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Imperative role of applied potential and inorganic carbon source on acetate production through microbial electrosynthesis

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ABSTRACT

Microbial electrosynthesis (MES) is a novel technology that produces organic molecules from the reduction of carbon dioxide (CO_2) at biocathode. MES system is a hybrid device that combines components of biological and fuel cells in a single system for chemicals/energy generation from inexpensive substrates. Present study evaluates the influence of cathodic potentials (−800 mV and −600 mV) on reduction of CO_2 to acetate using enriched acetogenic bacteria as the biocatalyst at 30 °C using graphite and VITO carbon electrodes as cathode and anode respectively. The first stage of evaluation of bicarbonate as carbon source was continued to second stage where gaseous CO_2 used as C source. In both the stages −800 mV showed higher acetate production efficiency. MES reactor with cathodic potential of −800 mV showed 4.05 and 5.45 g acetate/L respectively during first and second stage. Changing the carbon source of the systems from bicarbonate to CO_2 positively influence the performance. Moreover, change in operation mode from continuous to batch resulted in improved acetate production rate, which also proved that the performance was reproducible and stable. Continuous CO_2 supply maintained the pH near neutral which might explain the traces of ethanol produced in the system. Higher coulombic efficiency was also registered with −800 mV operation than −600 mV.

1. Introduction

Carbon capture and utilization is one of the major challenges of the present times. Minimizing the CO_2 emissions into atmosphere and simultaneous utilization of captured CO_2 to value added products requires novel ideas that also can assure the future generations with sustainable development. Microbial electrosynthesis (MES) is a novel biocathode-driven production technology for the reduction of CO_2 to chemicals and biofuels [10,8]. MES which implies the use of biocatalysts to achieve electricity driven product synthesis can be performed in bioelectrochemical systems operating in microbial electrolysis cells (MEC) mode with biocathodic configuration [18,16,17,20]. The reducing equivalents, electrons and protons are mobilized from anode to cathode through external circuit and ion exchange membrane, respectively. In the cathodic chamber, the terminal electron acceptor is converted to products by reduction process with electrons and protons [15,13,14]. Mild external potentials that are required to apply on working electrode/cathode depend on the reduction process occurring at the biocathodes. This mechanism is mainly determined by three factors namely, the type of biocatalyst, the applied potential and the nature of the terminal electron acceptor. Acetate, ethanol, H_2, methane, butanol, succinate, xylitol, propanol and polyhydroxybutyrate (PHB) were identified as possible products through MES process having a biocathode [18]. Among these, methane, acetate, butyrate, propionic acid, ethanol and acetone were reported as being produced through MES process [21,24,15,20,7].

The biocatalyst present on biocathode also should have a feasible metabolism for the product that is aimed through MES process. A suitable terminal electron acceptor should be available for the reduction reaction. Finally, cathodic or applied potential that breaks thermodynamic barrier of biological reaction should be applied for successful reduction reaction in MES. Homoacetogenic bacteria can efficiently converts CO_2 to acetate, which is a major intermediate molecule for biochemicals production [2]. Thermodynamically, conversion of CO_2 to acetate requires −280 mV cathodic potential. However, under practical conditions much lower applied potential is required to overcome potential losses due to the components of bioelectrochemical system. Apart from above factors, several other factors such as electrode materials, reactor design, mediators in electron transfer etc., influence the

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overall process efficiency [5,26,9]. At present MES technology is at infancy and mainly focused on proof of concept studies in multiple dimensions [11].

With this background, the present study was aimed at understanding the influence of the applied potential on biocathodic reduction reaction for acetate production. Two dual chambered/H-type MES reactors with well adapted electrochemically active biofilms were used for the study. The first stage of the study focused on the bioelectrochemical reduction of bicarbonate as inorganic carbon source for the production of acetate at −600 mV and −800 mV. In the second stage, the inorganic carbon source was changed to CO_2 gas to check the imperative role of the type of carbon source. Furthermore, the reproducibility of the results obtained was validated by operating the same reactors under batch mode operation during both the stages of operation. Since, the same reactors with active biofilms were used for long term (about 6 months), the stability of the homoacetogenic biofilms can also be identified.

2. Materials and methods

2.1. Design of dual chamber MES reactor

Two completely identical H-type MES reactors fabricated with glass were used in this study to evaluate the influence of electrode potential and type of carbon source on bioelectrochemical production of acetate. Cathode and anode chambers were having total and working volumes of 0.65 L and 0.5 L, respectively. Both the chambers were separated by a pre-treated Nafion 117® proton exchange membrane [1]. Several provisions or ports for electrode insertion, gas sampling, water sampling, reference electrode and gas supply were designed to each chamber of reactor. In both the reactors, graphite was used as cathode and VITO-CoRE™ derived carbon electrodes were used as anode. Both the electrodes had similar total (37.5 cm^2) and active surface areas (30.0 cm^2). The total surface area and the active surface area (the area that is submerged) were 37.5 cm^2 and 30.0 cm^2 [14]. Ag/AgCl (3.0 M KCl) reference electrode was placed in cathode chamber. Both cathode and anode electrodes were placed in respective chamber from the top of the reactor. A fine stainless steel wire was weaved through the stainless less steel current collector of VITO-CoRE™ electrode and extended through the airtight passage of the reactor cap. In the case of graphite rod, a 0.5 mm perforation was made, to which an insulated stainless steel wire was connected as described earlier [14]. At the bottom of anode and cathode chambers, crimp provision with rubber septa was present to provide gas supply from the bottom of the chamber. Anode chamber was connected to N_2 gas, whereas cathode chamber was connected to mixture of CO_2 and N_2 (20:80).

2.2. Operation

The MES reactors having electroactive cathodic biofilm that developed using bicarbonates as substrate in batch mode operation were engaged in this study. This study was followed by two different stages of operation (Table 1). In the first stage, two cathodic potentials such as −600 mV (MES1) and −800 mV (MES2) were optimized using bicarbonates as inorganic carbon substrate. In the second stage, same reactors were continued to evaluate their performance using carbon dioxide as the substrate. Electroactive cathodic biofilm developed using enriched homoacetogenic consortia. Based on the bicarbonate consumption rate, acetate production rate and reduction current data from preliminary operation, 5 days was considered as the optimal time for substrate consumption (data not shown). So 5 days' time was maintained to provide new substrate for both the reactors. Both the reactors were operated at 30 ± 1 °C on a magnetic stirrer (100 rpm) for catholyte. Before every feed change event, the magnetic stirrer was stopped for 30 min to allow the suspended biomass to settle. The supernatant was then carefully replaced with siphon flow that avoids biofilm damage. Chronoamperometric (CA) technique was used to analyze the reduction reaction happening in the system. Both, MES1 and MES2 were continuously poised at −600 mV and −800 mV vs. Ag/AgCl respectively through CA technique using potentiostat (BioLogic-VMP3 model, France). All the assays were performed *in situ* by considering cathode as working electrode and anode as counter electrode against Ag/AgCl (3.0 M KCl) reference electrode. All the potentials mentioned further in the manuscript are vs. Ag/AgCl reference electrode, unless otherwise stated.

2.2.1. Bicarbonate as inorganic carbon source

Phosphate buffer media containing NH_4Cl of 200 mg/L, $MgCl_2{\cdot}6H_2O$ of 200 mg/L, Yeast Extract of 10 mg/L along with the trace elements solution (per litre, Nitrilotriacetic acid, 1.5 g; $MgSO_4 \times 7\,H_2O$, 3.0 g; $MnSO_4{\cdot}H_2O$, 0.5 g; NaCl, 1.0 g; $FeSO_4{\cdot}7H_2O$, 0.1 g; $CoSO_4{\cdot}7H_2O$, 0.18 g; $CaCl_2{\cdot}2H_2O$, 0.1 g; $ZnSO_4{\cdot}7H_2O$, 0.18 g; $CuSO4{\cdot}5H_2O$, 0.01 g; $KAl(SO_4)\ 2 \times 12H_2O$, 0.02 g; H_3BO_3, 0.01 g; $Na_2MoO_4{\cdot}2H_2O$, 0.01 g; $NiCl_2{\cdot}6H_2O$, 0.03 g; $Na_2SeO_3{\cdot}5H_2O$, 0.30 mg) and vitamin solution (per litre, biotin, 2 mg; pantothenic acid, 5 mg; B-12, 0.1 mg; *p*-aminobenzoic acid, 0.5 mg; thioctic acid (alpha lipoic), 5 mg; nicotinic acid, 5 mg; thiamine, 5 mg; riboflavin, 5 mg; pyridoxine HCl, 10 mg; folic acid, 2 mg) was considered as the basic media [14]. In both the reactors, 90% of the catholyte was replaced with fresh feed containing bicarbonate equivalents of 2.5 g/L (3.44 g/L Sodium bicarbonate) and continued first feeding operation under respective cathodic potentials. To inhibit the possible methanogenic activity, 0.5 g/L concentration of bromoethanesulfonic acid (BESA) was added to the medium [3,22,23]. The inlet pH of the catholyte was maintained at 7.0. In catholyte, fresh

Table 1
Experimental scheme of the study for acetate production from bicarbonates (HCO_3^-) and carbon dioxide (CO_2) at two different cathodic potentials.

Description	MES1	MES2	Time/no of spikes/feeding events	Remarks
	−600 mV	−800 mV		
Stage 0: HCO_3^- feed Batch mode	Biofilm development with HCO_3 2.5 g/L of HCO_3^- feed		43 days	N_2 sparging for anaerobic conditions (*data not shown*)
Stage 1: HCO_3^- feed Feed Spike mode	90% feed replacement during startup of stage 1 2.5 g/L of HCO_3^- feed spiking for every 5 days: addition of nutrients		60 days/12 Spikes	N_2 sparging for anaerobic conditions
Stage 1: HCO_3^- feed Batch mode	with 90% feed replacement for every cycle of 5 days addition of nutrients		15 days/3 Feeding events	To evaluate reproducibility and stability N_2 sparging for anaerobic conditions
Stage 2: $CO_2 + N_2$ supply Continuous mode	90% feed replacement during startup of stage 2 addition of nutrients		60 days	$CO_2 + N_2$ supply also maintains anaerobic conditions
Stage 2: $CO_2 + N_2$ supply Batch mode	90% feed replacement for every cycle of 5 days addition of nutrients		15 days/3 Feeding events	To evaluate reproducibility and stability Phosphate buffer saturated with CO_2 was used

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40× concentrated bicarbonate solution along with vitamin and mineral solution was spiked to maintain 2.5 g/L HCO_3^- concentration. Similar protocol was continued for 12 cycles of operation (60 days). The subsequent three cycles were operated in batch mode by replacing 90% of the fresh feed to check the reproducibility of bioelectrochemical reduction process under specific conditions. The liquid samples were collected and stored to estimate the acetate and bicarbonate concentration. Anode chamber was filled with phosphate buffer solution. Both anode and cathode chambers were supplied with low rate of N_2 gas supply from the bottom of the reactor to maintain anaerobic conditions.

2.2.2. Carbon dioxide as inorganic carbon source

After evaluating bioelectrochemical reduction of bicarbonates, the carbon source of both MES1 and MES2 reactors were shifted to CO_2 and N_2 mixture (20:80). Initially, 90% of the feed was changed with fresh phosphate buffer solution along with vitamin and mineral solutions. No bicarbonate was added here. Mixture of CO_2 and N_2 was continuously supplied through a needle from the bottom of the reactor (through a rubber septum provided). Intermittently, trace elements solution and vitamin solution was injected to catholyte under anaerobic conditions. No specific N_2 gas supply was done from the bottom of catholyte. Mixture of CO_2 and N_2 helps to maintain anaerobic conditions. Whereas for anode, N_2 gas supply was continued throughout operation. Liquid samples were collected from catholyte for every 5 days of operation to analyze dissolved inorganic carbon concentration and acetate concentration. Continuous operation of MES1 and MES2 reactor with CO_2 was done for 60 days (similar to bicarbonate stage) and then three cycles were run by replacing 90% of the carbonated fresh

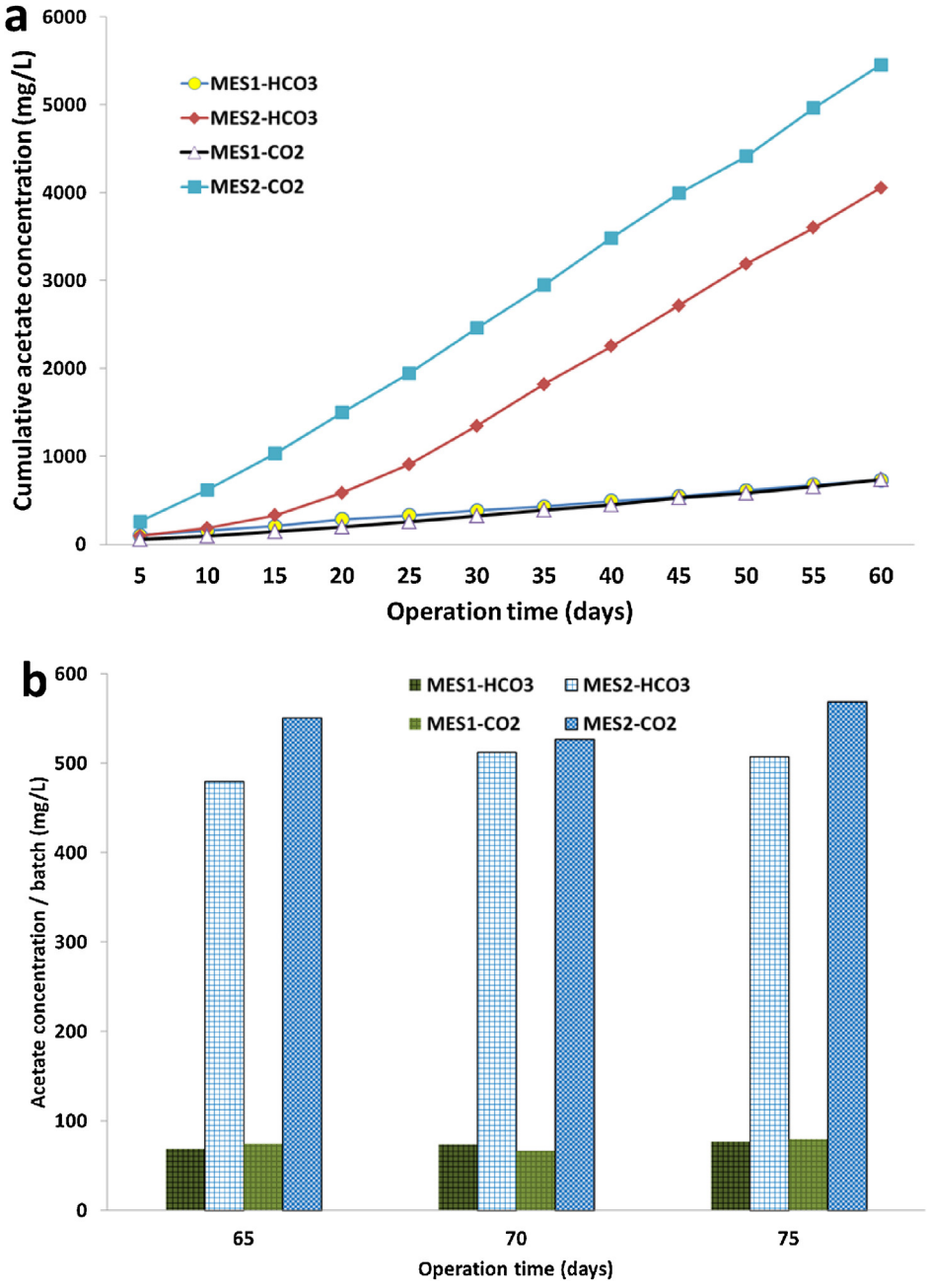

Fig. 1. Microbial electrosynthesis of acetate from bicarbonate and CO_2 under two different cathodic potentials studied in two stages. (a) Cumulative acetate production (mg/L) in 60 days of operation in two stages and (b) Total acetate production (mg/L) per batch cycle during reproducibility evaluation phase.

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feed. Carbonated fresh feed was prepared in a separate glass container by continuously supplying of CO_2 and N_2 to phosphate buffer solution at 10 mL/min flow rate for 5 days (control experiments showed CO_2 dissolution saturation within 5 days). After feed replacement, trace metal solution and vitamin solution were added to support bacterial metabolism in cathode/catholyte. In this stage also, CO_2 and N_2 mixture was continuously supplied.

2.3. Analysis

The liquid samples collected from MES1 and MES2 reactors were immediately stored in −20 °C freezer. The samples were analysed for volatile fatty acids (VFAs) such as formic acid, acetic acid, propionic acid and butyric acid, along with ethanol and pH. Prior to analysis for VFAs and ethanol, the samples were filtered through 0.45 μm Acrodisc syringe filters. HPLC analyses was performed using RID detector (Agilent 1260) connected to Agilent HPLC 1200 at a set wavelength of 215 nm. Agilent Hi-Plex column 8u (3000 mm × 7.7 mm) was used and operated at 60 °C equipped with a guard column of same material. Phosphoric acid (0.05% in isocratic gradient) was used as eluent at a flow rate of 1 mL/min. Standard injection volume of the samples was 20 μL. EZchrom software of Agilent was used for data analysis. pH was analyzed with regularly calibrated WTW Multi 340i pH meter. The bicarbonates or dissolved CO_2 was analysed by indirect estimation. Total inorganic carbon (TIC) provides the bicarbonates concentration in liquid samples. According to the methodology developed by ISO 8245, TOC analyzer (Multi N/C 3100 of Analytik Jena) with auto-sampler (APG 49 of Analytik Jena) was used for TIC analysis.

3. Results and discussion

3.1. Influence of cathodic potential on CO_2 reduction

3.1.1. Bioelectrosynthesis—bicarbonate as C source

The applied potential that is maintained at biocathode drives the reduction reaction of microbial electrosynthesis process. Based on the theoretical Gibbs free energy data, −280 mV (vs. SHE) is the cathodic potential required for reduction of bicarbonate to acetate [18]. Due to over potentials and losses associated with MES design and electrolytes, higher potential is required than standard potentials. Several researchers performed CO_2 reduction to acetate production at different cathodic potentials in the range of −600 mV to −1.1 V. In this direction, two reactors were evaluated at −600 mV (MES1) and −800 mV (MES2) for their imperative role on biocathodic reduction reaction for acetate production. Among two cathodic potentials evaluated, MES2 system operated with −800 mV showed superior function than MES1 (−600 mV). Soon after startup of the MES2 with 2.5 g HCO_3^-/L, bioelectrochemical reduction was observed which resulted in 94 mg/L acetate and current density of 14.14 A/m^2 in 5 days (Figs. 1 and 2). Addition of bicarbonate along with required nutrients and vitamins for every 5 days enhanced acetate concentration in catholyte. Current density also signified the bioelectrochemical reduction function. Acetate concentration was found to increase with every feeding event with significant improvement in acetate production rate and showed stable production by 6th feeding event, where the total acetate concentration was 1345 mg/L (Fig. 2). Acetate production rate was increased from 18.8 mg/L/day (first feeding event) to 87 mg/L/d (6th feeding event). Thereafter, stable production rates were observed with an average acetate production rate of 90.4 mg/L/d. On the whole from 12 feeding events (60 days), 4057 mg/L acetate was produced. From 60 days of operation, the average and maximum current densities were −39.11 and −58.60 A/m^2. It was demonstrated that higher production rates were associated with MES2 which was operated under hydrogen producing potential. Biologically induced hydrogen mediated the electron transfer to acetate which was reported as superior than direct electron transfer [16,17,4]. In other case (MES1), where direct electron transfer acted for acetate production the rate of production was lower.

In the case of MES1 reactor operated at −600 mV cathodic potential, the startup performance with respect to acetate concentration (102 mg/L in 5 days), and production rate (20.4 mg/L/d) were found similar to MES2 reactor. At the same time average current density (−1.49 A/m^2) was 10 times lesser than MES2. The higher concentration of acetate in 5 days operation is due to the residual acetate from the catholyte from adaptation phase. However subsequent feeding event was found to show less acetate production and respective current densities. From second to 12th feeding events, acetate production rate of MES1 was found to exhibit similar performance (11.42 mg/L/d), which is much less than MES2. By the end of 12th feeding event (60 days), cumulative

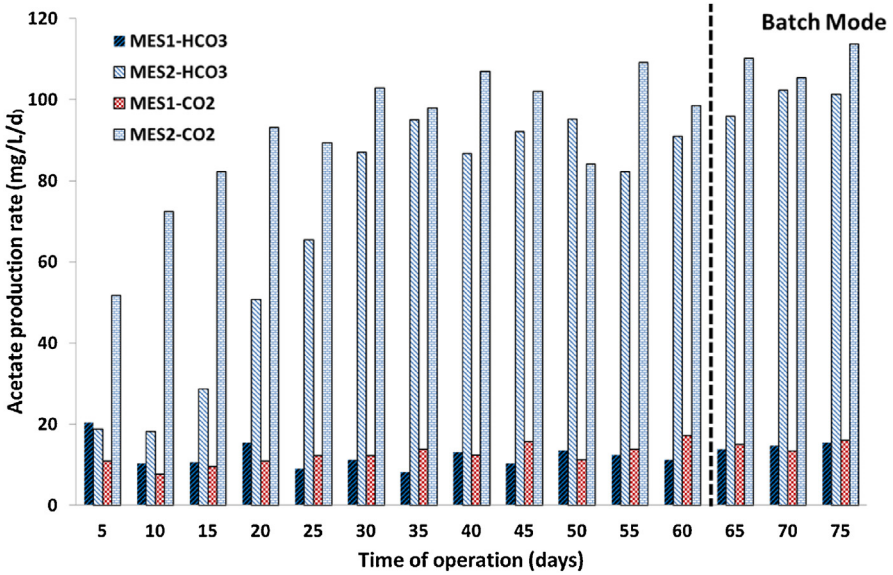

Fig. 2. Acetate production rate (mg/L/d) through microbial electrsynthesis from bicarbonate and CO_2 under two different cathodic potentials studied in two stages. (3 batch cycles operated were represented with dotted line).

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acetate production was registered as 730 mg/L. Contrary to the acetate production trend, higher reduction current was observed. During 60 days of MES1 reactor operation, maximum and average current densities of MES1 registered as 20.25 and 12.56 A/m^2 (Fig. 3a). The superior performance of MES reactor with cathodic potential can be attributed to effective electron transfer higher counter electrode (anode) potentials prevailing in the system. Counter electrode potentials of MES1 and MES2 reactors were 0.94 V and 1.78 V, respectively. The electrochemical reductive equivalents such as electrons and protons required for bicarbonate conversion to acetate generate more at higher potential than lower potential. Based on the visible observation, it was observed that cathodic biofilm of MES2 is thicker than MES1, which led us to hypothesize that higher cathodic potential is favorable for electroactive biofilm formation on cathode surface.

3.1.2. Bioelectrosynthesis—CO_2 as C source

After uninterrupted operation of MES1 and MES2 reactors for 12 feeding events and 3 batch mode operations with bicarbonate, both the reactors were shifted to CO_2 gas as C source for acetate production (Table 1). CO_2 and N_2 mixture (20:80) was supplied through fine needle from the bottom of reactor at 10 mL/min, showed solubility rate of 92 mg inorganic carbon/L/d. This is equivalent to 375 mg/L bicarbonates (HCO_3^-). This was determined from the continuous supply of CO_2 and N_2 mixture at 10 mL/min flow rate in control reactor for 10 days. This showed solubility saturation in 4 days at 1500 mg HCO_3^-/L. Similar to first stage, this stage also showed higher acetate production efficiency with MES2 reactor than MES1 reactor. As both the cathodic biofilms of both the reactors were operated for long time, adaptation to CO_2 supply condition was rapid and the attained stable acetate production rates quicker. Even though the performance is similar with first stage, marginal improvements in acetate production rates were observed. Since the CO_2 supply was continuous, no specific feeding can be identified. For comparable sampling and analysis intervals, liquid samples were collected for every 5 days. Acetate production rate of MES2 reactor was 51.8 mg/L in first 5 days (Fig. 2). Gradual improvement in acetate production rate was observed with time and registered maximum in 30 days of operation (103 mg/L). The maximum current density during this period was registered as 42.97 A/m^2 (Fig. 3d). During the later phase of operation (from 30 to 60 days), the cumulative acetate production was 5455 mg/L (100 mg/L/d). During this period, maximum and average current densities were registered as −44.89 and −34.53 A/m^2. In the case of MES1, the acetate production rate (12.3 mg/L) and current densities (maximum, 11.26 and average, 4.92 A/m^2) were at nearly stable performance from the beginning. Cumulative acetate produced from 60 days of operation was 740 mg/L. When the total performance is compared between bicarbonate and CO_2 conditions for MES2, CO_2 showed higher performance. In case of MES1 both the C source conditions were found more comparable. No limitation can be attributed to lower solubility rates of CO_2 in catholyte because acetate production rate is lesser than CO_2 solubility rate. This was determined by comparing CO_2 solubility rate and maximum acetate production rates. Average CO_2 solubility is 375 mg HCO_3^-/L/d. On theoretical equivalents evaluation, maximum production rate of 181.3 mg acetate/L/d (122 g of HCO_3^- required for 59 g of acetate) can be achieved. However the maximum acetate production rate of the present reactor is 113.8 mg acetate/L/d, which is considerably less than practically reachable rate. It was hypothesized that internal and external diffusion limitations play key role in biofilm based processes [19]. Here, internal diffusion is related to CO_2 that was continuously supplied to the system. So, saturated CO_2 levels were maintained in catholyte during second stage of operation. Another diffusion limitation is external

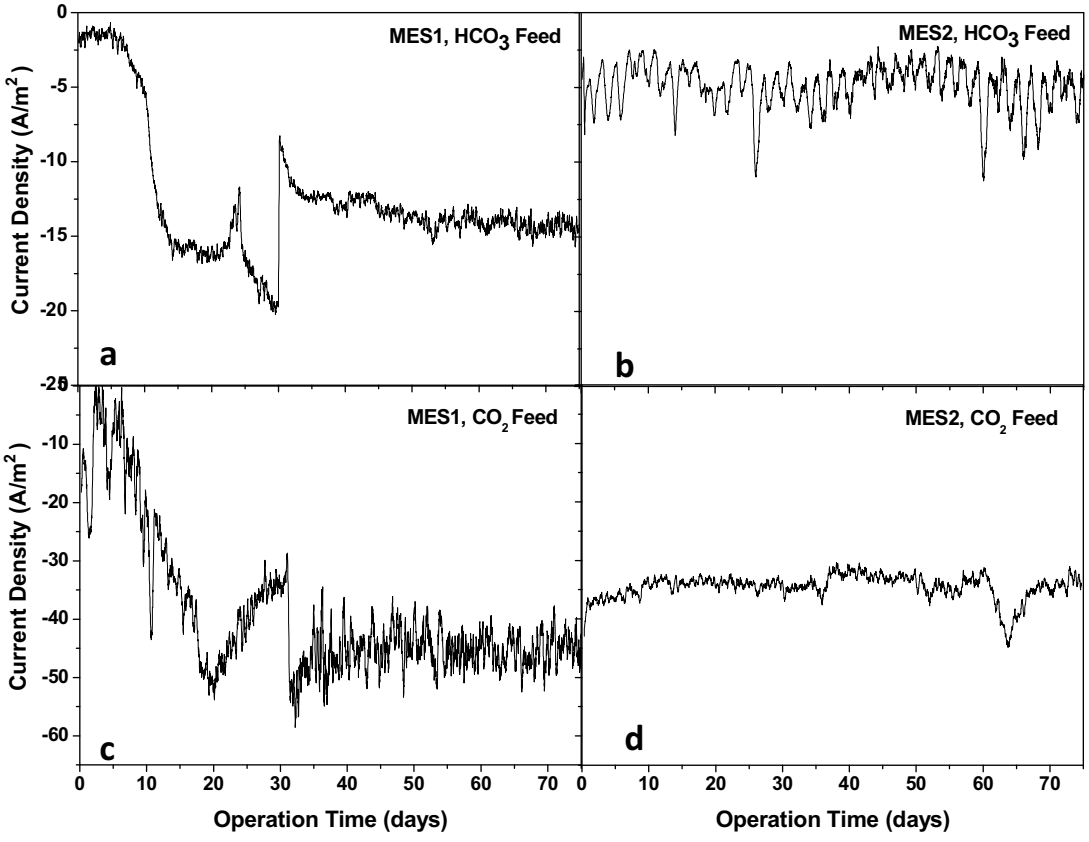

Fig. 3. Chronoamperometry depicting reduction current density (A/m^2) pattern observed during four variations studied for acetate production from inorganic carbon source.

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diffusion which ascertained to bring substrates into and products out of the biofilm [19]. In the present study also external diffusion limitations may be present. A detailed analysis on three dimensional structures of cathodic biofilm and diffusion rates of acetate through biofilm may provide answers for it. When the ratio of biofilm surface to total volume increases, internal and external limitations will be dominant.

3.2. Reproducibility and stability evaluation

Reproducibility of the results under minor change of operational conditions is crucial for the dependability of any technology or process. Use of bicarbonate and CO_2 as C source in first phase and second phase respectively evidenced −800 mV (MES2) of cathodic potential as more productive than −600 mV (MES1). Bicarbonate feed was given intermittently without changing bulk liquid catholyte. In case of CO_2 feed, CO_2 and N_2 mixture was continuously supplied. The reproducibility of the results was well demonstrated under batch mode operation. Moreover, small improvement in productivities was observed in case of MES1 and MES2 under bicarbonate and CO_2 conditions. MES1 and bicarbonate case showed improved acetate production rate from 13.6 to 15.4 mg/L/d, whereas MES1 and CO_2 case showed similar performance. In case of MES2, bicarbonate (95.2–102.4 mg acetate/L/d) and MES2, CO_2 cases (109.2–113.8 mg acetate/L/d) marginal improvement in acetate production rate were observed. Both the reactors were operated continuously for more than 150 days under changing operational conditions and two different types of feed illustrating the stability of the reactors towards bioelectrochemical conversion of acetate. The biofilms formed under both hydrogen mediating and direct electron transfer conditions were showed equal stability and reproducibility. To explain the potential mechanisms for electron transfer in the milieu of MES from CO_2 by biocathode several studies made an attempt [4,12]. Extensive studies towards identification of bioelectrochemical hydrogen production and/or non-biological/electrochemical hydrogen production is required. Along with hydrogen, bacterial cell wall embedded cytochromes are also involved in electron transfer mechanism.

3.3. pH influence on acetate production

pH is one of the major crucial factors that influence the biocathodic reduction reaction. The biocatalyst present on cathode and planktonic biomass are sensitive to catholyte pH. In our previous study [14], it was identified that pH more than 9.0 is detrimental for biofilm which leads to inhibition or truncation of whole process. Except for MES2 of second stage, in all the cases, gradual increase in pH was observed. In the present study, the catholyte pH of bicarbonate conditions varied in the range of 7.15–7.53 for MES1 and 7.25–7.84 for MES2 (Fig. 4). Whereas in MES1 of second stage, pH was increased from 7.0 to 7.18 only. Contrary to the above three conditions, second stage of MES2 showed almost stable pH was observed. Only in few cycles of operation, negligible drop was observed. In this case the pH was maintained in a narrow range (6.89 and 7.12). pH supply to the reactor medium provides buffering capacity [6,19]. This might be one of the reasons for low fluctuation in pH during second phase where continuous supply was provided. One of the crucial operating parameters for any biological process is stable pH. It can be controlled by increasing buffering capacity of media or reactor contents. As CO_2 is a good buffering agent, it is advantageous to use it as substrate in MES that helps to provides buffering nature catholyte. Drastic increase in pH was observed when bicarbonate was used as substrate. The product of present process, acetate creates mild acidic conditions. Here, the system was successful in maintaining the pH around 7.0. The change in pH during batch mode operation of each variation is similar to the trend followed for main study. Previous study hypothesized that the reason for the fragility of biofilm is high pH. Catholyte pH more than 9.0, caused instability of system in 127 days of operation [14]. In the present case, the pH did not exceed 7.8 and the system showed stable efficiency for more than 150 days of operation. The major variations between present study and previous study are reactor configuration and cathodic potential which were found to influence the stability of the electroactive cathodic biofilm. The continuous supply of CO_2 as the substrate maintained the pH near to neutral, whereas bicarbonate showed alkaline pH conditions prevailing in the catholyte.

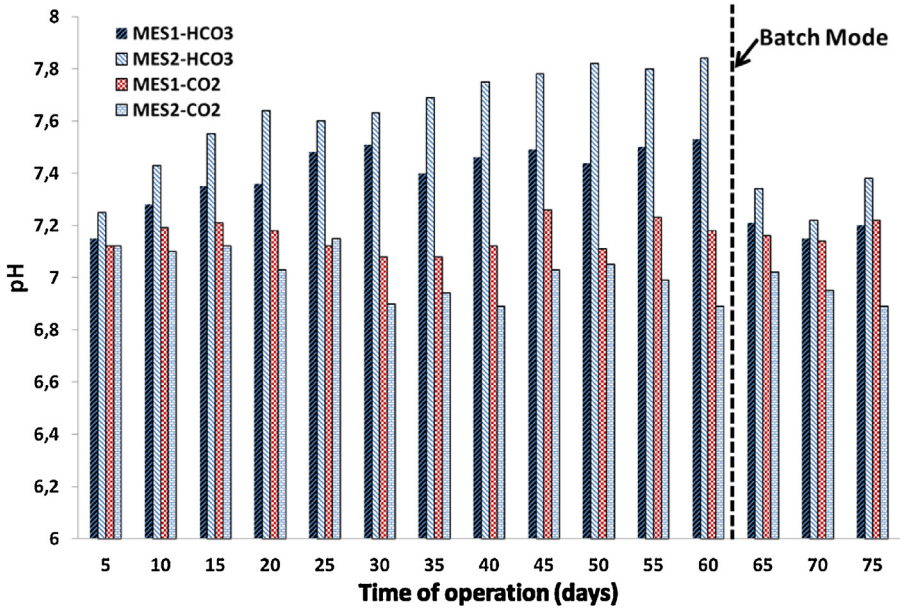

Fig. 4. pH variation during microbial electrosynthesis of acetate from bicarbonate and CO_2 with two different cathodic potentials studied in two stages. Inlet pH was constantly maintained at 7.0 for all conditions. Dotted line represents 3 batch cycles operated for reproducibility evaluation.

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3.4. Product specificity

Enriched homoacetogenic mixed culture that developed from anaerobic consortia [14] was used for the electroactive biofilm formation to convert CO_2 to acetate. The diverse metabolism of bacteria and mixed culture nature triggers other products such as ethanol and organic acids along with acetate. Methane is one of the major by-products that can be produced from methanogenic function on produced acetate, which negatively influence the overall process. Direct conversion of methane from bioelectrochemical reduction of CO_2 has been reported recently [25]. Ethanol can be produced from the acetate reduction with hydrogen [21], which was found in negligible conditions when enriched homoacetogenic consortia was used [14]. If the by-product generation metabolism is not controlled, the electron diverts to other products and decreases the total performance. This also influences the downstream process economics. BESA used in the catholyte was successful in inhibiting the methanogenic activity in all four operational variations, which was confirmed by gaseous analysis. During second stage, MES2 evidenced small concentrations of ethanol (between 15 and 81 mg/L), whereas MES1 registered 18–43 mg/L of ethanol production. Compared to second stage, first stage of operation showed less ethanol concentrations. It was observed in the range of 10–22 mg/L and 10–26 mg/L respectively for MES1 and MES2 reactor. According to [21], mild acidic conditions are favorable for ethanol conversion from acetate. In the present study, the catholyte pH was alkaline (7.2–7.8) in first stage. Whereas in the second stage, near neutral and mild acidic conditions prevailed. This demonstrated the influence of operational pH of catholyte for the inhibition of ethanol products. Apart from ethanol, no other by-products like propionic acid and butyric acid were produced justifying no suitable conditions were present in the bioreactors for chain elongation.

3.5. MES process efficiencies

Apart from product specificity, carbon conversion efficiency, coulombic efficiency, specific production rate and concentration of product also determine microbial electrosynthesis. Coulombic efficiency (CE%) signifies the ratio between total charge (coulombs) that are consumed for reduction and the actual charge contributed in conversion of product of interest. The formula for CE (%) is, C_P/C_T. where, C_P is the product of b (number of electrons consumed for the product, in case of acetate, it is 8), n (number of moles of product) and F (Faraday's constant (96,485C/mol). C_T is total coulombs consumed and they can be derived by integrating charge with the time [14,13]. Along with acetate, present study also evidenced ethanol production in small quantities. Compared to main product, ethanol was limited to approximately 1% of total concentration. So, the electrons consumed for ethanol formation were not considered for CE (%) calculation. Among the four operational variations studied, MES2 in second stage showed higher CE of 30.25% followed by MES2 in first stage (26.26%) and MES1 in first stage (19.05%). MES1 in second stage showed least CE of 15.45%. Even though the acetate concentration was comparable with state of the art, the CE values were comparatively less. The electrons might be diverted for biomass production. Identifying the loss of electrons will help to improve the methodology for higher efficiencies.

4. Conclusions

Microbial electrosynthesis of acetate from bicarbonate and CO_2 at two different potentials evidenced that higher potential where hydrogen production is possible is favorable than lower potentials. The electroactive biofilms showed consistent reduction of inorganic carbon source available in catholyte and produced acetate. pH of the system was influenced by type of carbon source provided in the system. It also helped to understand the hypothesis of pH influence on biofilm stability, which governs feasibility of MES process at industrial scales. This is the first study that evaluated optimum cathodic potential for bioelectrochemical acetate production. Further studies on the biomass growth kinetics, electron transfer mechanism in carbon conversion helps to identify the electron losses that resulted in lower coulombic efficiency.

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