. These electrochemical investigations were carried out in MES using a Potentiostat (Autolab, Metrohm, India). The anode was used as a counter electrode, and the biocathode was used as a working electrode, whereas the potentials were poised over an Ag/AgCl reference electrode (+288 mV vs. SHE, TES instruments, Chennai, India). CV analysis was carried out in potentiostatic mode with an

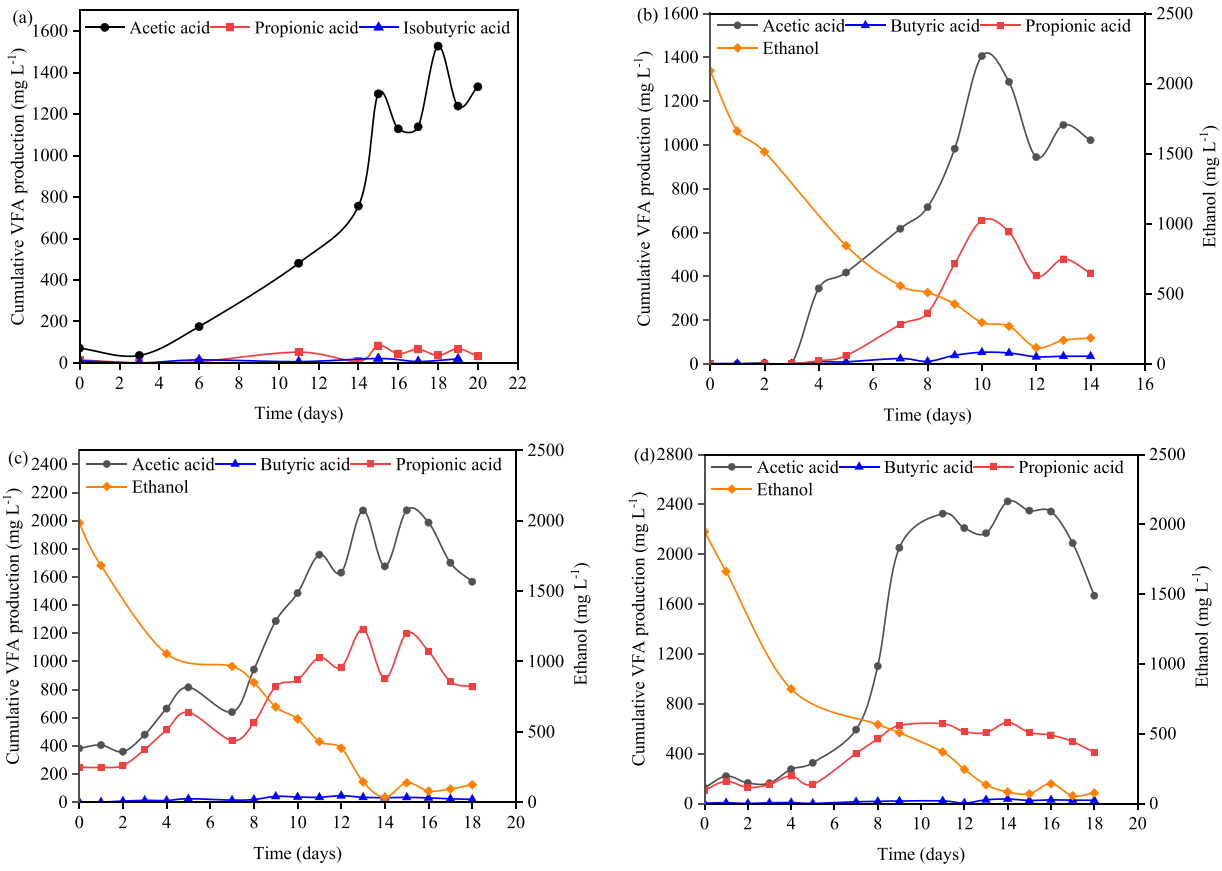

Fig. 1. VFA production and ethanol consumption profiles with time of operation in MES: (a) MES startup-pure CO_2 as carbon source (−0.8 V); (b) - 0.8 V, (c) − 1.0 V and (d) − 1.2 V vs Ag/AgCl (operating conditions for b, c and d: at initial pH of 7.15, ethanol concentration of 2318.7 mg L^{-1}, and CO_2 feeding 10 mL per min for 60 min for every 4 days).

applied potential window of − 1.2–1.2 V at a scanning rate of 1 mV/s. Electrochemical impedance spectroscopy (EIS) was conducted with an applied cathode potential of − 1.0 V vs. Ag/AgCl, with a voltage amplitude of 5 mV and a frequency range from 100 kHz to 10 mHz [32]. The EIS circuit fit circle method was employed in NOVA 2.0 software to analyze the solution and charge transfer resistance.

2.5. Scanning electron microscopy of biocathode

The surface morphology of the biofilm developed on the cathode in MES was examined using focused ion beam scanning electron microscopy (FIB-SEM, IIT Hyderabad, India). At the end of the experiments, the biocathode was carefully removed from MES, cut into small pieces, and prepared for biofilm imaging. The carbon felt sample was prepared as per the previous literature for FIB-SEM analysis [33].

Furthermore, the biofilm-containing bacteria were initially fixed by adding a few drops of 2% glutaraldehyde to each piece of biocathode and stored in a refrigerator at 4 °C for 24 h. After that, an alcohol-based dehydration method was adopted, using ethanol in different concentrations such as 25%, 50%, 75%, and 100% using milli pore water as the dehydrating agent. The ethanol-washed samples were allowed to dry in a petri dish for several hours. Later, gold was sputter deposited over each piece for 6 s. Then, the dry samples were placed on a pin mount using conductive carbon tape. FIB-SEM analysis was carried out at different magnification levels.

2.6. Analytical methods

Liquid samples of 5 mL were taken out of the cathode compartment through a rubber stopper using a 5 mL syringe. Firstly, the pH of each sample was determined using research grade pH meter (Hanna and HI5221). After that, the liquid samples were filtered through a 0.22-μ PVDF syringe filter and were stored at a temperature of − 20 °C. Sampling and replenishment of fresh medium were done during the CO_2 gas feeding to avoid O_2 interference. The amount of volatile fatty acids (VFAs) in liquid samples was measured using a gas chromatograph (GC-FID, Bruker SCION TQ) with a flame ionization detector and a 0.25 mm × 30 m column (DB SWAX) utilizing helium as the carrier gas. A temperature program of 250 °C, 200 °C (100 °C for 1 min, 100–200 °C with a ramping of 15 °C/min, holding 200 °C for 2 min), and 250 °C was used for the injector, column-oven and detector, respectively. Total Organic Carbon (TOC-L) Analyzer was used for the Total Inorganic Carbon (TIC) analysis and the bicarbonate consumption. Also, 5 mL gas samples were taken from the reactor headspace using a 5 mL gas-tight syringe. Gas samples were taken from gas bag connected to the outlet of the cathode chamber and analysed for gas composition using a gas chromatograph (Agilent micro GC 490, TCD detector). H_2, N_2, O_2 gases were analyzed using Mol Sieve 5 A with a temperature program of 70 °C, 40 °C, 40 °C used for the injector, column-oven and detector, respectively. In parallel, CO_2 was analysed in PoraPLOT U column with a temperature program of 52 °C, 130 °C, 130 °C used for the injector, column-oven and detector, respectively.

3. Results and discussion

3.1. Microbial electrosynthesis of acetate and propionate

The MES converted ethanol and CO_2 into organic chemicals, majorly acetic acid and propionic acid. The production of VFAs and the consumption profiles of ethanol at initial pH 7.15 and ethanol concentration

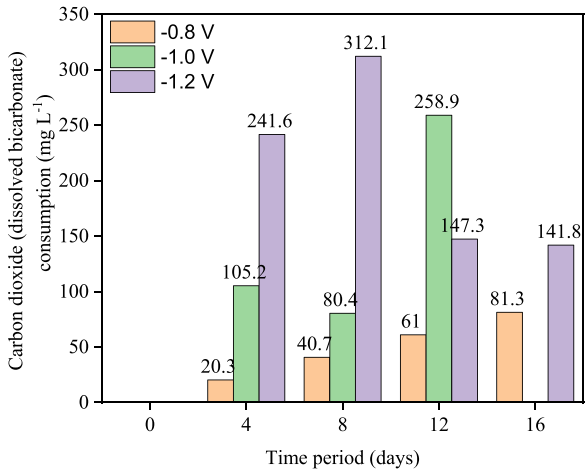

Fig. 2. Amount of bicarbonate consumption during each cycle of MES.

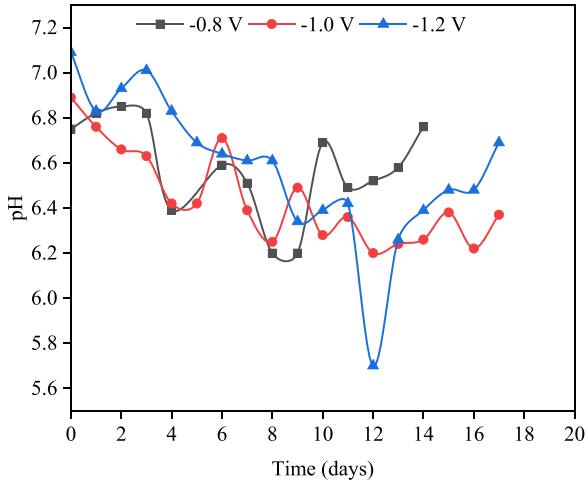

Fig. 3. pH profiles during MES operation at different applied voltages.

of 2318.7 mg L^{-1} are shown in Fig. 1. Before studying the influence of applied potential on VFA production, a batch experiment was performed with CO_2 as the sole carbon source. The investigation was named startup MES (Fig. 1a), which was inoculated with culture enriched in serum bottles as mentioned in the above Section 2.2. The negative applied voltage was decreased by 0.1 V daily from − 0.1 to − 0.8 V in eight days. The slight decrement of cathode voltage of 0.1 V was adopted to make the electroactive bacteria acclimated to the current [30]. Gas of composition CO_2:H_2 (1:1), v/v was supplied at a rate of 10 mL per minute to the cathodic chamber of MES on 0th, 4th and 7th days. After that, only CO_2 was fed for an hour at a rate of 10 mL per minute for every three days. The acetic acid production started from day 3 and steadily increased till day 16 (from 36 mg L^{-1} to 1128 mg L^{-1}). A rapid increase was observed from day 17 to day 18 (from 1138 mg L^{-1} to 1527 mg L^{-1}) and then slightly decreased with time. The main reason behind the decrement might be due to the consumption of acetic acid by VFA consuming bacteria in the mixed culture during MES [27]. The second reason might be due to lack of nutrients in the electrolyte solution at the end of the batch experiment, causing the inhibition of microbial activity and the bioconversion process. The maximum acetic acid production was 1527 mg L^{-1} (maximum rate of production 389 mg L^{-1} d^{-1}) which was observed on day 17. While acetic acid was the predominant product when pure CO_2 was used as the sole carbon source, trace amounts of propionic acid and isobutyric acid were also observed. The maximum production of isobutyric acid was 19.02 mg L^{-1} on day 15. Similarly, propionic acid production was observed on day 6 and then increased slowly as the operation progressed. It reached a maximum production of 67.67 mg L^{-1} on day 19.

After the startup, the MES reactor was operated at different negative applied voltages under optimal operating conditions. The applied voltages of − 0.8, − 1.0 and − 1.2 V vs Ag/AgCl (+288 mV vs SHE) were chosen based on our previous published statistical data analysis [34] and were named as first, second and third batch experiments, respectively.

In the first batch MES experiment, the cathodic chamber was inoculated with enriched mixed culture (25% of working volume), and this followed a negative cathode voltage of − 0.8 V which was imposed using chronoamperometry. This experiment was divided into four cycles, and each cycle lasted for 4 days, which was chosen based on the substrate (CO_2) feeding interval. Acetic acid production started on the 3rd day, and a steep increment was observed up to the 10th day, and then the VFA productions declined (Fig. 1b). The cumulative acetic acid production was 1406 mg L^{-1}, which was achieved within ten days. Similar to the MES startup, after 10th day, it was consumed by VFA consuming microbes in the mixed community [27]. Interestingly, in this batch experiment, the propionic acid production started on day 5th and reached a maximum production of 1023 mg/l (351 mg L^{-1} d^{-1}) on the same day as the highest acetic acid production was reported. Along with them, butyric acid (C4) was also observed but its concentration was below 100 mg L^{-1}.

Besides that, trace quantities of medium chain fatty acids like valeric acid (C5), caproic acid (C6), and heptanoic acids (C7) were observed. The amount of bicarbonate consumption in the cycle 1 was 20.33 mg L^{-1} and it increased to 81.33 mg L^{-1} in the cycle 4 during the VFA synthesis (Fig. 2). The electroactive bacteria consumed around 95% ethanol to produce VFAs in 12 days (Fig. 1b). The full utilization of ethanol for VFA production by bacteria, was in accordance with previous studies [36,37]. Vasudevan et al. [35] used a two-year-old enhanced mixed culture and reported full ethanol consumption at day 20 (11.4 g L^{-1}). According to Steinbusch et al. [36] an enriched mixed culture consumed all of the ethanol (4 g L^{-1}) within 40 days. In the present study, the results suggest that the VFA productions were obtained mostly from ethanol than CO_2 based on the carbon mass balance calculations given in Table S1. The combination of CO_2 and ethanol indicates the importance of ethanol addition to produce propionate. The production of the C3 compound from C1 and/or C2 might be due to the activity of chain elongation bacteria. The MES startup (CO_2 alone) comparison with the first batch experiment (ethanol and CO_2) confirmed that electroactive bacteria promoted chain elongation due to the addition of ethanol. Additionally, the MES batch experiment with ethanol resulted in reduction of startup time for chain elongation than CO_2 feeding alone (Fig. 1). In the first batch experiment, the pH changed from its initial value of 7.15–6.20 over eight days (Fig. 3). The decrement of pH was due to the production of VFAs during the MES process. In this study, the final pH didn't reach below 6, possibly due to bacterial metabolism and buffering capacity. Maybe due to the above reason, higher production rates of long-chain fatty acids were not observed. Between the days 9 and 14, the pH increased from 6.20 to 6.76; this might be due to VFA-consuming bacteria utilizing the early produced acids for their metabolism.

The next batch experiment started after finishing the first batch experiment with a fresh medium with the same optimal operational conditions. Like the first batch experiment, 25% of enriched inoculum was added to the cathodic chamber of MES for carrying out the second batch experiment. The MES experiment was run for 18 days with a cathode applied voltage of − 1.0 V vs Ag/AgCl. The second batch MES experiment achieved the maximum acetic acid production of 2074 mg L^{-1} (442 mg L^{-1} d^{-1}) and the highest amount of propionic acid production of 1228 mg L^{-1} (322 mg L^{-1} d^{-1}) over 13 days (Fig. 1c). The final ethanol concentration reached below 100 mg L^{-1} after 14 days of MES operation. Around 259 mg L^{-1} of bicarbonate was

Table 1
Results obtained in earlier studies performing MES of VFAs. All studies were performed using carbon felt (CF) as cathode.

References	Voltage/ potential (V) vs. SHE	Current density (A m^{-2})	Source of inoculum	Surface area of CF, cm^2	Maximum acetate titer, g L^{-1}	Acetate, mM d^{-1}	Other products
MES from CO_2							
Min et al.[37]	-0.903	-2.96	Sewage treatment plant	4.5 × 4.5	4.7	2.35	H_2
Jiang et al.[10]	-0.953	-19	Sewage treatment plant	7.0 × 7.0	0.095	6.58	H_2 and methane
Patil et al.[38]	-1.26	-5	Enriched mixed culture (UASB)	4	1.29	1	H_2
Song et al.[31]	3	4	Mixed culture	5 × 5	7.8	4	NA
Bajracharya et al. [26]	-0.9	10	Mixed culture	2 * 5 × 3	0.6	1.3	H_2 and CH_4
Bajracharya et al. [39]	-0.78	-15 mA cm^{-2}	Mixed culture	2 * 10 (circular)	2	2.34	Ethanol and butyrate
MES from CO_2 and ethanol							
Current study	-0.51	-0.01 mA cm^{-2}	Enriched mixed culture	5.7 × 3.5	1.4	7.05	H_2, propionic acid and butyric acid
	-0.71	-0.02 mA cm^{-2}			2.07	7.36	
	-0.91	-0.31 mA cm^{-2}			2.42	15.81	

Note: UASB: upflow anaerobic sludge blanket reactor; NA: Not available; SHE: Standard hydrogen electrode

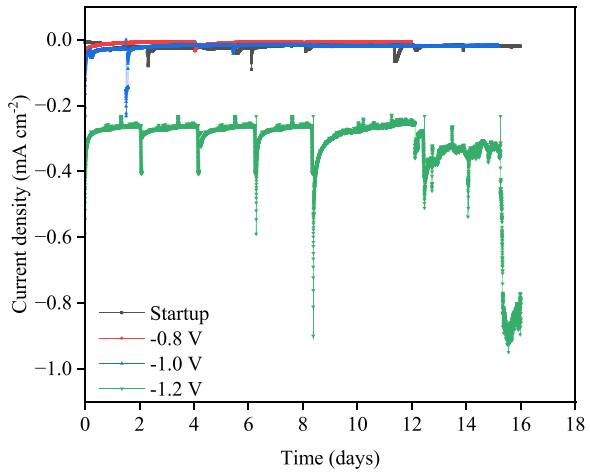

Fig. 4. Current density of MES during different applied voltage operation (Ag/AgCl (0.1 M KCl) as reference electrode).

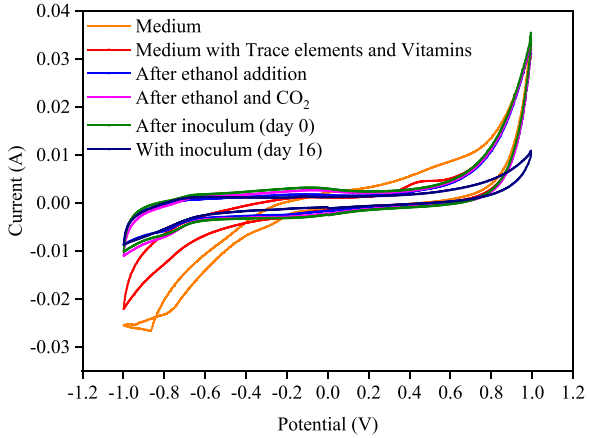

Fig. 5. Cyclic voltammetry graphs: for second batch MES operation at all stages (Scan rate of 1 mV/s recorded with respect to SHE as reference electrode).

consumed between 8 and 12 days, which was much higher than the first cycle (105 mg L^{-1}) and second cycle (80.37 mg L^{-1}). Like the first batch study, butyric acid concentration never went above 100 mg L^{-1}, and the other VFAs were produced in low quantities. The initial pH reduced to 6.25 from 7.15 after eight days of MES operation, which was due to the accumulation of VFAs in the system.

Like the first and second batch experiments, the same procedure was followed for the third batch experiment, and a cathode voltage of − 1.2 V was imposed. Production of acetic acid and propionic acid slowly started from day 4, it was consistent in production till day 7 and then suddenly increased to 2424 mg L^{-1} between days 7 and 10 (Fig. 1d). It depicted the highest acetic acid production rate of 949 mg L^{-1} d^{-1} when compared to first batch experiment (423 mg L^{-1} d^{-1}) and second batch (442 mg L^{-1} d^{-1}). The accumulation of acetic acid resulted in pH drop down to 5.7 from the initial pH 7.15 after 12 days of MES operation. Propionic acid production reached a maximum of 580 mg L^{-1} (maximum production rate of 110 mg L^{-1} d^{-1}), which was low compared to the second batch experiment. The ethanol consumption was around 97.35% over 18 days of MES operation. The bicarbonate consumption reached maximum of 312 mg L^{-1} in eight days, and this was the main reason for production of such a higher rate of acetic acid production during second cycle.

The carbon recovery was calculated using mass balance; carbon provided to the MES system and carbon present in the product (Table S1). The percentage of carbon recovery obtained in the present study was 100.5, 108.5 and 103.9 for the MES cells operated with − 0.8 V, − 1.0 V and − 1.2 V, respectively. The calculated percentage was higher than 100% due to consumption of CO_2 available in the gas bag connected to cathode chamber and direct utilization of the gas while feeding.

In the present study, a volumetric acetate production rate of 15.81 mM d^{-1} (0.95 g L^{-1} d^{-1}) was obtained with carbon felt electrode and made a comparison with previously published studies was mentioned in the Table 1.

This rate was higher than most of the other MES studies [32,38–40] but slightly lower than the MES cell used graphite granules 17.25 mM d^{-1} (1.04 g L^{-1} d^{-1}) [40]. The higher production rates and chain elongation was achieved due to (1) addition of external electron donor along with CO_2 for the electrosynthesis and (2) operation of MES reactor at optimized conditions.

Summarizing, the acetic acid production was higher in the case of MES operated at − 1.2 V than the other imposed potentials. Since this study targeted the production of higher chain carboxylic acids production, the cumulative production of propionic acid profiles suggests − 1.0 V as best imposed potential for the reduction of ethanol and CO_2 in a dual chambered MES.

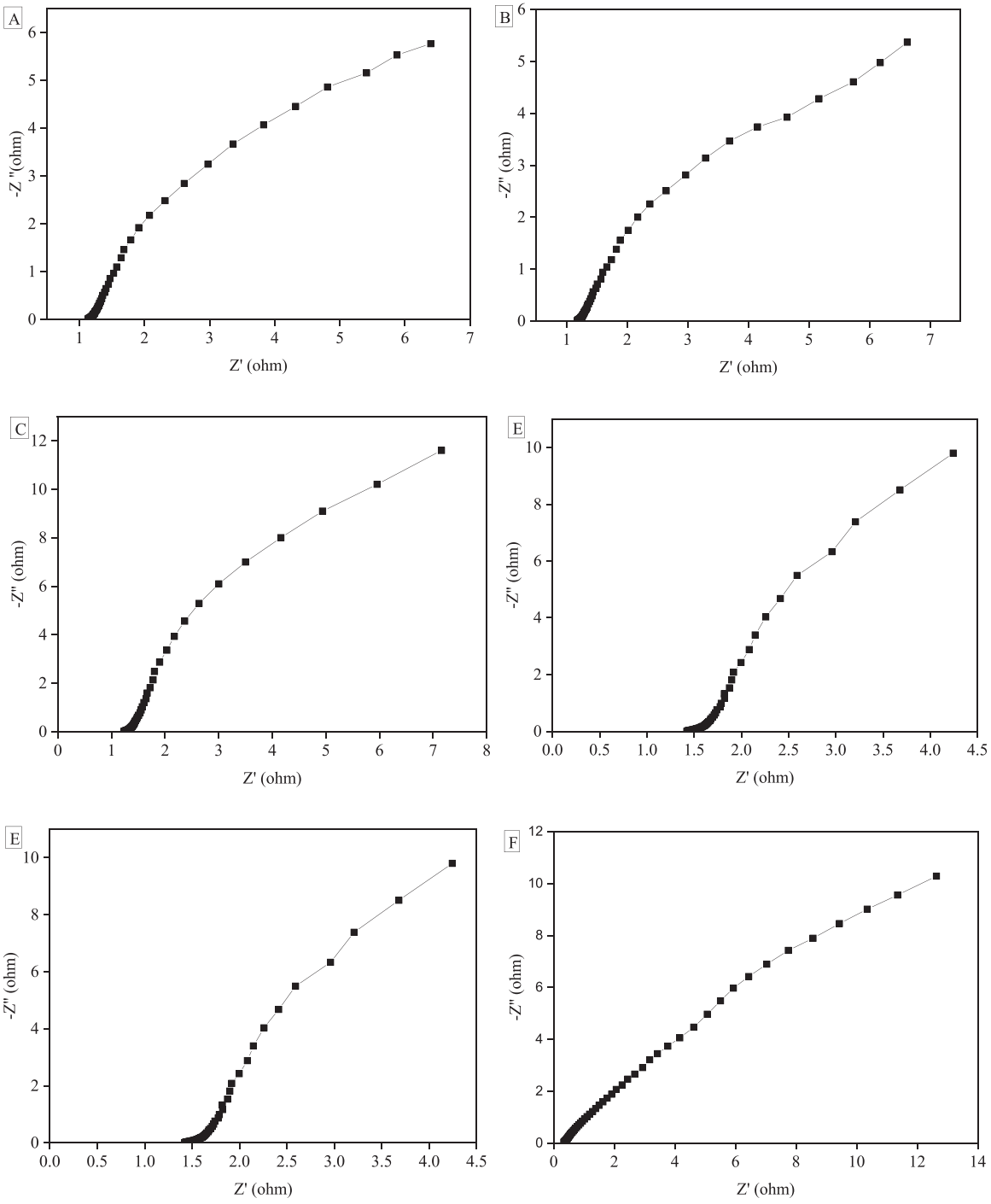

Fig. 6. Electrochemical impedance spectroscopy of cathode with: (a) Electrolyte; (b) Electrolyte with trace elements and vitamins; (c) Medium with ethanol; (d) Medium with ethanol and CO_2; (e) After inoculation on 0th day; (f) After inoculation on 16th day, for MES operated at − 1.0 V.

3.2. Influence of applied voltage on VFA synthesis

Imposed potential affects the metabolic activity of electroactive bacteria. A study by [41] described an increase in intensity in cell rupture and a reduction in cell metabolism with increase in imposed potential [42,43]. Increase of cathode potential from − 0.8 to − 1.2 V vs Ag/AgCl, in the current study, enhanced the maximum acetic acid production. The maximum acetic acid production (at −1.2 V) was 1.72 and 1.17 folds higher than in the MES operated at − 0.8 and − 1.0 V, respectively. In contrast, the highest propionic acid production was achieved at − 1.0 V which was 1.20 and 2.12 times higher than the MES operation at − 0.8 and − 1.2 V, respectively.

Also, the different negative applied voltage affects the beginning of VFA production (Fig. 1(b)-(d)). The more negative applied voltage had a higher lag time. These results indicate that higher H_2 evolution at the cathode due to more negative cathode potential caused pH variation (Fig. 3), inhibiting a few microbial activities and reducing biofilm formation. Low negative imposed potential has more butyric acid production from ethanol and CO_2 via microbial chain elongation. However, the product final concentration didn't reach more than 100 mg L^{-1}.

When operating at − 0.8 V, the MES reactor used less energy, or 1.53 kWh mol^{-1} of VFA produced, as opposed to 2.55 and 51.04 kWh mol^{-1}

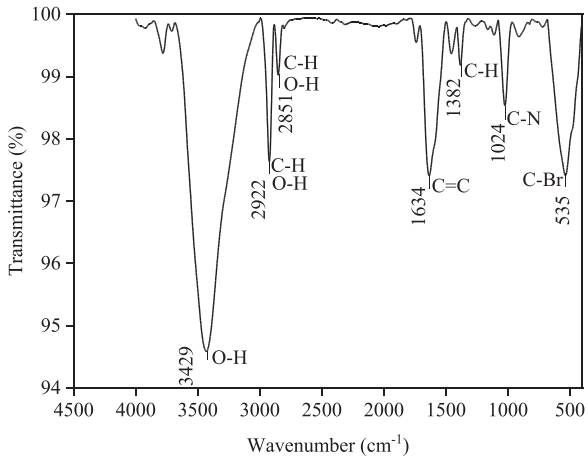

Fig. 7. FT-IR spectrum of carbon felt.

at − 1.0 and − 1.2 V, respectively (Table S2). These results demonstrate how well the MES utilized the energy input for the production of VFA.

The MES operated at − 0.8 V was observed to be more sustainable on utilization of coulombs to synthesize VFA. This batch experiment (−0.8 V) has achieved a coulombic efficiency of 47.37%, which was 1.6 and 15.7 folds higher than MES operated at − 1.0 V and − 1.2 V, respectively (Fig S2). These coulombic efficiencies were in agreement with previously reported studies, for instance Bajracharya et al. [39] obtained CE of 29.3% from CO_2: N_2 gas ratio (20:80), MES operated at − 0.8 V. Similarly, CE of 6.81% [6] and 38% [43] were obtained from bicarbonate in MES operated at − 0.8 V.

3.3. Current density performance in MES

The current density profiles of MES during operation at different negative applied voltages − 0.8, − 1.0 and − 1.2 V vs Ag/AgCl are shown in the Fig. 4. Increase in current density or current consumption led to the increased production of VFAs. After feeding CO_2 gas, the current increased suddenly and got stabilized in the same cycle due to the consumption of bicarbonate by microorganisms while producing VFAs.

The previous studies that used carbonate and/or CO_2 as a carbon source at neutral pH to explore the MES of VFA noticed that the pattern of increase in current following the addition of carbon source was found to be consistent [44,45].

The maximum current density was recorded from MES operation at a negative applied potential of − 1.2 V followed by − 1.0 and − 0.8 V, which suggest that − 1.2 V is a best applied for production of total VFAs from CO_2 and ethanol. The average current density was seen to be 0.009, 0.022, and 0.315 mA cm^{-2} in the MES operated with − 0.8, − 1.0 and − 1.2 V, respectively. The current consumption profiles of batch MESs correlate with the acetic acid production synthesized in this experimental study (Figs. 1 and 4).

3.4. Electrochemical characterization of cathode

Electrochemical characterization of cathode was done, using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), at different stages of MES operation. The maximum current response for bare electrode with medium and medium with trace elements and vitamins was 26.6 and 22 mA, respectively. The current response value was 1.16 folds higher after the inoculation of cathode chamber (day 0) compared to medium with ethanol. To further examine the electrochemical kinetics of the cathode at each stage of MES, onset potential was determined from Fig. 5. The value of onset potential is taken as − 1 mA which is the point of intersection of two tangents drawn from the starting point of the negative current and from the location when the CV curve changes direction [44].

The bare electrode with electrolyte and biocathode after 16 days were observed to have an onset potential of − 0.295 and − 0.178 V, respectively. The onset potential was lower for the biocathode compared to bare electrode with electrolyte which can be attributed to the high electrochemical activity of microorganisms in the cathodic biofilm.

Activation overpotential barriers in various stages at each stage of MES and the interfacial electron transport behaviour in the bioconversion reaction process were further studied using EIS. The real and imaginary components of impedance values are represented in the X and Y axes of a typical EIS plots, respectively.

The Nyquist plot provides the details of solution resistance (R_s) and charge transfer resistance (R_{ct}) by studying the interaction between the electrolyte and electrodes and the redox process. The maximum area of the semi-circle provides the value of R_{ct}, and the distance between the origin of the Nyquist plot and the occurrence of the first impedance value at the X-axes can be used to determine the magnitude of R_s. The system with the lowest R_{ct} value is regarded to have the lowest activation energy demand for redox reactions since the magnitude of R_{ct} is directly proportional to the activation energy. The R_{ct} and R_s values increased with increase in all the stages of MES operation except at the end of experiment (Fig. 6(a)-(f)). The R_{ct} value for the bare electrode with electrolyte system was observed as 17.79 Ω and it increased to 59.9 Ω after the addition of inoculum to medium. At the end of the second batch MES, the R_{ct} value was 66.69 Ω. On other hand, the R_s values obtained for bare electrode with electrolyte system and after inoculum were 1.17 Ω and 1.59 Ω, respectively. On 16th day the R_s value showed the lowest resistance around 0.33 Ω (Fig. 6f).

This might be due to continuous growth of biofilm thickness on the cathode, detachment and accumulation of dead cell biomass in the electrolyte. These R_s values were found to be less than those previously reported, which were 2.8 Ω or 5.06 Ω respectively from a dual chamber MES with a Ni-PHF or Ni-PHF/CNT cathode [45].

3.5. Characterization

3.5.1. Fourier-transform infrared spectroscopy (FTIR)

The functional groups of carbon felt was identified using FTIR spectroscopy and the results are shown in Fig. 7. The absorption peaks of carbon felt were obtained at 535 cm^{-1}, 1024 cm^{-1}, 1382 cm^{-1}, 1634 cm^{-1}, 2851 cm^{-1}, 2922 cm^{-1} and 3429 cm^{-1}. The absorption peak at 1380 cm^{-1} – 1385 cm^{-1} corresponds to C-H bending (medium, alkane), 1600 cm^{-1} – 1650 cm^{-1} corresponds to C═C stretching (conjugated alkene), 2840 cm^{-1} - 3000 cm^{-1} to C-H stretching (medium, alkane), 3200 cm^{-1}- 3550 cm^{-1} to O-H stretching (strong, alcohol).

3.5.2. Scanning electron microscopy

The structural morphology of the carbon felt before and after the use as cathode in MES is as shown in Fig. 8. The unused and pretreated carbon felt was having clean threads and no deposition on the fibers could be observed in the Fig. 8(a)-(b). The FIB-SEM imaging (Fig. 8(c)-(d)) clearly shows biofilm growth, indicating a significant microbial attachment on the surface of the biocathode.

The biofilm was consistent on the biocathode and uniformly distributed over the electrode surface. Also, it consists of multilayers with dense microbial formation on the electrode (Fig. 8e). The porosity of carbon felt might have allowed their growth in depth from the surface. FIB-SEM image of the biofilm on the biocathode of MES showed strong interaction between the electrode surface and electroactive microbes (Fig. 8f). The formation of biofilm mainly due to applied potential on cathode positively affected the MES performance in terms of VFA production.

Typically, the electron transfer occurs in three ways such as direct electron transfer (DET) from electrode surface, indirect electron transfer (IDET) via H_2 and electron shuttles [3]. The absence of pili or nanowires

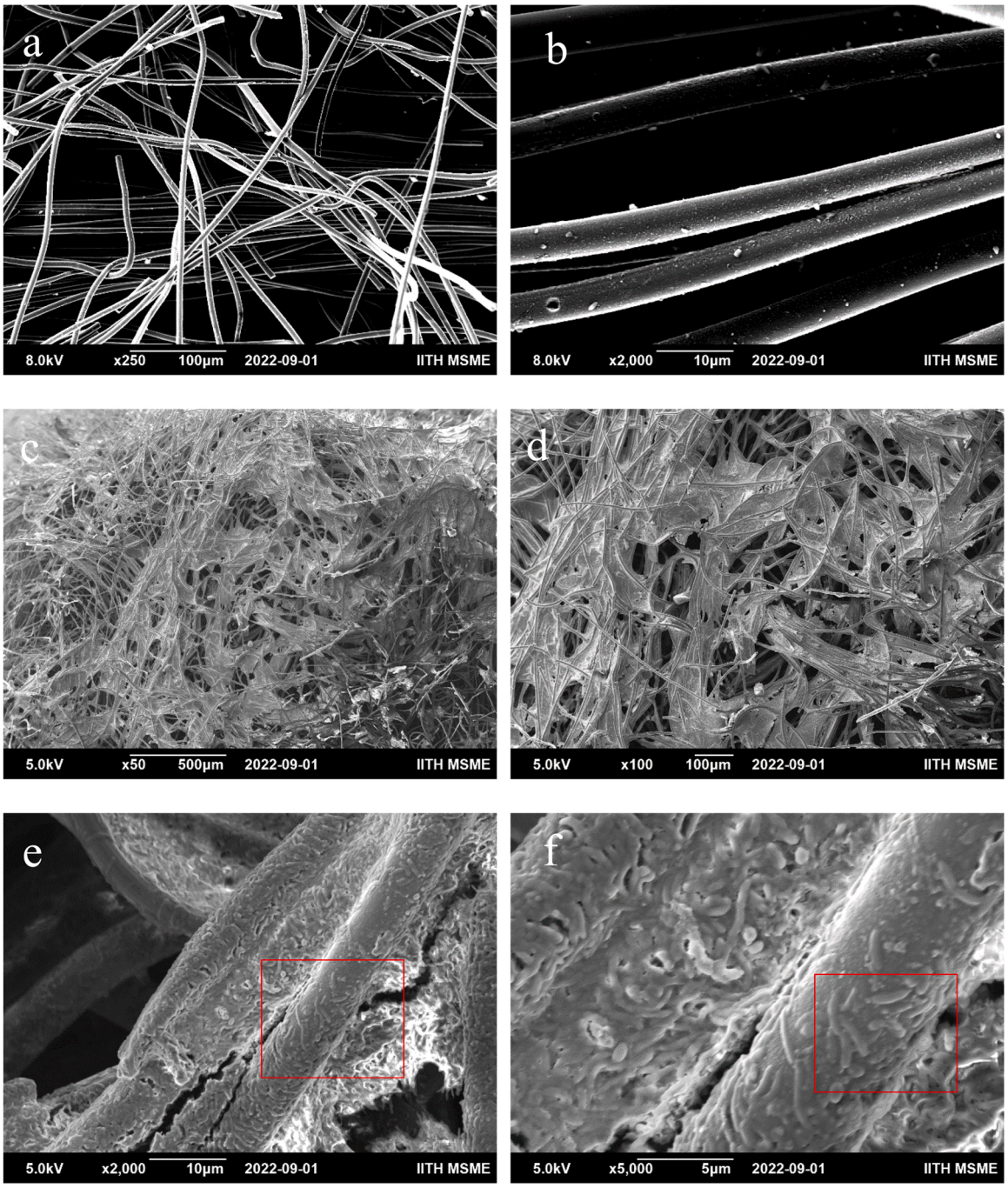

Fig. 8. FIB-SEM micrograph of (a) plain carbon felt, (b) magnified image of plain carbon felt, (c-d) biofilm on biocathode of MES and (e-f) magnified image of biofilm on biocathode of MES.

from the microorganisms in the biocathode may point to an indirect electron transfer mechanism in which H_2-mediated electron transfer is a potential route for electron uptake [44]. Also, hydrogen bubble formation and its continuous movement upwards on the electrode surface was observed physically. The presence of various sizes and shapes of microorganisms were clearly observed and depicted a different group of species involved in the transfer of electrons from electrode. However, for a clear understanding of the improvement in microbial electrosynthesis from ethanol and CO_2, more thorough and in-depth genomic investigations regarding the biofilm on the electrode would be necessary. In a study conducted by Li et al. [46] in MES with ethanol and CO_2, microbial community analysis at genus level showed that *Pseudoclavibacter* was dominant with a higher relative abundance of 24 ± 8%, which mainly guided chain elongation as reported by Cheng et al. [47]. Also, the presence of acetogens such as *Acetobacterium* (10 ± 6%) and *Acetoanaerobium* (1–6%), might have been involved in CO_2 conversion [48]. *Anaerocolumna* (9%) are reported to be capable of utilizing hydrogen [49] and *Rummellibacillus* are tolerant to ethanol and salt [50]. In the current study, there might be a similar existence of communities which might lead to the production of acetic acid and propionic acid from ethanol and CO_2.

3.6. Gas production and composition

For the headspace gas analysis, 5 mL of samples were collected using gas tight syringe, at the end of MES operation. The gas profiles of reactor

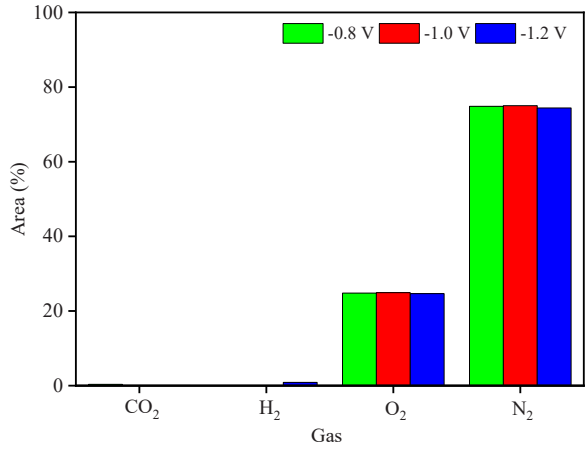

Fig. 9. Gases present in the headspace of MES reactor.

headspace gases are shown in the Fig. 9. The gas analysis revealed that there was no methane production in MES over 16 days of operation which might be due to the short time operation of MES and the change in medium pH from alkaline to acidic pH within six days due to VFA production and accumulation in the catholyte which inhibited the methanogenesis. Additionally, the H_2 evolution at the surface of the cathode was seen continuously during the experiments. But H_2 was not observed in the headspace, maybe due to leakage. CO_2 and N_2 were observed in the headspace. The oxygen generated at the anode was continuously expelled using N_2 gas feeding. However, the existence of oxygen in the cathode indicates the possibility of cross over from anode to cathode.

4. Conclusions

This study investigated the influence of different negative applied voltage such as - 0.8 V, - 1.0 V and - 1.2 V on the MES of carboxylic acids production from CO_2 and ethanol. MES operated under optimal conditions viz pH 7.15, ethanol concentration 2318.7 mg L^{-1}, obtained from serum bottles study, could produce acetic acid and propionic acid as main organic compounds. The applied voltage of − 1.2 V reported the highest amount of acetic acid production with production rates of 949 mg L^{-1} d^{-1} (15.81 mM d^{-1}). Furthermore, the highest amount of propionic acid with the production rates of 322 mg L^{-1} d^{-1} (4.34 mM d^{-1}) was obtained at - 1.0 V applied voltage. The biofilm formation on the cathode enhanced the production rates and showed higher current densities. This study helps in selecting the optimal voltage for synthesis of chain elongated products from CO_2 and ethanol in a dual chambered MES cell. Further studies on real industrial effluent containing ethanol, and under continuous MES operation, will be necessary to verify the products achieved here under batch operating conditions with synthetic wastewater. Current study demonstrated chain elongation from ethanol and CO_2 using enriched mixed culture in a synthetically prepared medium. Real wastewater might pose more challenges which are not explored in the current study. A simultaneous bio-utilization of CO_2 and ethanol has not been proven yet. Higher pressures can improve chain elongation, however the reactor design used in the current study could not withstand higher pressures. Further experimental research is needed for modified reactor design.

CRediT authorship contribution statement

Narnepati Krishna Chaitanya: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft. **Pritha Chatterjee:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision. **Akanksha Rajpurohit and Pavithra S Nair:** Investigation, Formal Analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was supported by Science and Engineering Research Board (SERB), Government of India funded project (Grant reference, SRG/2019/000757). Also, the authors would like to thank Department of Civil Engineering (IIT Hyderabad) for their financial support and Laboratory provided for this project.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bej.2023.108896.

References

[1] H. Seelajaroen, et al., "Enhanced bio-electrochemical reduction of carbon dioxide by using neutral red as a redox mediator,", ChemBioChem vol. 20 (9) (2019) 1196–1205, https://doi.org/10.1002/cbic.201800784.
[2] S. Cheng, D. Xing, D.F. Call, B.E. Logan, "Direct biological conversion of electrical current into methane by electromethanogenesis,", Environ. Sci. Technol. vol. 43 (10) (2009) 3953–3958, https://doi.org/10.1021/es803531g.
[3] K. Rabaey, R.A. Rozendal, "Microbial electrosynthesis - Revisiting the electrical route for microbial production,", Nat. Rev. Microbiol. vol. 8 (10) (2010) 706–716, https://doi.org/10.1038/nrmicro2422.
[4] X. Li, I. Angelidaki, Y. Zhang, "Salinity-gradient energy driven microbial electrosynthesis of value-added chemicals from CO_2 reduction,", Water Res vol. 142 (2018) 396–404, https://doi.org/10.1016/j.watres.2018.06.013.
[5] H.D. May, P.J. Evans, E.V. LaBelle, "The bioelectrosynthesis of acetate,", Curr. Opin. Biotechnol. vol. 42 (2016) 225–233, https://doi.org/10.1016/j.copbio.2016.09.004.
[6] G. Mohanakrishna, I.M. Abu Reesh, K. Vanbroekhoven, D. Pant, "Microbial electrosynthesis feasibility evaluation at high bicarbonate concentrations with enriched homoacetogenic biocathode,", Sci. Total Environ. vol. 715 (2020), 137003 https://doi.org/10.1016/j.scitotenv.2020.137003.
[7] K.P. Nevin, T.L. Woodard, A.E. Franks, "Microb. Electro.: Feed. Microb. Electro.: Feed. Microbes Electr. Convert," vol. 1 (2) (2010) 1–4, https://doi.org/10.1128/mBio.00103-10.Editor.
[8] C.W. Marshall, D.E. Ross, E.B. Fichot, R.S. Norman, H.D. May, "Electrosynthesis of commodity chemicals by an autotrophic microbial community,", Appl. Environ. Microbiol. vol. 78 (23) (2012) 8412–8420, https://doi.org/10.1128/AEM.02401-12.
[9] T. Zhang, et al., Improved cathode materials for microbial electrosynthesis, Energy Environ. Sci. vol. 6 (1) (2013) 217–224, https://doi.org/10.1039/c2ee23350a.
[10] Y. Jiang, M. Su, Y. Zhang, G. Zhan, Y. Tao, D. Li, "Bioelectrochemical systems for simultaneously production of methane and acetate from carbon dioxide at relatively high rate,", Int. J. Hydrog. Energy vol. 38 (8) (2013) 3497–3502, https://doi.org/10.1016/j.ijhydene.2012.12.107.
[11] L. Jourdin, et al., A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis, J. Mater. Chem. A vol. 2 (32) (2014) 13093–13102, https://doi.org/10.1039/c4ta03101f.
[12] E.V. LaBelle, H.D. May, "Energy efficiency and productivity enhancement of microbial electrosynthesis of acetate,", Front. Microbiol. vol. 8 (2017) 1–3, https://doi.org/10.3389/fmicb.2017.00756.
[13] Z. Zaybak, J.M. Pisciotta, J.C. Tokash, B.E. Logan, "Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems,", J. Biotechnol. vol. 168 (4) (2013) 478–485, https://doi.org/10.1016/j.jbiotec.2013.10.001.
[14] P. Batlle-Vilanova, et al., Microbial electrosynthesis of butyrate from carbon dioxide: Production and extraction, Bioelectrochemistry vol. 117 (2017) 57–64, https://doi.org/10.1016/j.bioelechem.2017.06.004.
[15] X. Christodoulou, T. Okoroafor, S. Parry, S.B. Velasquez-Orta, "The use of carbon dioxide in microbial electrosynthesis: Advancements, sustainability and economic feasibility,", J. CO_2 Util. vol. 18 (2017) 390–399, https://doi.org/10.1016/j.jcou.2017.01.027.
[16] P. Dessì, et al., Microbial electrosynthesis: Towards sustainable biorefineries for production of green chemicals from CO_2 emissions, Biotechnol. Adv. vol. 46 (2020) 2021, https://doi.org/10.1016/j.biotechadv.2020.107675.

[17] R. Ganigué, S. Puig, P. Batlle-Vilanova, M.D. Balaguer, J. Colprim, "Microbial electrosynthesis of butyrate from carbon dioxide,", Chem. Commun. vol. 51 (15) (2015) 3235–3238, https://doi.org/10.1039/c4cc10121a.
[18] I. Vassilev, et al., Microbial electrosynthesis of isobutyric, butyric, caproic acids, and corresponding alcohols from carbon dioxide, ACS Sustain. Chem. Eng. vol. 6 (7) (2018) 8485–8493, https://doi.org/10.1021/acssuschemeng.8b00739.
[19] Y. Jiang, N. Chu, D.K. Qian, R. Jianxiong Zeng, "Microbial electrochemical stimulation of caproate production from ethanol and carbon dioxide,", Bioresour. Technol. vol. 295 (2020), 122266 https://doi.org/10.1016/j.biortech.2019.122266.
[20] S.M.T. Raes, L. Jourdin, C.J.N. Buisman, D.P.B.T.B. Strik, "Continuous long-term bioelectrochemical chain elongation to butyrate,", ChemElectroChem vol. 4 (2) (2017) 386–395, https://doi.org/10.1002/celc.201600587.
[21] Q. Wu, et al., Medium chain carboxylic acids production from waste biomass: Current advances and perspectives, Biotechnol. Adv. vol. 37 (5) (2019) 599–615, https://doi.org/10.1016/j.biotechadv.2019.03.003.
[22] H. Seedorf, et al., "The genome of Clostridium kluyveri, a strict anaerobe with unique metabolic features,", Proc. Natl. Acad. Sci. U. S. A. vol. 105 (6) (2008) 2128–2133, https://doi.org/10.1073/pnas.0711093105.
[23] S. Das, I. Das, M.M. Ghangrekar, "Role of applied potential on microbial electrosynthesis of organic compounds through carbon dioxide sequestration,", J. Environ. Chem. Eng. vol. 8 (4) (2020), 104028 https://doi.org/10.1016/j.jece.2020.104028.
[24] S. Das, L. Diels, D. Pant, S.A. Patil, M.M. Ghangrekar, "Review—microbial electrosynthesis: a way towards the production of electro-commodities through carbon sequestration with microbes as biocatalysts,", J. Electrochem. Soc. vol. 167 (15) (2020), 155510 https://doi.org/10.1149/1945-7111/abb836.
[25] M.C.A.A. Van Eerten-Jansen, et al., Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures, ACS Sustain. Chem. Eng. vol. 1 (5) (2013) 513–518, https://doi.org/10.1021/sc300168z.
[26] S. Bajracharya, et al., Carbon dioxide reduction by mixed and pure cultures in microbial electrosynthesis using an assembly of graphite felt and stainless steel as a cathode, Bioresour. Technol. vol. 195 (2015) 14–24, https://doi.org/10.1016/j.biortech.2015.05.081.
[27] J. Annie Modestra, B. Navaneeth, S. Venkata Mohan, "Bio-electrocatalytic reduction of CO_2: Enrichment of homoacetogens and pH optimization towards enhancement of carboxylic acids biosynthesis,", J. CO_2 Util. vol. 10 (2015) 78–87, https://doi.org/10.1016/j.jcou.2015.04.001.
[28] A.B.T. Nelabhotla, C. Dinamarca, "Bioelectrochemical CO 2 reduction to methane: MES integration in biogas production processes,", Appl. Sci. vol. 9 (6) (2019) 16–18, https://doi.org/10.3390/app9061056.
[29] B. Baghchehsaraee, G. Nakhla, D. Karamanev, A. Margaritis, G. Reid, "The effect of heat pretreatment temperature on fermentative hydrogen production using mixed cultures,", Int. J. Hydrog. Energy vol. 33 (15) (2008) 4064–4073, https://doi.org/10.1016/j.ijhydene.2008.05.069.
[30] S. Das, M.M. Ghangrekar, "Value added product recovery and carbon dioxide sequestration from biogas using microbial electrosynthesis,", Indian J. Exp. Biol. vol. 56 (7) (2018) 470–478.
[31] T. shun Song, G. Wang, H. Wang, Q. Huang, J. Xie, "Experimental evaluation of the influential factors of acetate production driven by a DC power system via CO_2 reduction through microbial electrosynthesis,", Bioresour. Bioprocess. vol. 6 (1) (2019) https://doi.org/10.1186/s40643-019-0265-5.
[32] P. Liu, et al., Stimulated electron transfer inside electroactive biofilm by magnetite for increased performance microbial fuel cell (no. February), Appl. Energy vol. 216 (2018) 382–388, https://doi.org/10.1016/j.apenergy.2018.01.073.
[33] L. Jourdin, S.M.T. Raes, C.J.N. Buisman, D.P.B.T.B. Strik, "Critical biofilm growth throughout unmodified carbon felts allows continuous bioelectrochemical chain elongation from CO_2 up to caproate at high current density," (no. MAR), Front. Energy Res. vol. 6 (2018) 1–15, https://doi.org/10.3389/fenrg.2018.00007.
[34] N.K. Chaitanya, A. Tripathi, P. Chatterjee, in: L. Singh, D.M. B. T.-D. L.-C. B., B. R. Mahapatra (Eds.), "Chapter 6 - Microbial Electrosynthesis: Recovery of High-value Volatile Fatty Acids from CO_2,", Elsevier, 2021, pp. 123–142.
[35] D. Vasudevan, H. Richter, L.T. Angenent, "Upgrading dilute ethanol from syngas fermentation to n-caproate with reactor microbiomes,", Bioresour. Technol. vol. 151 (2014) 378–382, https://doi.org/10.1016/j.biortech.2013.09.105.
[36] K.J.J. Steinbusch, H.V.M. Hamelers, C.M. Plugge, C.J.N. Buisman, "Biological formation of caproate and caprylate from acetate: Fuel and chemical production from low grade biomass,", Energy Environ. Sci. vol. 4 (1) (2011) 216–224, https://doi.org/10.1039/c0ee00282h.
[37] S. Min, Y. Jiang, D. Li, "Production of acetate from carbon dioxide in bioelectrochemical systems based on autotrophic mixed culture,", J. Microbiol. Biotechnol. vol. 23 (8) (2013) 1140–1146, https://doi.org/10.4014/jmb.1304.04039.
[38] S.A. Patil et al., "Selective Enrichment Establishes a Stable Performing Community for Microbial Electrosynthesis of Acetate from CO 2," 2015, doi: 10.1021/es506149d.
[39] S. Bajracharya, K. Vanbroekhoven, C.J.N. Buisman, D.P.B.T.B. Strik, D. Pant, "Bioelectrochemical conversion of CO_2 to chemicals: CO_2 as a next generation feedstock for electricity-driven bioproduction in batch and continuous modes,", Faraday Discuss. vol. 202 (2017) 433–449, https://doi.org/10.1039/c7fd00050b.
[40] C.W. Marshall, D.E. Ross, E.B. Fichot, R.S. Norman, H.D. May, "Long-term operation of microbial electrosynthesis systems improves acetate production by autotrophic microbiomes,", Environ. Sci. Technol. vol. 47 (11) (2013) 6023–6029, https://doi.org/10.1021/es400341b.
[41] K. Wang, Y. Sheng, H. Cao, K. Yan, Y. Zhang, "Impact of applied current on sulfate-rich wastewater treatment and microbial biodiversity in the cathode chamber of microbial electrolysis cell (MEC) reactor,", Chem. Eng. J. vol. 307 (2017) 150–158, https://doi.org/10.1016/j.cej.2016.07.106.
[42] J.L. Varanasi, R. Veerubhotla, S. Pandit, D. Das, Biohydrogen Production Using Microbial Electrolysis Cell: Recent Advances and Future Prospects, Elsevier B.V,, 2018.
[43] J.A. Modestra, S.V. Mohan, "Microbial electrosynthesis of carboxylic acids through CO 2 reduction with selectively enriched biocatalyst: Microbial dynamics,", J. CO_2 Util. vol. 20 (2017) 190–199, https://doi.org/10.1016/j.jcou.2017.05.011.
[44] M.T. Noori, S.V. Mohan, B. Min, "Microbial electrosynthesis of multi-carbon volatile fatty acids under the influence of different imposed potentials,", Sustain. Energy Technol. Assess. vol. 45 (2020) 2021, https://doi.org/10.1016/j.seta.2021.101118.
[45] B. Bian, et al., "Porous nickel hollow fiber cathodes coated with CNTs for efficient microbial electrosynthesis of acetate from CO_2 using: Sporomusa ovata,", J. Mater. Chem. A vol. 6 (35) (2018) 17201–17211, https://doi.org/10.1039/c8ta05322g.
[46] Z. Li, et al., Efficient production of medium chain fatty acids in microbial electrosynthesis with simultaneous bio-utilization of carbon dioxide and ethanol, Bioresour. Technol. vol. 352 (2022), 127101, https://doi.org/10.1016/j.biortech.2022.127101.
[47] S. Cheng, et al., Elucidating the microbial ecological mechanisms on the electro-fermentation of caproate production from acetate via ethanol-driven chain elongation, Environ. Res. vol. 203 (2022), 111875, https://doi.org/10.1016/j.envres.2021.111875.
[48] S. Mills, et al., A meta-analysis of acetogenic and methanogenic microbiomes in microbial electrosynthesis, npj Biofilms Micro vol. 8 (1) (2022), https://doi.org/10.1038/s41522-022-00337-5.
[49] T. Gao, H. Zhang, X. Xu, J. Teng, "Integrating microbial electrolysis cell based on electrochemical carbon dioxide reduction into anaerobic osmosis membrane reactor for biogas upgrading,", Water Res vol. 190 (2021), 116679 https://doi.org/10.1016/j.watres.2020.116679.
[50] M. Li, Y. Li, X. Fan, Y. Qin, and Y. He, "crossm a Physiologically Recalcitrant Bacterium with High Ethanol," no. April, pp. 6–8, 2019.