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ARTICLE

Impact of Dissolved Carbon Dioxide Concentration on Process Parameters during its Conversion to Acetate through Microbial Electrosynthesis

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Reduction of carbon dioxide (CO_2) released from the industries helps to reduce the greenhouse gases (GHGs) emissions to the atmosphere while producing value added chemicals and contributes to carbon fixation. Microbial electrosynthesis (MES) is a recent process which accomplishes this idea by using cathodic bacteria at the expense of minimum energy. In this study, enriched mixed homoacetogenic bacteria as cathodic biocatalyst for the reduction of CO_2 with five different concentrations were evaluated to produce acetate at a constant potential. Increasing the carbon concentration showed improved acetate production rate and carbon conversion efficiency. A maximum acetate production rate of 142.2 mg L^{-1} day^{-1} and maximum carbon conversion efficiency of 84% were achieved respectively at 4.0 and 2.5 g HCO_3^- L^{-1}. The changes in pH due to interactive reactions between bicarbonate (substrate) and acetate (products) were able to create buffering nature in the catholyte that control operating parameters of microbial electrosynthesis (MES) process such as pH and substrate specificity. Higher acetate production shifted catholyte pH towards acidic conditions which further triggered favorable conditions for the bioelectrochemical reduction of acetate to ethanol.

Introduction

Bioelectrochemical systems (BESs) are one of promising emerging technologies for the reduction of carbon dioxide (CO_2) to multicarbon compounds through biocathodic reactions using specific bacteria or microbial consortia as biocatalyst. This process is named as microbial electrosynthesis (MES) or bioelectrochemical synthesis[1-2]. Tremendous research focus is being provided to MES by the global research community due to its potential application in conversion of CO_2 into multicarbon and value added compounds at the expense of minor amount of energy [3-5]. The advantages of the process also can be stated in terms of CO_2 reduction from the atmosphere, biofuels and chemicals production from the GHGs, low energy input for the production and renewable and low-cost biocatalyst application in the process [6-8]. The energy required to drive the bioelectrochemical reduction can be generated by the renewable energy sources such as solar cells/photovoltaics, wind power, geothermal heat, etc.[8] The electrical energy generating from the treatment of wastewater using microbial fuel cells (MFCs) can also be integrated with microbial electrosynthesis of multicarbon organic compounds for making the process even more sustainable [10-13]. Integration of MFCs for energy or biochemicals production by utilizing negative-valued waste streams combines integrated wastewater management and energy recovery. This can solve the energy crisis and environmental pollution simultaneously [14-15]. Several products such as acetate, methane, ethanol, butyrate etc., have been produced through MES process at lab scale in several proof of concept studies, among which acetate was the most studied product [16-18]. Various types of biocatalysts such as pure cultures, mixed cultures, enriched cultures were used for the production of acetate. It was also identified that reactor configuration, electrode materials etc., influences the process efficiency [2,19-22].

Acetate production rate, carbon conversion efficiency and coulombic efficiency are the major process parameters which can be considered to evaluate the efficiency of this process[21]. Acetate production rate was influenced by type of bacteria/biocatalyst employed, carbon availability in catholyte and electron transfer efficiency. Current density, product specificity and production rate are the major factors influencing coulombic efficiency (CE). However, all such parameters were majorly governed by the operating conditions such as substrate availability, catholyte pH, cathodic reduction potential, biocatalyst used for biocathode (microbial strain involved in electrochemical reduction), electrode surface area, etc. [22-24]. Most of the research groups working on the

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acetate production through CO_2 conversion, reported maximum production rate of around 0.5 to 780 mg of acetate L^{-1} h^{-1} [6,27-32]. Improving acetate production rate and operating MES system up to higher concentrations is one of the major options for improved economics and energy efficiencies. Higher acetate concentration has negative influence on biofilm stability and on catholyte pH conditions [6,33]. It is also important to operate MES with higher CO_2 concentrations for maximum availability of the substrate, thus the increased CO_2 conversion rates can be expected. At higher acetate concentration, the acidic pH conditions triggers further conversion of acetate to ethanol, that hampers the purity of the desired product and also the CE of the process[6,34].

In this regard, the present study was aimed for the elucidation of increasing concentrations of bicarbonates in catholyte and its influence on reduction reaction. It was also aimed to evaluate the direct impact of bicarbonate concentration on the catholyte buffering nature during acetate production and bicarbonate consumption. Based on this, a total of nine different concentrations of bicarbonate were evaluated at the range of 1.0 to 15.0 g HCO_3^- L^{-1} (1.0, 1.5, 2.0, 2.5, 4.0, 8.0, 12.0 and 15.0 g HCO_3^- L^{-1}, by gradual increasing in concentration). All the concentrations were aimed to evaluate with same cathodic potential under similar operational conditions. However, the system has exhibited inhibition after 4.0 g HCO_3^- L^{-1}. So, the present manuscript was explained the results and behavior of MES system in the range of 1.0 to 4.0 g HCO_3^- L^{-1}. In-situ developed and enriched homoacetogenic bacterial consortium was used as the biocatalyst in dual chambered MES system. The research findings were used to explain the pH influence on CO_2 reduction process.

Materials and Methods

Blueprint of MES reactor

H-type bioelectrochemical reactor fabricated with glass bottles was used to study the influence of inorganic carbon concentration. Anode and cathode chambers were separated by Nafion 117® proton exchange membrane (PEM)[6] (Fig 1). Total and working volumes of each chamber were considered as 0.65 L and 0.5 L, respectively. In-house-fabricated, activated carbon based VITO-CoRE® electrodes with active surface area of 30.0 cm^2 (total surface area, 37.5 cm^2) was used as both cathode (working) and anode (counter) electrodes. Stainless steel mesh that was used as the current collector in VITO-CoRE® electrode, was extended outside the chamber for connection with potentiostat system. Electrodes were placed in the respective chambers from the top and sealed to maintain the reactor airtight. Both the chambers of the MES reactor were equipped with sampling ports for water and gas separately[6]. Crimped rubber septum at the bottom of the reactor was used to supply N_2 to maintain the system anaerobic. Standard Ag/AgCl (3.0 M KCl) reference electrode was placed in cathode chamber as the reference electrode.

Figure 1: Experimental setup used for the evaluation of dissolved CO_2 concentration influence on biocathodic reduction for acetate production

MES modus operandi

The MES reactor having electroactive cathodic biofilm that developed on VITO-CORE® electrodes using bicarbonates as substrate in batch mode operation were engaged in this study [5]. MES reactor was operated with bicarbonate as substrate and the substrate concentration was gradually increased after 7 cycles of operation with each concentration (from 1.0 and 4.0 g HCO_3^- L^{-1}). The subsequent concentrations such 8.0 and 12.0 g HCO_3^- L^{-1} operated for 2 cycles only. In the case of 15.0 g HCO_3^- L^{-1}, only single cycle was operated due to inefficiency at higher bicarbonate concentrations. Out of eight different concentrations were studied, the MES system was found show reduction property only in the range of 1.0 and 4.0 g HCO_3^- L^{-1}. Hence, the discussion was confined this range only (brief details of higher concentrations were presented in Supporting Information). Shifting the operation from one concentration to next higher concentration was done over 7 consecutive batch operations. Seven cycles were considered as time for adaptation and also as replicates. The average values of these replicates plotted for results. Optimized cathodic potential of -800 mV (*vs*. Ag/AgCl) was constantly used for the whole study. Hydraulic retention time (HRT) of 5 days for each batch was optimized based on the reduction current signal at 1.0 g L^{-1} concentration and the same HRT was continued uniformly for rest of the variations and operated at room temperature (22 ±2 °C) on magnetic stirrer (100 rpm for catholyte). Every feed change (catholyte) event was preceded by settling of the suspended biomass/biocatalyst under non stirring conditions, and then 90% of feed was replaced with fresh feed carefully. BioLogic potentiostat (model: VMP3, France) was used to apply the controlled cathodic potential of -800 mV *vs* Ag/AgCl through chronoamperometry (CA) technique. Rest of the manuscript mentions potentials *vs* Ag/AgCl reference electrode, unless otherwise stated.

Electrolyte and substrate

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According to the design of operation, 500 mL of electrolyte was used for anode and cathode. Phosphate buffer solution (PBS, 25 mM) along with bicarbonate source and trace elements solution was prepared for catholyte, as reported elsewhere [5,28] As bicarbonate strongly influences the pH of the solution, mild phosphate buffer was used to control the sudden fluctuations in the catholyte pH. For different concentrations of carbon source, both bicarbonate equivalents (from sodium bicarbonate) and trace element concentrations were changed proportionally. Similarly, anode was filled with 500 mL of 25 mM phosphate buffer. Anaerobic condition was assured by supplying N_2 gas at a flow rate of 2 mL min^{-1}. As enriched mixed consortium is the catalyst in biocathode, methanogenic activity can be developed on long time operation. Methanogenic activity may decrease the acetate concentration by converting it to methane. Bromoethanesulfonic acid (BESA) which is inhibitor for methanogenic activity, was added at a concentration of 500 mg L^{-1} [35,36] to overcome the methanogenic activity. The inlet pH of the catholyte was maintained at 7.0. However, due to the 10% of that left in the cathode chamber was influenced the initial pH in the reactor. Fresh bicarbonate solution with same concentrations along with vitamin and mineral solution was replaced in MES system. Similar protocol was continued for all the study. Liquid samples were collected and stored to estimate the acetate and bicarbonate concentration. Feed change and sampling operations were done under N_2 environment to ensure the anaerobic microenvironment in the reactor.

The role of bicarbonate concentration on the bioelectrochemical reduction of CO_2 was evaluated at various carbon concentrations using bicarbonate as substitute for dissolved CO_2 gas. This helps to avoid the operational challenges associated with CO_2 gas.

Analysis

Liquid samples were collected from the reactors for the quantitative analysis of volatile fatty acids (VFA), ethanol and bicarbonates and pH at pre-defined time intervals. Each time, only 5 mL of volume was drawn and same volume of fresh media was injected to maintain the total volume constant. This may lead to change in the composition of catholyte, however, the change was considered negligible. The collected samples were immediately transferred to -20 °C freezer and stored till further analysis. VFAs such as formic acid, acetic acid, propionic acid and butyric acid, along with ethanol were analyzed through HPLC using RID detector (Agilent 1260) connected to Agilent HPLC 1200 at a set wavelength of 215 nm. Agilent Hi-Plex column 8u (3000 mm X 7.7 mm) was used and operated at 60 °C equipped with a guard column of same material. Isocratic gradient phosphoric acid of 0.05% was used as eluent at a flow rate of 1 mL min^{-1}. Standard injection volume for each sample in HPLC was 20 µl. EZchrom software of Agilent was used for data analysis. Calibrated WTW Multi 340i pH meter was used for pH analysis. The bicarbonates or dissolved CO_2 was quantified by indirect estimation method using total inorganic carbon (TIC) analysis. The stored liquid samples were thawed and analyzed by adapting 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.

Results and Discussion

Substrate concentration influence on acetate production rate

The solubility of CO_2 in water is limited to 1.685 g CO_2 L^{-1} (0.0383 M) under standard pressure and temperature[37-38]. The solubility rate of CO_2 depends on several factors such as purging conditions, pH of the catholyte or buffer used and rate of CO_2 reduction due to the bioelectrochemical reaction. Reaction kinetics is also influenced by the substrate concentrations present in the catholyte. Different concentrations of bicarbonate evaluated showed influence on the bioelectrochemical reduction reaction. Each concentration variation was run for consecutive of 7 batch cycles with fresh bicarbonate solution to the MES system considering 5 days as the operation time for each batch. Acetate production rate exhibited concomitant adaptation of MES to each substrate variation along with the number of operating cycles. Acetate production rate was increased constantly in each cycle and reached a stable performance phase by fourth cycle (Fig 2a). Among the experimental variations studied, the rate of bicarbonate reduction to acetate production was found to be correlating with the availability of the substrate in catholyte. Here, biocathode reduction potential was also found to depend on the bicarbonate concentration. Highest acetate production rate of 142.2 mg L^{-1} d^{-1} was identified with 4.0 g HCO_3^- L^{-1} (711.1 mg L^{-1} $cycle^{-1}$, average) and lowest of 35.5 mg L^{-1} d^{-1} with 1.0 g HCO_3^- L^{-1} (177.3 mg L^{-1}/cycle, average). Whereas at 2.5 g HCO_3^- L^{-1}, both acetate production rate and concentration per cycle were limited to 102.4 mg L^{-1} d^{-1} and 512.0 mg L^{-1}/cycle (average), respectively. Cumulative acetate production was also documented maximum with 4.0 g HCO_3^- L^{-1} (4.98 g acetate L^{-1}), followed by 2.5 g HCO_3^- L^{-1} (3.58 g L^{-1}), 2.0 g HCO_3^- L^{-1} (2.15 g L^{-1}) and 1.5 g HCO_3^- L^{-1} (1.45 g L^{-1}) (Fig 2b). Minimum acetate production of 1.24 g L^{-1} was reported with 1.0 g HCO_3^- L^{-1}.

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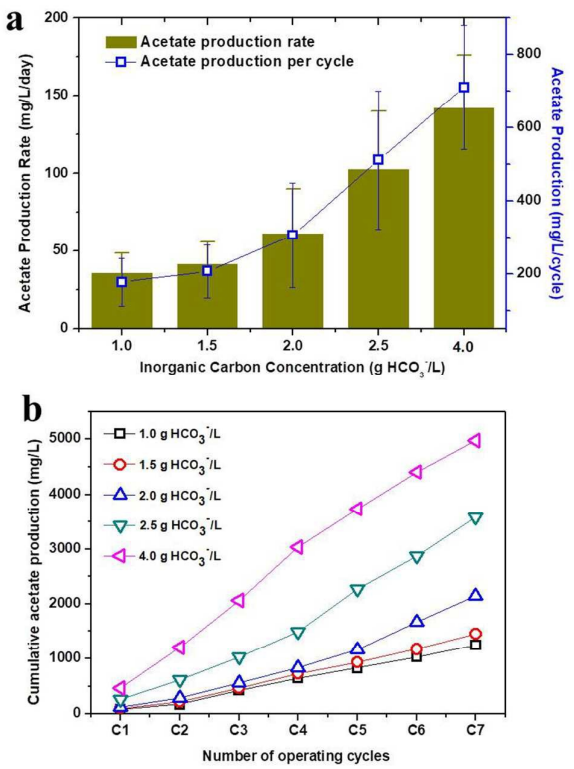

Figure 2: Bioelectrochemical reduction of carbon to acetate at different bicarbonate concentrations. (a) Both acetate production rate and acetate production in each cycle were represented along with the standard deviation; (b) cumulative acetate production from 7 consecutive batch cycles.

Conversion Efficiencies

Reduction Current and Coulombic Efficiency

Biocathodic reduction for the production of acetate was indicated by the reduction current at cathode. Chronoamperometry was used to quantify the reduction current and to correlate with the product in the cathode chamber. Current density was found to increase with time during startup of each experimental variation and showed stable current density from the third or fourth cycle of operation. Current density can be directly related to the reduction reaction that occurs at cathode involving primarily in acetate production[20]. Recorded current density values during 5 concentrations were increased with increase in the bicarbonate concentration (Fig 3). Similarly, acetate production rates were also increased with bicarbonate concentration in catholyte. Maximum current density of -101.2 mA m^{-2} (average, -91.8 mA m^{-2}) was recorded with 2.5 g HCO_3^- L^{-1} followed by 2.0 g HCO_3^- L^{-1} (-84 mA m^{-2}, average -76.20 m m^{-2}), 4.0 g HCO_3^- L^{-1} (-69.1 mA m^{-2}, average -53.8 mA m^{-2}), 1.5 g HCO_3^- L^{-1} (-63 mA m^{-2}, average -53.40 mA m^{-2}) and 1.0 g HCO_3^- L^{-1} (-42 mA m^{-2}, average -39 mA m^{-2}). Current density that resulted from the chronoamperometry was used to calculate the CE [5,29]. Among the five concentration variations, the average CEs of each concentration were found to vary. In case of 1.0 g HCO_3^- L^{-1} was showed 45.55% (average) of CE. When the bicarbonate concentration was shifted to 1.5 g HCO_3^- L^{-1}, a significant drop was recorded (39.56% CE). Subsequent gradual increase in bicarbonate concentration resulted in improvement in CE (2.0 g HCO_3^- L^{-1}, 40.37%; 2.5 g HCO_3^- L^{-1}, 56.25%). For 1.0 and 2.5 g HCO_3^- L^{-1}, CE was found stable among 7 operating cycles in each concentration depicting the stable performance of cathodic biofilm in accepting the electrons from the electrode surface to produce acetate. On the contrary, in the case of 4.0 g HCO_3^- L^{-1}, CE showed more fluctuations during seven cycles of operation. An improvement in the coulombic efficiency might be due to the availability in the bicarbonate concentrations in the catholyte. This might have helped to perform acetate producing biocathode more efficiently under suitable applied potentials. In another study, it was hypothesized that product selectivity and production rate can be influenced by the product concentrations and high current densities [39]. In the case of higher bicarbonate concentrations, where we obtained more acetate concentration, the reaction at the biocathode was more favorably disposed towards acetate production, most likely due to the higher concentration of bicarbonate which increased the conductivity [13] and facilitated the efficient use of hydrogen generated at cathode towards acetate production. Since this is the first study to test MES with increasing bicarbonate concentrations, further more detailed analysis is required to optimize the balance between the reduction current and its selective usage towards acetate production.

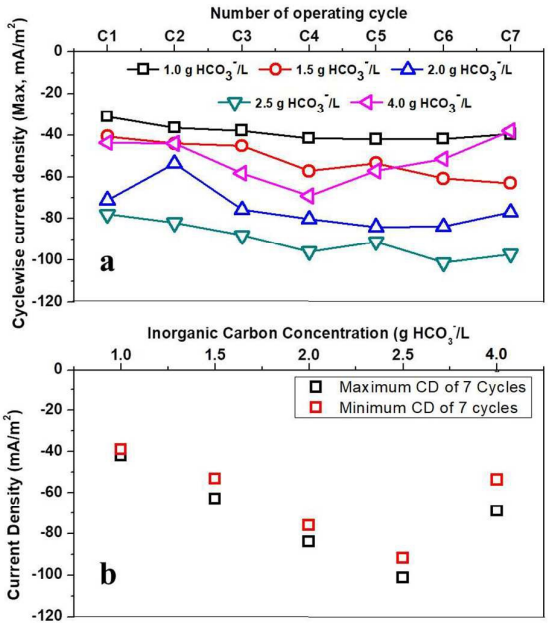

Figure 3: Maximum and average inorganic carbon conversion and conversion rate at each experimental variation.

Carbon Conversion Efficiency

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Carbon equivalents of bicarbonate were considered for the calculation of carbon conversion towards acetate production through biocathodic reduction reaction with the electron equivalents generated by applied potential. Carbon consumption during individual cycle was gradually increased with time which indicated that the acetate production was happening. The carbon consumption at the end of the 5 day of each cycle was increased with number of cycles of operation (Fig 4). The carbon consumption was found to increase with increase in the availability with substrate in the catholyte, which was in good correlation with acetate production. Highest average carbon consumption was recorded with 4.0 g HCO_3^- L^{-1} (600 mg L^{-1} $cycle^{-1}$) and the lowest was with 1.0 g HCO_3^- L^{-1} (123 mg L^{-1} $cycle^{-1}$). In the case of 1.5, 2.0 and 2.5 g HCO_3^- L^{-1} conditions, average carbon consumption were registered respectively as 138.8, 192.5 and 291.0 mg L^{-1} $cycle^{-1}$. Carbon equivalents calculated based on the produced acetate concentration in each cycle of operation and analyzed bicarbonate consumption were calculated through TOC were correlated to compute the carbon conversion efficiency (%). Except for 4.0 g HCO_3^- L^{-1}, all the experimental variations were documented more than 70% of the carbon bioelectrochemically reduced to acetate. However, the carbon conversion efficiency was found to vary based on the bicarbonate concentration. Average values from the 7 cycles of operation for each experimental variation helped for precise understanding of carbon conversion efficiency. All the average values were exhibited in a narrow range. Maximum carbon conversion efficiency of 84% was registered with 2.5 g HCO_3^- L^{-1}, followed by 2.0 g HCO_3^- L^{-1} (82%), 1.5 g HCO_3^- L^{-1} (75%), 1.0 g HCO_3^- L^{-1} (74%) and 4.0 g HCO_3^- L^{-1} (67%). The documented loss of bicarbonate majorly claimed for two factors. One, carbon conversion to acetate and another is subsequent conversion of acetate to biomass production in cathode chamber. It is also possible that higher evaporative loss of bicarbonate occurred at high concentrations due to the continuous sparging of N_2 at lower rate to catholyte to maintain anaerobic conditions.

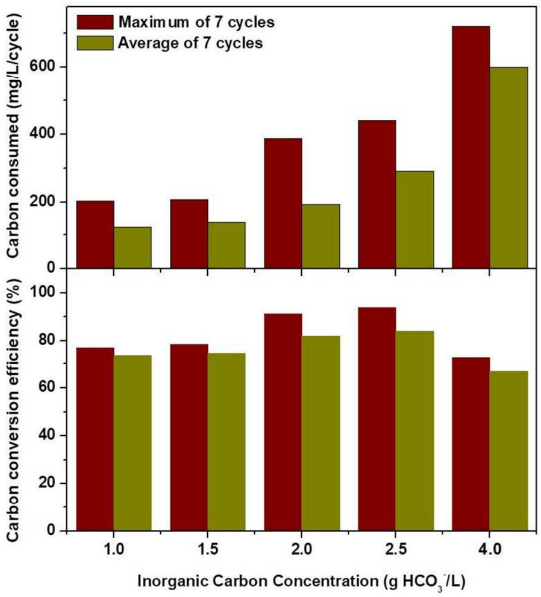

Figure 4: Carbon conversion and carbon conversion efficiency to acetate by the biocathodic reduction reaction at 5 different substrate concentrations

Product and substrate regulating cathodic pH

pH of the electrolyte is another critical factor that regulating bio-electrochemical reduction reaction. The optimum pH for homoacetogenic bacteria was identified as neutral to mild acidic conditions [38-39]. In case of bicarbonates also similar phenomenon follows. However, use of bicarbonates as substrate in the mild phosphate buffer in batch mode operation helps to control the pH as favorable for the biocathodic reduction reaction. Electrochemical potential window for both acetate and ethanol production from the CO_2 is found in a narrow range of 280 and 310 mV *vs* SHE (Eq 1 and 2). The produced acetate further can be converted to ethanol at standard electrochemical potential of 390 mV (Eq 3). In the present study, inlet pH of the catholyte was set to 7. With the time of operation the pH of the system increased due to the consumption of CO_2 and leave hydroxide (OH^-) ions in the electrolyte that stimulate mild alkaline conditions. At the same time, bioelectrochemically consumed CO_2 produces acetate, which create acidic environment. Theoretically, OH^- ions are stronger alkaline environment compared to acidic environment that can be created by the acetate ions. Thus, the resultant pH of the catholyte will be overall slightly alkaline. Moreover, stoichiometric production of one mole of acetate leaves two moles of hydroxide ions in the catholyte. In the present study, during the biocathodic reduction process, inlet pH of catholyte 7.0 was changed differently depending on the concentrations of bicarbonate (Fig 5). In the case of lower bicarbonate concentrations such as 1.0 and 1.5 g HCO_3^- L^{-1}, pH of the catholyte was gradually increased toward alkaline

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conditions with time of operation. By end of the 5th day of operation, final pH reached 7.8±0.16 and 8.24±0.21 respectively for 1.0 and 1.5 g HCO_3^- L^{-1}. In the case of 2.0 conditions, the pH was increased at higher rate and reached 8.6±0.38. Interestingly, in the case of 2.5 and 4.0 HCO_3^- L^{-1}, pH was increased up to 30 h of operation (7.59±0.15, 2.5 HCO_3^- L^{-1} and 7.71±.20, 4.0 HCO_3^- L^{-1}). Later it showed a slow decrease in pH and stabilized at neutral to mild acidic conditions (at the end of the cycle, 6.98±0.41, 2.5 g HCO_3^- L^{-1} and 6.76±0.25, 4.0 g HCO_3^- L^{-1}) (Fig 5). This might be due to the high amount of acetate production which can triggers acidic conditions in the system. Compared to 2.5 g HCO_3^- L^{-1} condition, 4.0 g HCO_3^- L^{-1} condition has showed high and rapid drop in pH, where it exhibited acidic conditions by 48 h of operation. Both substrate and product concentrations were found to be regulating the pH of the catholyte for the favorable biocathodic reduction conditions, which also suggested that operating at 2.5 and 4.0 g HCO_3^- L^{-1} suitable for acetate production from inorganic carbon source. It was also identified that more alkaline pH is detrimental to the cathodic biofilm and biofilm stability can be increased at neutral to mild acidic conditions [5].

$$2\,CO_2^- + 8\,e^- + 8\,H^+ \rightarrow CH_3COOH + 2H_2O \quad (-280\ mV\ vs\ SHE) \quad \text{----- (Eq 1)}$$

$$2CO_3^- + 12\,e^- + 12\,H^+ \rightarrow CH_3CH_2OH + 3\,H_2O \quad (-310\ mV\ vs\ SHE) \quad \text{--- (Eq 2)}$$

$$CH_3COOH + 4\,e^- + H^+ \rightarrow CH_3CH_2OH + H_2O \quad (-390\ mV\ vs\ SHE) \quad \text{----- (Eq 3)}$$

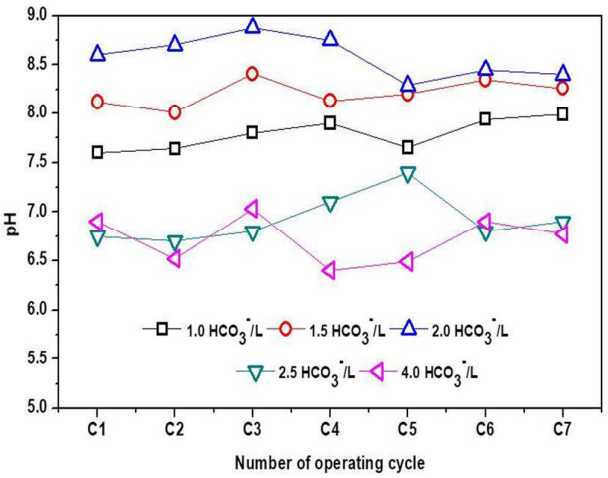

Figure 5: Cycle wise changes in the pH of the catholyte during bicarbonate reduction to acetate for five substrate concentrations studied.

It was understood that substrate and product concentrations influence the pH conditions of the catholyte. Neutral pH was found to be favorable for the effective substrate conversion to product[43]. Acidic conditions of catholyte also elicit solventogenesis. The tendency of the catholyte towards acidic conditions was higher when at higher bicarbonate concentration and increase acetate concentration in a cycle of operation. Apart from acidic pH, acetate concentration also triggers the solventogenesis. Here, ethanol was produced in catholyte at minor concentrations (Fig 6). At lower concentrations of bicarbonates, ethanol production was found to be negligible (1.5 g HCO_3^- L^{-1}, 8±5 mg ethanol L^{-1}; 2.0 g HCO_3^- L^{-1}, 16±4 mg ethanol L^{-1} at 5th day of operation). However, at 1.0 g HCO_3^- L^{-1}, ethanol concentration was not detectable. In the case of 2.5 g HCO_3^- L^{-1}, ethanol concentration increased to 18±8 mg L^{-1}. Maximum ethanol production of 65±15 mg L^{-1} was identified at 4.0 g HCO_3^- L^{-1} (Fig 6). Sudden increase in ethanol concentration with 4.0 g HCO_3^- L^{-1} might be due to the above mentioned two factors *viz*, acidic pH and higher acetate concentration that triggers solventogenesis. In the biochemical evaluation for VFA and ethanol through HPLC analysis, no other multicarbon organic acids such as propionate and butyrate were found in the catholyte suggesting that the system was not supported for chain elongation [44]. Production of ethanol along with the acetate hampers the product specificity and coulombic efficiency. Homoacetogenic bacteria that developed from mixed consortia used as the biocathode for acetate production is effective in neutral to mild alkaline pH range [45]. However, prevailing mild acidic conditions at 2.5 and 4.0 g HCO_3^- L^{-1} conditions are favorable for ethanol production promotes further conversion of acetate to ethanol. Along with solventogenesis, it is also possible that direct conversion of CO_2 to ethanol in bioelectrochemical way[1,13,46].

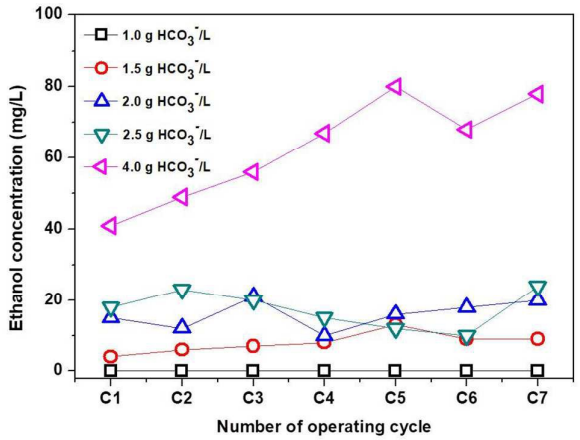

Figure 6: By-product production along with acetate production during bioelectrochemical reduction of bicarbonate.

Adaptability of biocathode at varied substrate conditions and its stability

The stability of electroactive biofilm is very much important for the bioelectrochemical systems. Along with the biofilms, it was identified that bacterial cells present in suspension also contributes for the electrochemical reduction reaction [8,17,33,47]. Biofilm formed on the cathode acts as catalyst and triggers all the bioelectrochemical reactions. It was observed that bioelectrochemical reactions are effective with biofilm formed

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on electrode rather than biomass present in the suspension. Stable biofilm which can sustain at varied environmental conditions is crucial and limits the overall performance of the MES system. After operating the MES with 4.0 g HCO_3^- L^{-1} and identified that drop in performance, the system shifted back to 2.5 g HCO_3^- L^{-1} and operated under similar conditions to regain the performance. Soon after recovery, gradually the system was operated with the higher bicarbonate concentrations such as 4.0, 8.0 and 12.0 g HCO_3^- L^{-1}. At the bicarbonate concentrations of 8.0 and 15.0 g HCO_3^- L^{-1}, the system was found to inhibit completely. Further, same system was operated at 2.5 g HCO_3^- L^{-1}, so the system was shifted back to 2.5 g HCO_3^- L^{-1}, where it regained the performance and showed similar productivity in terms of acetate production rate, coulombic efficiency, carbon conversion efficiency. However, it took 4 cycles of operation (20 days) to regain the process.

The system was also analyzed for the adaptability of the MES process during gradual changing the feed to higher concentration. Gradual increment in bicarbonate concentration in the system showed faster adaptability. The adaptability was majorly considered for stable current density and acetate production rate. In case of 1.0 to 1.5 g HCO_3^- L^{-1}, the system showed stable acetate production rate within 3 cycles of operation. In the case of bicarbonate concentrations of 2.0 and 2.5 g HCO_3^- L^{-1}, stable system performance was attained by 4^{th} cycle of operation. In contrast to the lower bicarbonate concentrations, 4.0 g HCO_3^- L^{-1} didn't show stability even up to 7 cycles of operation.

Conclusions

Microbial electrosynthesis of acetate with five substrate concentrations evidenced that acetate production rate was influenced by the available bicarbonate in the catholyte. Bicarbonate concentration and the produced acetate concentration in the catholyte were regulating the pH and buffering nature of the catholyte. Neutral pHs were sustained with 2.5 g HCO_3 L^{-1} concentrations. As the bicarbonate concentration was increased to 4.0 g HCO_3 L^{-1}, total performance of the system was hampered and it led to an increased ethanol production. The coulombic and carbon conversion efficiencies were also found to depend on the substrate loading conditions. Biofilm stability and product specificity with different loading conditions signified practical feasibility of the bioelectrosynthesis process. Further increase in higher carbon loading conditions might lead to instable process. However, to achieve improved process efficiencies at high carbon loading rates, it is required to focus the research on microbial consortia that can tolerate high carbon conditions or hypersaline environment.

Conflicts of interest

There are no conflicts to declare.

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References

1 K. Rabaey, R. A. Rozendal, *Nat. Rev. Microbiol.* 2010, **8**(10), 706-716.
2 S. Bajracharya, R. Yuliasni, K. Vanbroekhoven, C. J. Buisman, D.P. Strik, D. Pant, *Bioelectrochem.* 2017, **113**, 26-34.
3 T. Ueki, K.P. Nevin, T.L. Woodard, D.R. Lovley, *MBio*, 2014, **5**(5), e01636-14.
4 F. Kracke, J. O. Krömer, *BMC bioinformatics*, 2014, **15**(1), 410.
5 G. Mohanakrishna, J. S. Seelam, K. Vanbroekhoven, D. Pant, *Faraday Disc.* 2015, **183**, 445-462.
6 G. Mohanakrishna, K. Vanbroekhoven, D. Pant, *J CO_2 Utilization*. 2016, **15**, 57-64.
7 N. Faraghiparapari, K. Zengler, *J. Chem. Technol. Biotechnol.* 2017, **92**(2), 375-381.
8 S. Bajracharya, S. Srikanth, G. Mohanakrishna, R. Zacharia, D. P. Strik, D. Pant, *J. Power Sources*, 2017, **356**, 256-273.
9 K. P. Nevin, S.A. Hensley, A. E. Franks, Z.M. Summers, J. Ou, T. L. Woodard, D.R. Lovley, *Appl. Environ. Microbiol.* 2011, **77**(9), 2882-2886.
10 Y. Gong, A. Ebrahim, A. M. Feist, M. Embree, T. Zhang, D. Lovley, K. Zengler, *Environ. Sci. Technol.* 2012, **47**(1), 568-573.
11 M. Sun, L.F. Zhai, W.W. Li, H.Q. Yu, *Chem. Soc. Rev.* 2016, **45**(10), 2847-2870.
12 D. Liu, T. Zheng, C.J. Buisman, A. Ter Heijne, *ACS Sustainable Chem. Eng.* 2017, **5** (12), 11346–11353.
13 H. Luo, P. Xu, T.M. Roane, P.E. Jenkins, Z. Ren, *Bioresour. Technol.* 2012, **105**, 60-66.
14 R. Ganigué, S. Puig, P. Batlle-Vilanova, M.D. Balaguer, J. Colprim, *Chem. Comm.* 2015, **51**(15), 3235-3238.
15 J. Sadhukhan, J.R. Lloyd, K. Scott, G. C. Premier, H.Y. Eileen, T. Curtis, I.M. Head, *Renew. Sustainable Energy Rev.* 2016, **56**, 116-132.
16 M. Siegert, M.D. Yates, A.M. Spormann, B.E. Logan, *ACS Sustainable Chem. Eng.* 2015, **3**(7), 1668-1676.
17 P. Dürre, B.J. Eikmanns, *Curr. Opin. Biotechnol.* 2015, **35**, 63-72.
18 May, H. D.; Evans, P. J.; LaBelle, E. V. *Curr. Opin. Biotechnol.* 2016, **42**, 225-233.
19 S. Gildemyn, K. Verbeeck, R. Slabbinck, S.J. Andersen, A. Prévoteau, K. Rabaey, *Environ. Sci. Technol. Lett.* 2015, **2**(11), 325-328.
20 C. G. Giddings, K.P. Nevin, T. Woodward, D.R. Lovley, C.S. Butler, *Frontiers Microbiol.* 2015, 6. doi: 10.3389/fmicb.2015.00468.
21 M. Cui, H. Nie, T. Zhang, D. Lovley, T. P. Russell, *Sustainable Energy Fuels*. 2017, 1, 1171-1176.
22 S. Roy, A. Schievano, D. Pant, *Bioresour. Technol.* 2016, **213**, 129-139.
23 S. A. Patil, S. Gildemyn, D. Pant, K. Zengler, B. E. Logan, K. A. Rabaey, *Biotechnol. Adv.* 2015, **33**(6), 736-744.
24 J. Desloover, J.B. Arends, T. Hennebel, K. Rabaey, *Biochem. Soc. Trans.* 2012, **40**, 2133-1238.
25 R. Moscoviz, J. Toledo-Alarcón, E. Trably, N. Bernet, *Trends in Biotechnol.* 2016, **34**(11), 856-865.

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DOI: 10.1039/C7RE00220C

26 M. Sharma, S. Bajracharya, S. Gildemyn, S.A. Patil, Y. Alvarez-Gallego, D. Pant, *Electrochimica Acta* 2014, **140**, 191-208.
27 C.W. Marshall, D.E. Ross, E.B. Fichot, R.S. Norman, H.D. May, *Appl. Environ. Microbiol.* 2012, **78**(23), 8412-8420.
28 S. Bajracharya, M. Sharma, G. Mohanakrishna, X. D. Benneton, D.P. Strik, P.M. Sarma, D. Pant, *Renew. Energy* 2016, **98**, 153-170.
29 L. Jourdin, S. Freguia, B. C. Donose, J. Chen, G.G. Wallace, J. Keller, V. Flexer, *J. Mat. Chem. A*, 2014, 2(32), 13093-13102.
30 K. P. Nevin, T. L. Woodard, A. E. Franks, Z. M. Summers, D.R. Lovley, *MBio* 2010, **1**(2), e00103-10.
31 C. W. Marshall, D. E. Ross, E. B. Fichot, R. S. Norman, H. D. May, *Environ. Sci. Technol.* 2013, **47**(11), 6023-6029.
32 E.V. LaBelle, H.D. May, *Frontiers in Microbial.* 2017, **8**. *Article 756.*
33 J. A. Modestra, S.V. Mohan, *J. of CO_2 Utilization* 2017, **20**, 190-199.
34 S. A. Patil, J. B. Arends, I. Vanwonterghem, J. Van Meerbergen, K. Guo, G. W. Tyson, K. Rabaey, *Environ. Sci. Technol.* 2015, **49**(14), 8833-8843.
35 S.V. Mohan, G. Mohanakrishna, S.V. Raghavulu, P.N. Sarma, *Int. J. Hydrogen Energy* 2007, **32**(15), 3284-3292.
36 S. Bajracharya, A. ter Heijne, X.D. Benetton, K. Vanbroekhoven, C.J. Buisman, D.P. Strik, D. Pant, *Bioresour. Technol.* 2015, **195**, 14-24.
37 D.R. Lide, *Handbook of Chemistry and Physics,* 81st Ed; CRC, Boca Raton: FL, U.S.A., 2000.
38 C. Costentin, M. Robert, J.M. Savéant, A. Tatin, *PNAS*. 2015, **112**(22), 6882-6886.
39 S.D. Molenaar, P. Saha, A.R. Mol, T.H. Sleutels, A. ter Heijne, C.J. Buisman, *Int. J. molecular sci.* 2017, **18**, 204.
40 S.E. Oh, S. Van Ginkel, B.E. Logan, *Environ. Sci. Technol.* 2003, **37**(22), 5186-5190.
41 G. Mohanakrishna, S.V. Mohan, *Appl. Energy.* 2013,**107**, 244-254.
42 K. Rabaey, P. Girguis, L.K. Nielsen, *Curr. Opin. Biotechnol.* 2011, **22**(3), 371-377.
43 E.V. LaBelle, C.W. Marshall, J.A. Gilbert, H.D. May, *PLoS One*. 2014, **9**(10), e109935.
44 A. Schievano, T.P. Sciarria, K. Vanbroekhoven, H. De Wever, S. Puig, S.J. Andersen, D. Pant, *Trend. Biotechnol.* 2016, **34**(11), 866-878.
45 F. Ammam, P.L. Tremblay, D.M. Lizak, T. Zhang, *Biotechnol. for Biofuels*. 2016, **9**(1), 163.
46 D.R. Lovley, K.P. Nevin, *Curr. Opin. Biotechnol*. 2013**, 24**(3), 385-390.
47 S. Bajracharya, K. Vanbroekhoven, C.J. Buisman, D. Pant, D.P. Strik, *Environ. Sci. Pollution Res.* 2016, **23**(22), 22292-22308.