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Long-term operation of electro-biocatalytic reactor for carbon dioxide transformation into organic molecules

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Abstract

Electro-biocatalytic reactor was operated using selectively enriched mixed culture biofilm for about 320 days with CO_2/bicarbonate as C-source. Biocathode consumed higher current (-16.2±0.3 A/m^2) for bicarbonate transformation yielding high product synthesis (0.74 g/l/day) compared to CO_2 (-9.5±2.8 A/m^2; 0.41 g/l/day). Product slate includes butanol and butyric acid when CO_2 gets transformed but propionic acid replaced both when bicarbonate gets transformed. Based on electroanalysis, the electron transfer might be H_2 mediated along with direct transfer under bicarbonate turnover conditions, while it was restricted to direct under CO_2. Efficiency and stability of biofilm was tested by removing the planktonic cells, and also confirmed in terms of Coulombic (85-97%) and carbon conversion efficiencies (42-48%) along with production rate (1.2-1.7 kg/m^2 electrode) using bicarbonate as substrate. Selective enrichment of microbes and their growth as biofilm along with soluble CO_2 have helped in efficient transformation of CO_2 upto C4 organic molecules.

Keywords: Microbial electrosynthesis (MES); bio-electrochemical system (BES); CO_2 capture and reduction; Butanol; Butyric acid

1.0 Introduction

Increasing pollution loads due to the industrial revolution resulted in about 40% rise in the atmospheric concentration of carbon dioxide (CO_2) (IPCC, 2013). Main source of CO_2 is from anthropogenic human activities, i.e combustion of conventional fossil fuels such as wood, coal, oil, and natural gas, etc (Bajracharya et al., 2017a; Leung et al., 2014). In this context, researchers across the globe are in search of carbon neutral ways to meet the escalating energy demands as well as reducing the pollution loads on environment through capturing renewable energy and valorizing the waste organics (Srikanth et al., 2016). Alternatively, CO_2 capture and transformation is also being considered very seriously to reduce the CO_2 emissions at source itself. Among various strategies adapted for the CO_2 transformation, electro-biocatalysis is a novel and promising approach to store the electrical energy into chemical energy (Jourdin et al., 2014; Marshall et al., 2013a). Supply of small amount of external energy from renewable sources like solar, wind, etc., can alter the microbial redox reactions and help the bacteria to meet the thermodynamic energy required to transform CO_2 resulting in different product formations (Zaybak et al., 2013). This electro-biocatalysis is termed as microbial electrosynthesis (MES) and is a process driven by the synergistic action of biotechnology and electrochemistry. The chemoautotrophic electro-active microbes uptake the electrons from electrodes to transform CO_2 to value-added multi-carbon products (Lewis and Nocera, 2006; Lovley and Nevin, 2013; Nevin et al., 2011; Rabaey et al., 2011; Rabaey and Rozendal, 2010).

After the inception of this concept in 2010 (Nevin et al., 2010), several studies till date have been reported the product synthesis using CO_2 as sole C-source but major products reported include acetic acid (Marshall et al., 2013b, 2012; Steinbusch et al., 2010), where the higher product titre reported went upto 11 g/l using mixed culture (Jourdin et al., 2016). Thermodynamic equilibrium

suggests that microbes can produce longer chain organic compounds from CO_2 through electro-biocatalysis (Rabaey and Rozendal, 2010; Venkata Mohan et al., 2014; Aryal et al., 2017) but till date, the production of C4 compounds only demonstrated that too as minor constituents along with acetate and ethanol (Bajracharya et al., 2017b, 2016; Batlle-Vilanova et al., 2017; Ganigue et al., 2015; Arends et al., 2017). CO_2 bioavailability, reducing equivalents availability (Batlle-Vilanova et al., 2017; Mohanakrishna et al., 2016) as well as the selective microbial consortia (Modestra et al., 2015; Mohanakrishna et al., 2015; Patil et al., 2015a), operating pH, retention time (Ganigue et al., 2015; Arends et al., 2017), etc., are the key factors controlling the product slate and titers. Producing longer chain alcohols and organic acids can address the energy issue along with reducing more CO_2. The longer chain alcohols like ethanol and butanol can be used as drop in fuels in the existing fuel sources. Similarly, it was believed that the role of planktonic cells is crucial for H_2 evolution during electro-biocatalysis (Modestra et al., 2015; Bajracharya et al., 2015; Mohanakrishna et al., 2015), which results in lower coulombic efficiencies (CE) and mass transfer issues. In this direction, an attempt was made in the current study to grow a stable biofilm which can work effectively for the CO2 transformation into organic moities. To the best of our knowledge, present work depicted the possibility of producing multiple products in higher titres from CO_2, for the first time.

2. Materials and methods

2.1. Biocatalyst

The source inoculum was obtained from corroded metal surface and was initially enriched on specific media of sulfate reducing bacteria (SRB) and iron reducing bacteria (IRB) separately for 3 repeated cycles each with 48h retention time. SRB is known for the alcohol production (Sharma et al., 2013), while the IRB is known for the electron exchange (Bond and Lovley, 2003; Nevin et al., 2008; Schröder, 2007) with solid electron acceptors. Further, both these

cultures mixed in equal ratio (1:1) and were selectively enriched using specific media having trace metals (NH4Cl - 0.5 g/l, MgSO4 - 0.3 g/l, CoCl2 -25 mg/l, ZnSO4 -11.5 mg/l, CuSO4-10.5 mg/l, CaSO4- 5 mg/l, MnSO4 - 15 mg/l; NiSO4 -16 mg/l; FeSO4-25 mg/l) and bicarbonate as C-source, formic acid as energy carrier under CO_2 partial pressure. The enrichment was carried out for 6 cycles each with 72 h and the enriched culture was pelletted out into phosphate buffer (PBS, pH 7.4) before inoculation into the working electrode chamber.

2.2. Reactor construction

Dual chambered custom made glass reactor (total/ working volume, 0.28/0.20 l) was used for the experiment. Plain graphite plate loaded with carbon powder (29.8 cm^2) was used as working electrode (working electrode, cathode) and activated carbon cloth attached to graphite rod (54.2 cm^2) was used as counter electrode (counter electrode, anode). Proton exchange membrane (Nafion117, Sigma-Aldrich) was used to separate both the chambers. Stainless steel wires were used as current collectors for both the electrodes. Leak proof sealing was employed to maintain anaerobic microenvironment in the working electrode compartment. Provision was made in the design for sampling ports. Both the working electrode and counter electrode chambers were equipped with Ag/AgCl (3 M KCl) reference electrode (0.197 V vs. SHE). Reactor operation was carried out for about 320 days and the output was monitored in terms of current consumption in chronoamperometry (CA) and product formation. The details of the reactor configuration and operational parameters of the experiment were presented in Table 1.

2.3. Start-up and operation

The working electrode compartment of reactor was inoculated with enriched mixed culture along with PBS and trace metal solution. PBS added with NaCl (1 g/l) was used as electrolyte in the counter electrode (pH 6±0.1). Both the electrodes were completely immersed in the respective

electrolytes. CO_2 was sparged for about 30 min and then the inoculum was added to working electrode chamber. Experiments were carried out under strict anaerobic condition at ambient temperature (29 ± 2^0C). Every day prior to start-up of CO_2 sparging, pH of the electrolyte in working electrode chamber was monitored and adjusted to 8±0.1 using 1N NaOH. Experiment was carried out in 4 phases, viz., biofilm stabilization phase, CO_2 phase, bicarbonate phase and confirmation phase. During both biofilm stabilization and CO_2 phases, CO_2 was used as sole C-source, while bicarbonate was used as C-source during bicarbonate and confirmation phases. The whole content of the working electrode chamber was replaced with fresh PBS before each phase of operation for first 3 phases, while the content of the working electrode chamber was replaced with fresh PBS after every 25 days during confirmation phase to assess the biofilm capability of CO_2 transformation. However, the trace metal solution was added at 15 days interval irrespective of the phase of operation. The working electrode chamber was continuously purged with CO_2 (99.999%) at a rate of 15 ml/min for 8h in a day during first two phases as C-source and it also helped to maintain anaerobic conditions. During next two phases (bicarbonate phase and confirmation phase), bicarbonate was used as C-source (at a rate of 1.2 g CO_2 equivalents/day) and sparged with N_2 (99.999%) to maintain anaerobic conditions. An outlet was provided at working electrode chamber for the removal of excess gas and the gas tube was immersed in 15 cm water column to maintain the gas pressure in the chamber and to avoid the gas exchange. Contents of both working and counter electrodes were mixed continuously on a magnetic stirrer at 350 rpm. Experiments were carried out in duplicate and the average of the results was presented here in the manuscript. Current consumption and product formation are the major criteria to assess the performance of BES. Electrosynthesis experiments were carried out in potentiostat control using potentiostat-galvanostat (Ivium, The Netherlands) system and the

working electrode potential was maintained at -1V vs Ag/AgCl (-795 mV Vs SHE). Unless stated otherwise, all potentials provided in this manuscript were against Ag/AgCl reference electrode (3 M KCl, 0.201 V vs SHE; ALS, Japan). Abiotic and biotic controls were also carried out along with the experiment, where all the operating conditions were similar to experiment except that the abiotic control was not inoculated with biocatalyst and the biotic control was not connected to the potentiostat for power supply.

2.4. Analysis and calculations

BES performance in terms of current consumption was monitored based on the current density obtained on CA at applied potential of -1 V. Electrochemical analysis of the BES was carried out through cyclic voltammetry (CV) and linear sweep voltammetry (LSV) at a scan rate of 1 mV/s in order to get mechanistic insights into the electrochemical reactions occurring at the working electrode. All the electrochemical analysis was done under turn-over (constant CO_2 purging or bicarbonate load) conditions. Both CV and LSV were performed over a range of -0.8 V to 0.4 V vs. Ag/AgCl at a scan rate of 1 mV/s using a potentiostat-galvanostat (IviumStat, The Netherlands). Electrolyte pH was monitored on daily basis prior to adjusting to 8.0 and before CO_2 sparging, using pH/conductivity meter. TOC/TIC was analyzed using TOC analyzer (Multi N/C 2100S from M/s Analytic jena) and the product analysis was done using gas chromatography (GC). A Perkin Elmer Clarus 500 GC with flame ionization detector (FID) was used for the carbon number distribution of control and bio treated samples. A non-polar capillary metal column 100 % dimethyl polysiloxane (60 m x 0.32 mm id x 0.25 µm film thickness) was used for the desired separation. The temperature programme was 35°C (0.5min)-10°C- 450°C (20min) to achieve separation using injector temperature 350^{o}C and detector temperature 350^{o}C with 2.0 ml/min column flow (He). Head-space gas was analyzed on a Gas Chromatograph

(NAT GAS-B analyzer) equipped with a thermal conductivity detector (TID) and 1.8 × 3.2 nm stainless-steel columns packed with molecular sieve 5A. Helium gas was used as carrier gas.

The CE, carbon conversion efficiency (CCE) and energy conversion were calculated as described in literature (Patil et al., 2015b). Integrated coulombs consumption over time relative to the maximum coulombs required to produce the exact quantity of each product formed was calculated as CE, while the ratio of carbon equivalents of CO_2/bicarbonate provided to the carbon equivalents of the formed product was calculated as CCE.

3.0 Results and Discussion

Electro-biocatalytic reactor was operated for 320 days in different phases using selectively enriched mixed culture as biocatalyst. During initial phase, the biocatalyst was allowed to form an efficient CO_2 transforming biofilm on electrode. Further, the efficiency of the biofilm was evaluated using CO_2 or direct bicarbonate as C-source.

3.1 Current consumption

Abiotic control operation showed about -0.8±0.3 A/m^2 without any significant change during 15 days of operation which is the catalytic current due to the active surface groups of the electrode. No product formation during this abiotic control operation strongly supports this observation. On the contrary, test experiment showed significant variation in current consumption on CA with respect to the experimental condition, time of operation and C-source. Immediately after start-up the current consumption was similar to the catalytic current observed during abiotic operation (-0.9±0.2 A/m^2) for about 10 days and then started varying due to the start-up of biocatalytic activities (Fig 1). Till 45 days, no significant increment in current consumption was observed except during 13th day and 31st day. Sudden increment was observed during these two days for

few hours and reached back normal due to the start-up of reduction reaction. After 45 days of operation, the current consumption was increased gradually and reached a maximum value (-13.8±0.9 A/m^2) between 85-95 days of operation. Increase in electron consumption pattern can be correlated to biofilm growth on cathode (Jourdin et al., 2014). However, there is constant fluctuation in current consumption throughout operation during this phase due to the varying reduction reactions because of the limitation in C-source solubility/availability to the biocatalyst. The current consumption started decreasing gradually from 98th day and reached to a lower value (-1.7±0.8 A/m^2) by 105 days of operation. This is because of product accumulation and change in buffering capacity. Till this day, the operation was considered as biofilm formation phase and further operation was continued using the biofilm grown on working electrode. Once, the current consumption was decreased, the whole content of working electrode chamber was replaced with new electrolyte (PBS at pH 8.0 along with trace metal solution) under aseptic anaerobic condition. This phase of operation was considered as CO_2 phase, where the CO_2 was used as sole C-source and the biofilm grown on electrode acted as biocatalyst. Immediately after a day of electrolyte change, the current consumption started increasing which indicates the active role of biofilm in reduction reaction. The reduction current gradually increased till 130th day and maintained the same (-7.6±2.5 A/m^2) till 150th day of operation followed by decrement for 4 days and increased again to a highest value (-13.7±1.6 A/m^2) of this phase by 165th day. However, the fluctuation in current consumption remained similar during this phase as well indicating the necessity of CO_2 solubility and constant availability to the biofilm. Further to this phase, the whole content of the working electrode chamber was again replaced with fresh electrolyte (PBS at pH 8.0 along with trace metal solution) along with bicarbonate as C-source instead CO_2 and considered this phase as bicarbonate phase. Bicarbonate spiking was continued

at regular time intervals to maintain the C-source availability. Immediately after the change in electrolyte and C-source, the current consumption increased (from -3.8 A/m^2 to -7.4 A/m^2) in a day indicating the impact of change in C-source availability to the biofilm. The current consumption remained then similar for about 25 days (-7.6±0.4 A/m^2) without any significant change. A gradual increment in reduction current started from 192nd day of operation and continued till 204th day reaching a maximum value of total operation (-16.4 A/m^2). Thereafter, no significant change in reduction current (-15.9±0.6 A/m^2) was observed till 250th day of operation. To confirm the ability of the biofilm stability, whole content of electrolyte from working electrode chamber was replaced again with the fresh electrolyte and bicarbonate was spiked. The current consumption was almost continued at same value as of it is a continuous mode operation till 275th day. The same was repeated again for two more cycles and observed the similar current consumption indicating the stability of the biofilm.

3.2 Microbial electrosynthesis of products

3.2.1 CO_2 as sole C-source

Product formation was monitored at regular time intervals throughout the operation irrespective of the experimental condition to assess the CO_2 transforming ability of the system (Fig 2). Samples were analyzed at every 3rd or 4th day of operation and a cumulative of 15 days was considered for discussion. Both the abiotic (without inoculums under applied potentials similar to test) and biotic (with similar biocatalyst but without applied potential) control experiments did not show any product formation within 15 days of operation. This is due to the non-availability of electron source for the reduction of CO_2 in case of biotic control and the absence of (bio)catalyst for utilizing the electron source to transform CO_2 into products in the case of abiotic control. The test experiment also didn't show any product till 50th day of operation but depicted

good amount of acetic acid (1.4 g/l) and ethanol (0.8 g/l) on 65th day indicating the start-up of CO_2 transformation. This was continued further and by 85th day different acids and alcohols were produced in significant quantity (g/l- methanol, 0.2; ethanol, 1.2; 1-butanol, 2; acetic acid, 0.8; butyric acid, 0.82). Further continuation of operation has resulted in accumulation of these products and by 120th day, 23.58 g/l of total products were accumulated (g/l- methanol, 3.2; ethanol, 0.98; 1-butanol, 3.6; acetic acid, 12.2; butyric acid, 3.6). At this juncture, whole content of the working electrode chamber was replaced with fresh PBS and operation continued. This allowed all the suspended bacterial population to be removed and the 120 days old biofilm started catalyzing the CO_2 transformation. This phase is considered as CO_2 phase where the biofilm on the electrode will be fed with CO_2 as sole C-source and its transformation was studied. After start-up of this phase, 3.6 g/l of total product including methanol (1.1 g/l) and acetic acid (2.5 g/l) was observed within 15 days supporting the activity of chemolithoautotrophic biofilm formed during 1st phase. Further operation has showed accumulation of about 18.2 g/l of total product (g/l- methanol, 1.4; ethanol, 5.2; 1-butanol, 2.8; acetic acid, 7.4; butyric acid, 1.4) in 45 days. Though, a very good amount of product formation was observed during this phase, the coulombic and carbon conversion efficiencies (CE and CCE) as well as product yields should also be considered for the assessment of effectiveness of the biocathode developed. The product yield as well as CE and CCE were calculated at regular time intervals based on total product formed and individual product titres respectively (Fig 3). Maximum product yield obtained during this phase was 1.36% with a CE and CCE of 84.87% and 1.01% respectively (Table 2). These values clearly indicated the requirement of solubilizing the CO_2 to enhance the product formation as well as improve the yields. Further to this,

bicarbonate was used as sole C-source in place of CO_2 to evaluate the system efficiency with solubilized CO_2.

3.2.2. Bicarbonate as sole C-source

The working electrode chamber was emptied completely and filled with fresh PBS along with trace metals and spiked with bicarbonate (1.2 g equivalents/day). Significant change in product concentrations and profile was observed after shifting from CO_2 to bicarbonate as C-source, depicting the impact of CO_2 solubilization (Fig 2). After 20 days of operation with bicarbonate as substrate the total product was about 8.4 g/l (g/l- methanol, 2.2; ethanol, 1.6; acetic acid, 2.4; propionic acid: 2.2) which further increased to 17.2 g/l (g/l- methanol, 6.4; ethanol, 3.7; acetic acid, 4.2; propionic acid: 2.9) in 35 days. Further operation has resulted in accumulation of 33.4 g/l product (g/l- methanol, 9.8; ethanol, 7.4; acetic acid, 11.4; propionic acid: 4.8) in 55 days which increased to 55.4 g/l (g/l- methanol, 15.7; ethanol, 13.0; acetic acid, 20.1; propionic acid: 6.6) in 75 days. However, further operation of BES has showed a decreasing product formation of 52.8 g/l (g/l- methanol, 16.1; ethanol, 9.7; acetic acid, 19.8; propionic acid: 7.2) at 85th day. The product yield during this phase started around 18% and increased to 30% in 60 days with a CE of 87-93% and CCE of 20-35% (Fig 3). At this juncture, whole content of the working electrode chamber was replaced with fresh PBS along with the trace metals to confirm the change in product concentrations and profile with bicarbonate as sole C-source. This phase was considered as confirmation phase and 3 cycles were operated each with 25 days of operation time for 2 cycles and 20 days for 3rd cycle. This time period was considered based on the product formation rate, which was analyzed at every 5 day interval time. The total product formed during the 1st and 2nd cycle was 25.4 g/l (g/l- methanol, 8.8; ethanol, 5.4; acetic acid, 9.1; propionic acid: 2.1) and 24.2 g/l (g/l- methanol, 9.1; ethanol, 4.8; acetic acid, 8.1; propionic acid: 2.2)

respectively, while in the 3rd cycle it was 17.9 g/l (g/l- methanol, 7.1; ethanol, 3.3; acetic acid, 5.7; propionic acid: 1.8) because of the reduced time of operation. Product yield during this phase was 37.5±1.6% along with the CE and CCE of 95±2.9% and 47.2±1.7% respectively.

3.3 Electroanalysis

Change in electrochemical behavior of BES with time was evaluated *in situ* employing cyclic voltammetry (CV). CV helps to detect the redox signals and permits the elucidation of possible oxidation and reduction reactions happening at the solution electrode interface and the electrochemical reactions occurring at the electrode surface (Patil et al., 2012; Srikanth and Mohan, 2012). Synergistic interaction of the biocatalyst with the electrode is the key aspect behind the electron exchange at electrode interface (Srikanth et al., 2011). CVs were periodically obtained under turnover conditions to electrochemically characterize the BES with respect to the operating conditions. Figure 4 represents CVs performed under turnover conditions, corresponding to the biofilm formation phase, CO_2 phase when the fully developed biofilm was given with CO_2 as sole C-source followed by the bicarbonate phase where the bicarbonate was provided as C-source. The other three CVs obtained from the confirmation phase, where the bicarbonate was used as substrate and the CO_2 transformation abilty of the biofilm was checked every 25 days by replacing the whole content of the working electrode chamber. Except the first CV, rest of all the CVs obtained during the phase where the biofilm was fully developed and BES is producing organic acids as well as alcohols from CO_2. Voltammograms (vs Ag/AgCl) visualized marked variation in the redox currents with the function of biofilm development, adaptability of the biofilm to form of CO_2 and its solubility as well as bioavailability. Reduction current was higher compared the oxidation current irrespective of the operating condition. Significant variation in the reduction and oxidation currents was observed between the CVs

obtained during biofilm phase, CO_2 phase and bicarbonate phases as well as confirmation phase. Both oxidation and reduction currents were near baseline during biofilm formation phase but a small increment current consumption was observed at -0.66 V. Aftre the biofilm development, the CV obtained with CO_2 as C-source has showed an increased current demand at -0.4 V. A sudden increase of the current demand was observed on both the oxidation and reduction side, when the C-source shifted from CO_2 to bicarbonate but after stabilization, in the confirmation, the oxidation current demand reduced near to base line and the steep increment in reduction current observed at -0.2 V. The CV shape obtained during confirmation phase is typically linked to the catalytic production of H_2 (Batlle-Vilanova et al., 2017; Jourdin et al., 2015), which was not in the case of the other phases of operation. This can be attributed to the CO_2 transformation via direct electron transfer during the CO_2 phase, while it was through hydrogen during bicarbonate and confirmation phases. With the time of adaption to bicarbonate as substrate, the reduction peak increased showing the H_2 evolution and its participation in the CO_2 transformation. Though, significant H_2 accumulation is not observed in the headspace of the reactors, this does not mean to exclude the possibility of H_2-mediated electron exchange between the electrode and microbe (Jourdin et al., 2015). Alternatively, electrons could also be delivered directly from the electrode or trough other soluble mediators.

3.4 Discussion

MES is a recent innovation, with only a few studies demonstrated the process at a lab scale using either pure cultures (Giddings et al., 2015; Nevin et al., 2010, 2011; Zhang et al., 2013) or mixed cultures (Modestra et al., 2015; Batlle-Vilanova et al., 2016; Jourdin et al., 2014; Marshall et al., 2013b; Mohanakrishna et al., 2015; Patil et al., 2015a; Su et al., 2013; Tremblay and Zhang, 2015). But the use of mixed culture is attractive because they are readily obtainable in large

quantities, are more tolerant to environmental stress and fluctuations and showed higher production rates till date over long-term operation (Bajracharya et al., 2017b; Marshall et al., 2013b; Arends et al., 2017; Modestra et al., 2017). Nature of biocatalyst and its metabolic efficiencies strongly influence the CO_2 transformation ability of BES. CO_2 transformation requires a strong energy source that can be obtained from hydrogen directly or hydrogen carrying primary/secondary metabolites or from redox equivalents (H^+ and e^-). Only few bacteria in nature have the ability to exchange electrons with solid electrodes, especially to uptake the electrons from external source. The microbe with ability to uptake electrons from external source as well as utilize CO_2 as C-source is prime requisite for this process. The electroactive biofilms of chemolithoautotrophic bacteria with strong CO_2 reducing ability are most suitable for MES. Different autotrophic bacteria such as Clostridium ljungdahlii, Moorella thermoacetica, Sporomusa ovate, etc., as well as selectively enriched homoacetogenic biofilms were reported for the successful production of acetate from electrode driven CO_2 fixation (Modestra et al., 2017; Marshall et al., 2012; Mohanakrishna et al., 2015; Nevin et al., 2010; Patil et al., 2015a). Most of the studies reported depicted invariantly acetate as major product along with ethanol and in very few studies, butanol or butyric acids or isopropanol were reported in negligible quantities (Zaybak et al., 2013; Ganigue et al., 2015; Arends et al., 2017). However, the present study has depicted diverse range of products including butanol, butyric acid and propionic acid in significantly higher quantities compared to literature. The selectively enriched biocatalyst used in the present study was having the ability of electron exchange and utilizing CO_2/bicarbonate as sole C-source along with formic acid as energy carrier. Providing the required nutrients on weekly basis helped the bacteria to meet the basic requirements of metabolic activities, which lead to more efficient product synthesis.

On the other hand, pH appeared to have strong influence on product synthesis rates as well as current demand (Batlle-Vilanova et al., 2016; LaBelle et al., 2014). Keeping the pH acidic was shown to increase the production of acid intermediates (Batlle-Vilanova et al., 2016), while basic pH increases the CO_2 solubility and bioavailability. Operating pH of the biocathode chamber was adjusted to near 8 on daily basis to maintain the CO_2 solubility and its availability to biocatalyst. However, the pH was observed to be decreasing every day before adjustment to 8.0. Figure 6 depicts the change over in the pH during operation, even after adjusting to 8.0, which is due to the producing acid intermediates as well as CO_2 sparging. This pH adjustment on daily basis helped in increased CO_2 solubility, which might have triggered different metabolic pathways of the microbes resulting in diverse product profile. The pH curve shows a gradual drop during biofilm formation phase and the drop in pH was faster once, the product synthesis started. After 85^{th} day of operation, the acetate production reached to 8.1 g/l and further to 12.2 g/l, which reflected in pH drop upto 5.5. In similar way, the change in product profile reflected in pH and this drop in pH also might have triggered new metabolic pathways towards more reduced end products like alcohols and higher carbon chain organics. The current consumption was also reduced, once the acid intermediates concentration increased, for instance after 150^{th} day during CO_2 phase. This decrement in current can be correlated to the drop in pH due to the accumulation of acid intermediates which results in lower CO_2 solubility and thus hindering the reduction reaction. This might have triggered the shifting of microbial metabolism towards alcohol production and then the current consumption increased rapidly resulting in alcohol synthesis. Accumulation of acid intermediates coupled with lower pH disturbs the cytosolic pH of microbes by free flow of undissociated acids into cytosol and creates a deficiency of available

redox shuttlers that ultimately leads to the triggering of solventogenesis (Srikanth and Venkata Mohan, 2014; Ganigue et al., 2016).

Detailed product profiling depicted a clear demarcation in the product synthesis with CO_2 as C-source and bicarbonate as C-source. Propionic acid was absent when the biocathode was fed with CO_2 as C-source but a high value butanol and butyric acid were produced. Acetate synthesis in MES was proven through reductive acetyl Co-A/Wood-Ljungdahl pathway (Schuchmann et al., 2014; Arends et al., 2017). Extending the carbon chain length to butyric acid and butanol via linear extension of acetyl moiety or through reverse β-oxidation was also reported (Raes et al., 2016). Moreover, the outlet for the head-space gas was immersed in a water column of 15 cm to maintain the partial pressure at the biocathode. Thus, the partial pressure of CO_2 was maintained at biocathode, when the CO_2 is used as C-source under which the chain elongation of the acetate to butyrate occurred and resulted in more pH drop. Contrast to this, when bicarbonate was used as C-source, the carbon chain length stopped at C3 (propionic acid) only and no butyric acid or butanol was produced. Unlike the first two phases of operation with CO_2 as C-source, during biocarbonate phase, a rapid or faster drop in pH was observed to a lower value, which doesn't allow the chain elongation and rather triggers the solventogenesis to regain the cytosolic pH. Moreover, when bicarbonate was used as C-source, H_2 production was also observed in head-space gas. The head-space gas was measured at regular time intervals and found H2 (1-1.5%) along with CO_2, when bicarbonate was used as C-source, while H2 was absent during CO_2 phase. This can be well correlated with the CVs obtained, where the H2 peak was visualized only when bicarbonate was used as C-source. Increased ionic strength of the electrolyte due to the Na+ ions present in the bicarbonate might be supporting the H_2 evolution, which was not in the

case of CO_2 sparging. The propionate is synthesized via acetoactyl Co-A after losing one CO_2 moiety which helped in maintaining the head-space CO_2. Hence, there is partial pressure maintenance of both CO_2 and H_2 at the head-space of working electrode chamber when bicarbonate was used as substrate. The availability of H_2 as energy carrier solventogenesis is easier to be triggered. Higher ethanol and methanol titres observed with bicarbonate than CO_2 are strong evident for this phenomenon. The methanol synthesis is one of the fastest routes for microbes to overcome the proton gradient issues and it occurs at -0.38 V vs SHE with consuming 6 reducing equivalents. When CO_2 and H_2 are available, synthesis of methanol is a three step reaction, where the CO_2 is converted to formic acid, then to formaldehyde and finally to methanol (Srikanth et al., 2017). The estimated inorganic carbon using TOC analyzer also showed higher value of available carbon during bicarbonate phase, as expected. Higher partial pressure of CO_2 and H_2 might also have triggered the methanol synthesis pathway to escape the burden on cytosolic pH.

In similar lines, if we consider the product titres, significant increment in product titres was observed when C-source shifted to bicarbonate. Percentage occupancy of different products showed that irrespective of the C-source, 50% of the product profile composed of alcohols but the butanol contributed for the make-up when CO_2 was used, while methanol and ethanol alone managed the alcohol fraction when bicarbonate was used. Methanol concentration was on higher side (35±5%) with bicarbonate, while it was low (17±5%) with CO_2 as C-source. The pattern of CE and CCE also considerably varied with time and C-source. The CCE was negligible when the CO_2 is considered as substrate because of the solubility and bioavailability issue. CCE was calculated based on the input CO_2 which is quite higher and hence, even the product formed was

on higher side compared to literature, the CCE depicted a lower value around 1%. When similar calculation was done using input bicarbonate, the CCE reached upto 46±2% by the time of stabilization.

On the contrary, the CE was always on higher side irrespective of the CO_2 or bicarbonate. CE was calculated based on the current demand and final concentration of products and it varied between 75-97% which is quite high and indicates that most of the coulombs consumed by the biocathode are more or less used for the product formation along with microbial growth. Two major electron sinks hypothesized for the electron loss in MES include, consumption of electrons for H2 production at cathode and scavenging due to oxygen (Batlle-Vilanova et al., 2017). In this view, the H2 production is low based on the head-space gas analysis and strict anaerobic condition at biocathode through maintaining partial pressure, contributed in reducing the electron losses and in turn increasing the CE. The head-space H2 was also considered for calculating the CE but was insignificant when compared to the coulombs consumed for the product synthesis. When CO_2 was used as C-source, the coulombs about 12-18% contributed for methanol synthesis, 5-30% for ethanol and 20-38% for butanol synthesis. In similar way, methanol synthesis has consumed about 25-35% and ethanol has consumed 20-30% coulombs, when bicarbonate used as C-source (Fig 5). In spite of higher CE, the CCE was on lower side, especially with CO_2 as C-source, because the optimized combination of coulombs and carbon supply should be achieved to make the process more energy efficient.

Interesting observations can be made based on the rate of product synthesis against operation time, reactor volume and biocathode projected surface area with respect to C-source. Unlike CCE, the difference in volumetric production rate was not very significant between CO_2 (0.4±0.02 kg/m3/day and 1.02±0.2 kg/m2 electrode) and bicarbonate (0.95±0.06 kg/m3/day and

1.65±0.05 kg/m2 electrode) as C-source (Table 2). But the product synthesis rate based on CO_2 input showed marked variation (0.0057±0.0002 kg/kgCO_2 with CO_2; 0.21±0.02 kg/kg CO_2 with bicarbonate) supporting the requirement of CO_2 solubility and bioavailability for efficient transformation. Most of the MES studies reported mainly acetate synthesis till date, however, a recent study also shown the simultaneous production of mixture of products composed of acetate, butyrate, ethanol and butanol using mixed culture. Overall, the reported performances of MES are insufficient for scaling MES to practical applications. The product titters obtained in the present study are giving a possible indication of upscaling the process. But still the notable MES performance can be achieved only after optimizing the microbe-electrode interaction through strong and efficient biofilm formation, increasing the electron transfer rate, increasing the CO_2 solubility and bioavailability, enhanced reaction rates, improved reactor design, etc. (Guo et al., 2015). Research in this direction is strongly required to design an efficient MES process for CO_2 transformation.

Conclusions

Electro-biocatalytic reactor for CO_2 transformation to organics was studied for 320 days using selectively enriched mixed culture. Stabilized biocathode, was tested for its efficiency using CO_2 or bicarbonate as C-source. Addition of bicarbonate resulted in higher current consumption and product titre compared to CO_2. Butanol and butyric acid were produced with CO_2 as substrate but propionic acid replaced both when direct bicarbonate was provided. Voltammogram showed H2 mediated electron transfer along with direct transfer under bicarbonate turnover conditions, while the electron transfer restricted to direct under CO_2 turnover. CE was high irrespective of the carbon nature but CCE significantly varied.

References

1. Arends, J.B.A., Patil, S.A., Roume, H., Rabaey, K., 2017. Continuous long-term electricity-driven bioproduction of carboxylates and isopropanol from CO2 with a mixed microbial communityJ. CO2 Util. 20, 141–149.
2. Bajracharya, S., ter Heijne, A., Dominguez Benetton, X., Vanbroekhoven, K., Buisman, C.J.N., Strik, D.P.B.T.B., Pant, D., 2015. 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. 195, 14–24.
3. Bajracharya, S., Vanbroekhoven, K., Buisman, C.J.N., Pant, D., Strik, D.P.B.T.B., 2016. Application of gas diffusion biocathode in microbial electrosynthesis from carbon dioxide. Environ. Sci. Pollut. Res. 23, 22292–22308.
4. Bajracharya, S., Srikanth, S., Mohanakrishna, G., Zacharia, R., Strik, D.P., Pant, D., 2017a. Biotransformation of carbon dioxide in bioelectrochemical systems: State of the art and future prospects. J. Power Sources 356, 256–273.
5. Bajracharya, S., Yuliasni, R., Vanbroekhoven, K., Buisman, C.J.N., Strik, D.P.B.T.B., Pant, D., 2017b. Long-term operation of microbial electrosynthesis cell reducing CO2 to multi-carbon chemicals with a mixed culture avoiding methanogenesis. Bioelectrochemistry 113, 26–34.
6. Batlle-Vilanova, P., Ganigué, R., Ramió-Pujol, S., Bañeras, L., Jiménez, G., Hidalgo, M., Balaguer, M.D., Colprim, J., Puig, S., 2017. Microbial electrosynthesis of butyrate from carbon dioxide: Production and extraction. Bioelectrochemistry 117, 57–64.
7. Batlle-Vilanova, P., Puig, S., Gonzalez-Olmos, R., Balaguer, M.D., Colprim, J., 2016. Continuous acetate production through microbial electrosynthesis from CO 2 with microbial

mixed culture. J. Chem. Technol. Biotechnol. 91, 921–927.

8. Bond, D.R., Lovley, D.R., 2003. Electricity production by Geobacter sulfurreducens attached to electrodes. Appl. Environ. Microbiol. 69, 1548–55.
9. Ganigue, R., Puig, S., Batlle-Vilanova, P., Balaguer, M.D., Colprim, J., Berton, J., Tsukamoto, J., Stevens, C. V., Rabaey, K., 2015. Microbial electrosynthesis of butyrate from carbon dioxide. Chem. Commun. 51, 3235–3238.
10. Ganigué, R., Sánchez-Paredes, P., Bañeras, L., Colprim, J., 2016. Low fermentation pH is a trigger to alcohol production, but a killer to chain elongation. Front. Microbiol. 7, 1-11.
11. Giddings, C.G.S., Nevin, K.P., Woodward, T., Lovley, D.R., Butler, C.S., 2015. Simplifying microbial electrosynthesis reactor design. Front. Microbiol. 6, 468.
12. Guo, K., Prevoteau, A., Patil, S.A., Rabaey, K., 2015. Engineering electrodes for microbial electrocatalysis. Curr. Opin. Biotechnol. 33, 149–156.
13. IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.
14. Jourdin, L., Freguia, S., Donose, B.C., Chen, J., Wallace, G.G., Keller, J., Flexer, V., Covalla, S.F., Franks, A.E., Liu, A., Lovley, D.R., 2014. A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis. J. Mater. Chem. A 2, 13093.
15. Jourdin, L., Freguia, S., Flexer, V., Keller, J., 2016. Bringing High-Rate, CO_2-Based Microbial Electrosynthesis Closer to Practical Implementation through Improved Electrode Design and Operating Conditions Environ. Sci. Technol., 50, 1982–1989.
16. Jourdin, L., Grieger, T., Monetti, J., Flexer, V., Freguia, S., Lu, Y., Chen, J., Romano, M., Wallace, G.G., Keller, J., 2015. High Acetic Acid Production Rate Obtained by Microbial

Electrosynthesis from Carbon Dioxide. Environ. Sci. Technol. 49, 13566–13574.

17. LaBelle, E. V., Marshall, C.W., Gilbert, J.A., May, H.D., Voordouw, G., 2014. Influence of Acidic pH on Hydrogen and Acetate Production by an Electrosynthetic Microbiome. PLoS One 9, e109935.
18. Leung, D.Y.C., Caramanna, G., Maroto-Valer, M.M., 2014. An overview of current status of carbon dioxide capture and storage technologies. Renew. Sustain. Energy Rev. 39, 426–443.
19. Lewis, N.S., Nocera, D.G., 2006. Powering the planet: chemical challenges in solar energy utilization. Proc. Natl. Acad. Sci. U. S. A. 103, 15729–35.
20. Lovley, D.R., Nevin, K.P., 2013. Electrobiocommodities: powering microbial production of fuels and commodity chemicals from carbon dioxide with electricity. Curr. Opin. Biotechnol. 24, 385–390.
21. Marshall, C.W., LaBelle, E. V, May, H.D., 2013a. Production of fuels and chemicals from waste by microbiomes. Curr. Opin. Biotechnol. 24, 391–397.
22. Marshall, C.W., Ross, D.E., Fichot, E.B., Norman, R.S., May, H.D., 2013b. Long-term operation of microbial electrosynthesis systems improves acetate production by autotrophic microbiomes. Environ. Sci. Technol. 47, 6023–9.
23. Marshall, C.W., Ross, D.E., Fichot, E.B., Norman, R.S., May, H.D., 2012. Electrosynthesis of commodity chemicals by an autotrophic microbial community. Appl. Environ. Microbiol. 78, 8412–20.
24. Modestra, J.A., Navaneeth, B., Venkata Mohan, S., 2015. Bio-electrocatalytic reduction of CO2: Enrichment of homoacetogens and pH optimization towards enhancement of carboxylic acids biosynthesis. J. CO2 Util. 10, 78–87.
25. Modestra, J.A., Venkata Mohan, S., 2017. Microbial electrosynthesis of carboxylic acids

through CO 2 reduction with selectively enriched biocatalyst: Microbial dynamics. J. CO2 Util. 20, 190-199

26. Mohanakrishna, G., Seelam, J.S., Vanbroekhoven, K., Pant, D., 2015. Enriched electroactive homoacetogenic biocathode improves the microbial electrosynthesis of acetate through carbon dioxide reduction. Faraday Discuss.
27. Mohanakrishna, G., Vanbroekhoven, K., Pant, D., 2016. Imperative role of applied potential and inorganic carbon source on acetate production through microbial electrosynthesis. J. CO2 Util. 15, 57–64.
28. Nevin, K., Woodard, T., Franks, A., Summers, Z., Lovley, D., 2010. Microbial Electrosynthesis : Feeding Microbes Electricity To Convert Carbon Dioxide and Water to Multicarbon Extracellular Organic Compounds. MBio 1, e00103-10-.
29. Nevin, K.P., Hensley, S.A., Franks, A.E., Summers, Z.M., Ou, J., Woodard, T.L., Snoeyenbos-West, O.L., Lovley, D.R., 2011. Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. Appl. Environ. Microbiol. 77, 2882–6.
30. Nevin, K.P., Richter, H., Covalla, S.F., Johnson, J.P., Woodard, T.L., Orloff, A.L., Jia, H., Zhang, M., Lovley, D.R., 2008. Power output and columbic efficiencies from biofilms of Geobacter sulfurreducens comparable to mixed community microbial fuel cells. Environ. Microbiol. 10, 2505–14.
31. Patil, S.A., Arends, J.B.A., Vanwonterghem, I., van Meerbergen, J., Guo, K., Tyson, G.W., Rabaey, K., 2015a. Selective Enrichment Establishes a Stable Performing Community for Microbial Electrosynthesis of Acetate from CO2. Environ. Sci. Technol. 49, 8833–43.
32. Patil, S.A., Gildemyn, S., Pant, D., Zengler, K., Logan, B.E., Rabaey, K., 2015b. A logical

data representation framework for electricity-driven bioproduction processes. Biotechnol. Adv. 33, 736–44.

33. Patil, S.A., Hägerhäll, C., Gorton, L., 2012. Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems. Bioanal. Rev. 4, 159–192.
34. Rabaey, K., Girguis, P., Nielsen, L.K., 2011. Metabolic and practical considerations on microbial electrosynthesis. Curr. Opin. Biotechnol. 22, 371–377.
35. Rabaey, K., Rozendal, R.A., 2010. Microbial electrosynthesis - revisiting the electrical route for microbial production. Nat. Rev. Microbiol. 8, 706–16.
36. Schröder, U., 2007. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. Phys. Chem. Chem. Phys. 9, 2619–29.
37. Sharma, M., Aryal, N., Sarma, P.M., Vanbroekhoven, K., Lal, B., Benetton, X.D., Pant, D., 2013. Bioelectrocatalyzed reduction of acetic and butyric acids via direct electron transfer using a mixed culture of sulfate-reducers drives electrosynthesis of alcohols and acetone. Chem. Commun. (Camb). 49, 6495–7.
38. Srikanth, S., Pavani, T., Sarma, P.N., Venkata Mohan, S., 2011. Synergistic interaction of biocatalyst with bio-anode as a function of electrode materials. Int. J. Hydrogen Energy. 36, 2271-2280
39. Srikanth, S., Mohan, S.V., 2012. Change in electrogenic activity of the microbial fuel cell (MFC) with the function of biocathode microenvironment as terminal electron accepting condition: influence on overpotentials and bio-electro kinetics. Bioresour. Technol. 119, 241–51.
40. Srikanth, S., Mohan, S.V., 2014. Regulating feedback inhibition caused by the accumulated acid intermediates during acidogenic hydrogen production through feed replacement. Int. J.

Hydrogen Energy. 39(19), 10028-10040.

41. Srikanth, S., Kumar, M., Singh, M.P., Das, B.P., 2016. Bioelectro Chemical Systems: A Sustainable and Potential Platform for Treating Waste. Procedia Environ. Sci. 35, 853–859.
42. Srikanth, S., Alvarez-Gallego, Y., Vanbroekhoven, K., Pant, D., 2017. Enzymatic electrosynthesis of formic acid through carbon dioxide reduction in a bioelectrochemical system: Effect of immobilization and carbonic anhydrase addition. ChemPhysChem, 18, 18 (22), 3174-3181
43. Steinbusch, K.J.J., Hamelers, H.V.M., Schaap, J.D., Kampman, C., Buisman, C.J.N., 2010. Bioelectrochemical Ethanol Production through Mediated Acetate Reduction by Mixed Cultures. Environ. Sci. Technol. 44, 513–517.
44. Su, M., Jiang, Y., Li, D., 2013. Production of acetate from carbon dioxide in bioelectrochemical systems based on autotrophic mixed culture. J. Microbiol. Biotechnol. 23, 1140–6.
45. Tremblay, P.-L., Zhang, T., 2015. Electrifying microbes for the production of chemicals. Front. Microbiol. 6, 201.
46. Venkata Mohan, S., Velvizhi, G., Annie Modestra, J., Srikanth, S., 2014. Microbial fuel cell: Critical factors regulating bio-catalyzed electrochemical process and recent advancements. Renew. Sustain. Energy Rev. 40, 779–797.
47. Zaybak, Z., Pisciotta, J.M., Tokash, J.C., Logan, B.E., 2013. Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems. J. Biotechnol. 168, 478–485.
48. Zhang, T., Nie, H., Bain, T.S., Lu, H., Cui, M., Snoeyenbos-West, O.L., Franks, A.E., Nevin, K.P., Russell, T.P., Lovley, D.R., Schroder, U., 2013. Improved cathode materials for

microbial electrosynthesis. Energy Environ. Sci. 6, 217–224.

49. Schuchmann, K., Müller, V., 2014. Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria. Nat. Rev. Microbiol. 12, 809–821

50. Raes, S.M.T., Jourdin, L., Buisman, C.J.N., Strik, D.P.B.T.B., 2016. Continuous Long-Term Bioelectrochemical Chain Elongation to Butyrate, ChemElectroChem 4(2), 386–395.

Caption for figures

Figure 1: Change in current density against operation time with the function of experimental variation

Figure 2: Product slate against operation time with the function of experimental variation

Figure 3: Total product along with Coulombic (CE) and carbon conversion efficiencies (CCE) during operation against time

Figure 4: Cyclic voltammograms obtained during operation with the function of experimental variations

Figure 5: Percentage occupancy of different products in total product with the function of experimental variation

Figure 6: Shifts in operating pH against time (dotted line indicates the adjusted pH on daily basis)

Table 1: Details of reactor design and operating conditions used during experiment

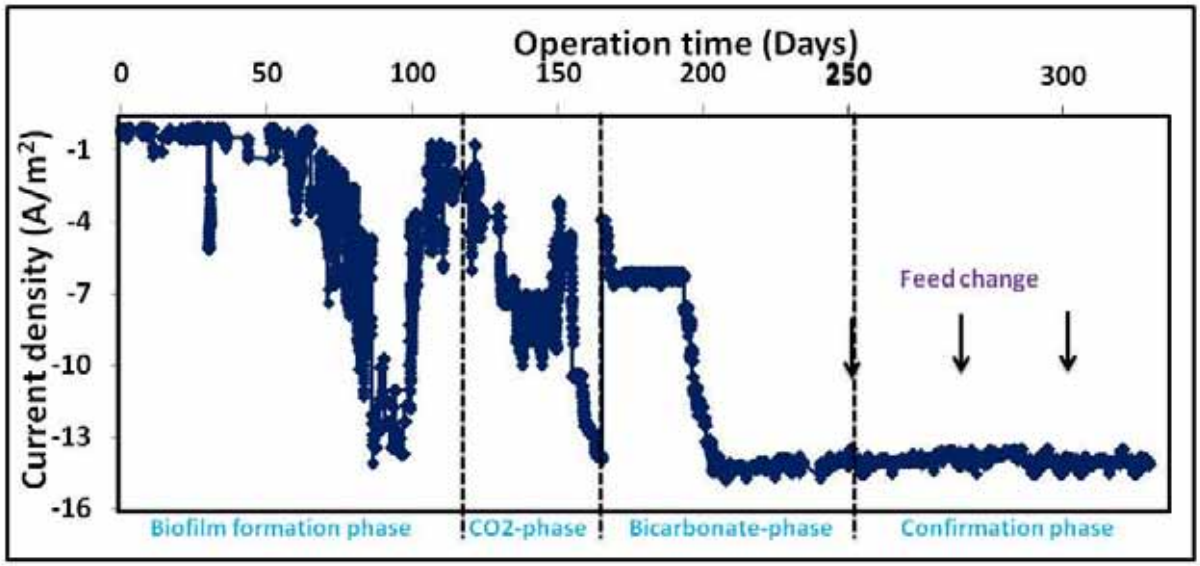
	Biofilm formation phase	**CO_2 phase**	**Bicarbonate phase**	**Confirmation phase**
Configuration and Volume	Dual chambered (total/ working volume, 0.28/0.20 l)			
WE/ Electrolyte	Graphite plate loaded with carbon powder/ PBS			
CE/ Electrolyte	Activate carbon cloth on graphite rod/ PBS added with 0.1% NaCl (w/v)			
Power source/ Applied potential	Potentiostat/ -1 V vs. Ag/AgCl to WE			
pH (WE/CE)	8/ 6			
Membrane/size	Nafion 117/ 25 mm diameter			
Biocatalyst	Mixed culture predominating with SRB and IRB			
C-source (concentration)	CO_2 (15 ml/min for 8h in a day)	CO_2 (15 ml/min for 8h in a day)	Bicarbonate (1.2g carbon equivalents/day)	Bicarbonate (1.2g carbon equivalents/day)

Table 2: Consolidated results obtained during operation with the function of different experimental conditions

Operation phase	Days	C-Source	Carbon Input (L or G)	Total product (g/L)	Yield (%)	CCE (%)	CE (%)	Production rate		
								kg/m^3/ day	kg/kg CO_2	kg/m^2 electrode
Biofilm formation phase	15	CO_2 (L)	108	0	0.00	0.00	0	0	0	0.00
	30	CO_2 (L)	216	0	0.00	0.00	0	0	0	0.00
	50	CO_2 (L)	360	0	0.00	0.00	0	0	0	0.00
	65	CO_2 (L)	468	2.20	0.10	0.08	72.61	0.034	0.0005	0.15
	85	CO_2 (L)	612	5.02	0.26	0.17	72.17	0.059	0.0008	0.34
	105	CO_2 (L)	756	19.50	0.63	0.45	85.60	0.185	0.0026	1.31
	120	CO_2 (L)	864	23.58	0.69	0.47	88.55	0.196	0.0028	1.58
CO_2 phase	135	CO_2 (L)	108	3.60	0.57	0.49	57.43	0.240	0.0034	0.24
	150	CO_2 (L)	216	12.20	1.21	0.97	98.97	0.407	0.0057	0.82
	165	CO_2 (L)	324	18.20	1.36	1.01	84.87	0.404	0.0057	1.22
Bicarbonate phase	185	Bicarbonate (G)	24.8	8.40	18.66	20.81	93.80	0.420	0.0933	0.56
	200	Bicarbonate (G)	43.4	17.20	19.61	23.91	99.75	0.491	0.1092	1.15
	220	Bicarbonate (G)	68.2	33.40	24.96	29.59	87.04	0.607	0.1349	2.24
	240	Bicarbonate (G)	93	55.40	30.12	35.97	93.71	0.738	0.1641	3.72
	250	Bicarbonate (G)	105.4	52.80	25.27	29.88	74.05	0.621	0.1380	3.54
Confirmation phase	275	Bicarbonate (G)	31	25.40	39.18	48.62	97.93	1.016	0.2256	1.70
	300	Bicarbonate (G)	31	24.20	36.87	46.13	92.81	0.968	0.2150	1.62
	320	Bicarbonate (G)	24.8	17.90	33.87	42.51	84.97	0.895	0.1988	1.20

Research Highlights

- Electro-biocatalytic CO_2 transformation to organics was studied for 320 days
- Stabilized biocathode of selectively enriched culture was studied for electro-synthesis
- CO2 availability in soluble form dictated the product slate
- Electron transfer is possibly H2 mediated and DET with bicarbonate but restricted to DET with CO2
- CE was high irrespective of carbon nature but CCE significantly varied for CO2 and bicarbonate



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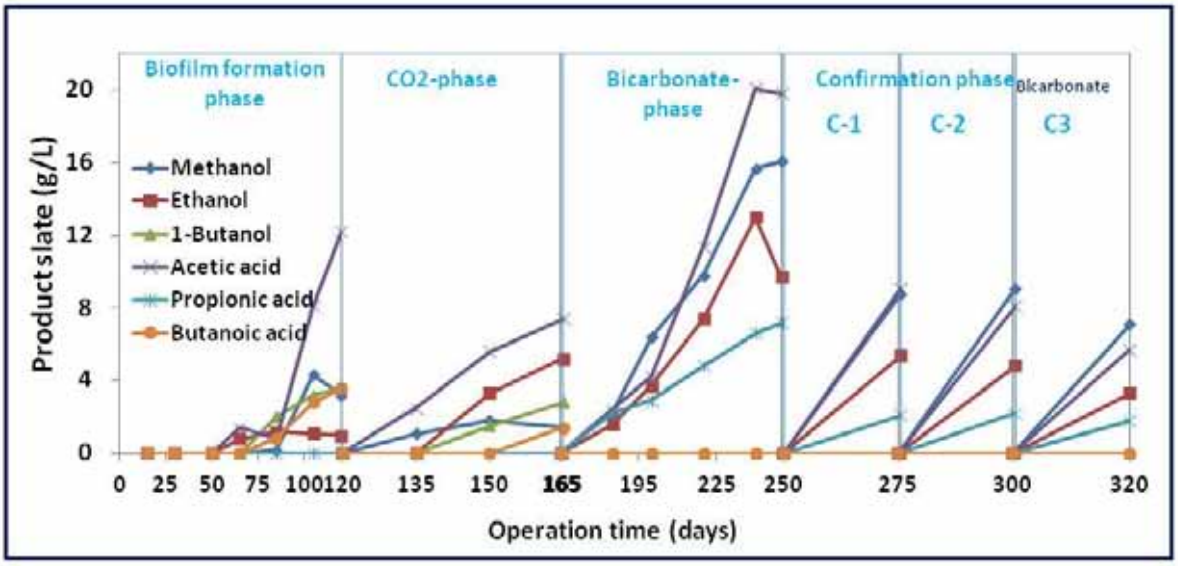

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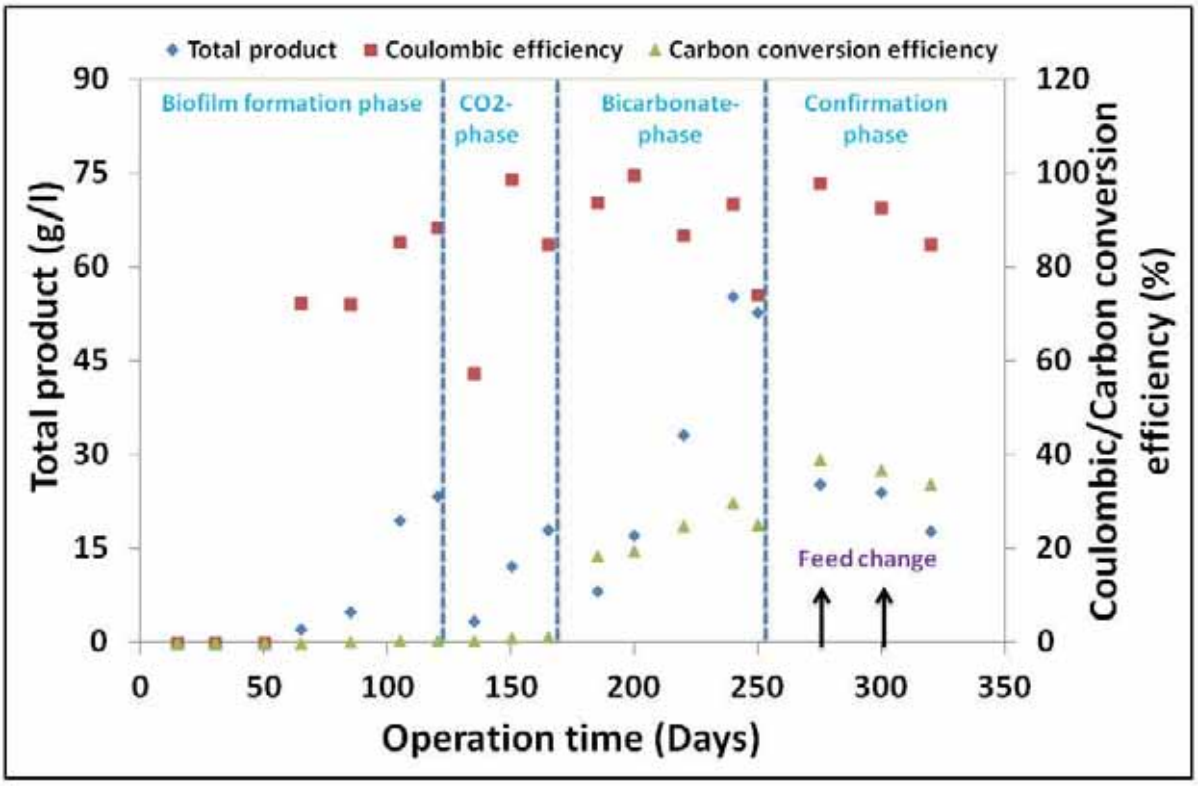

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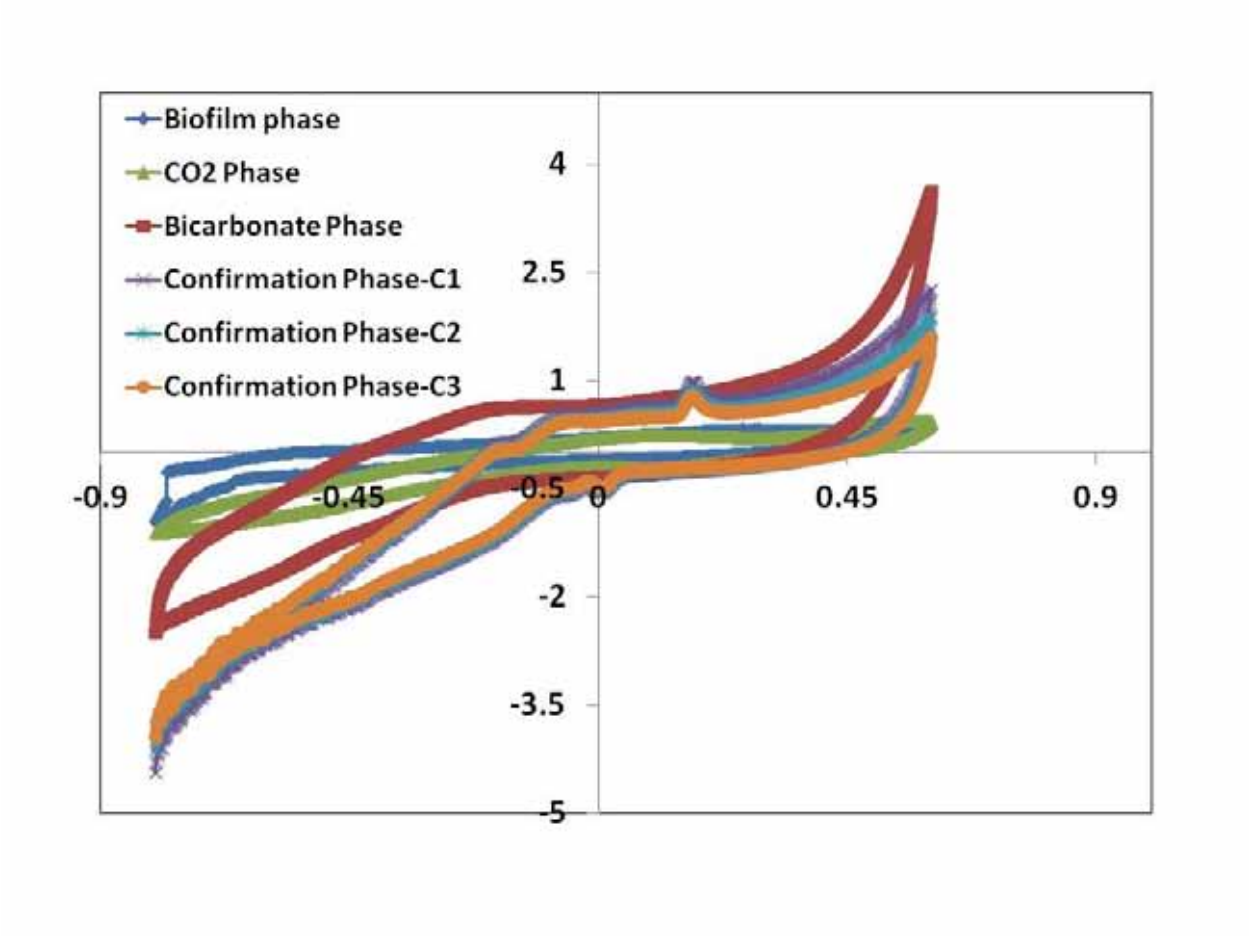

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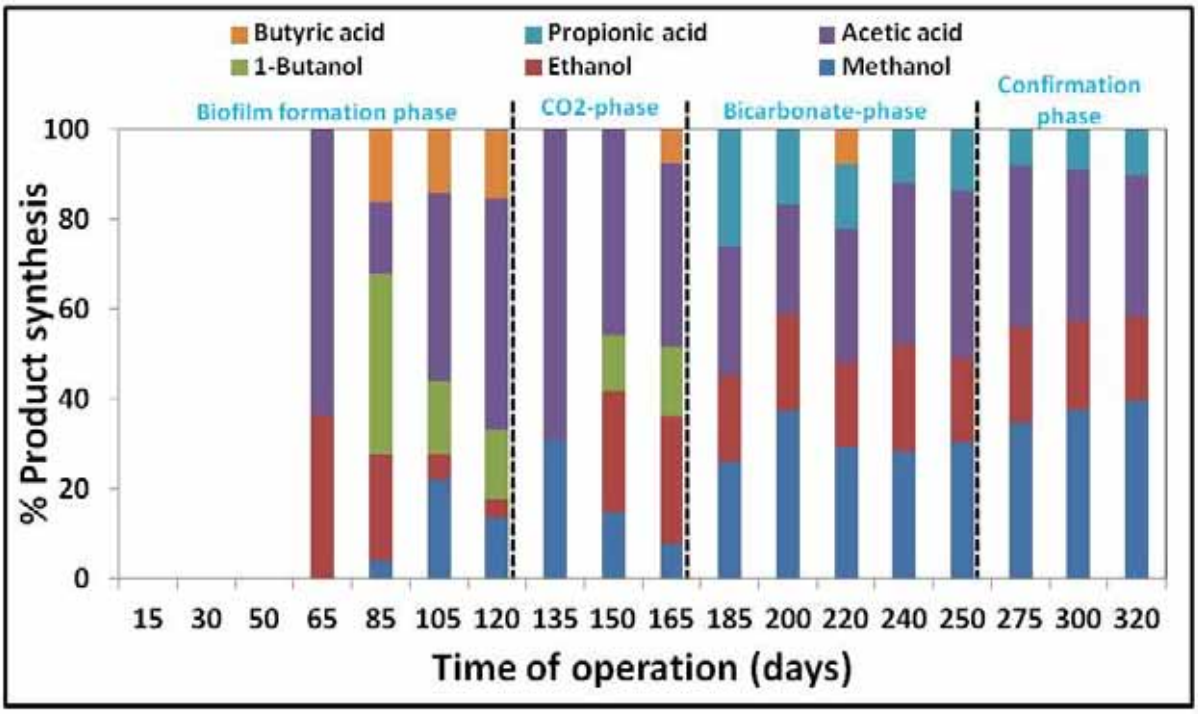

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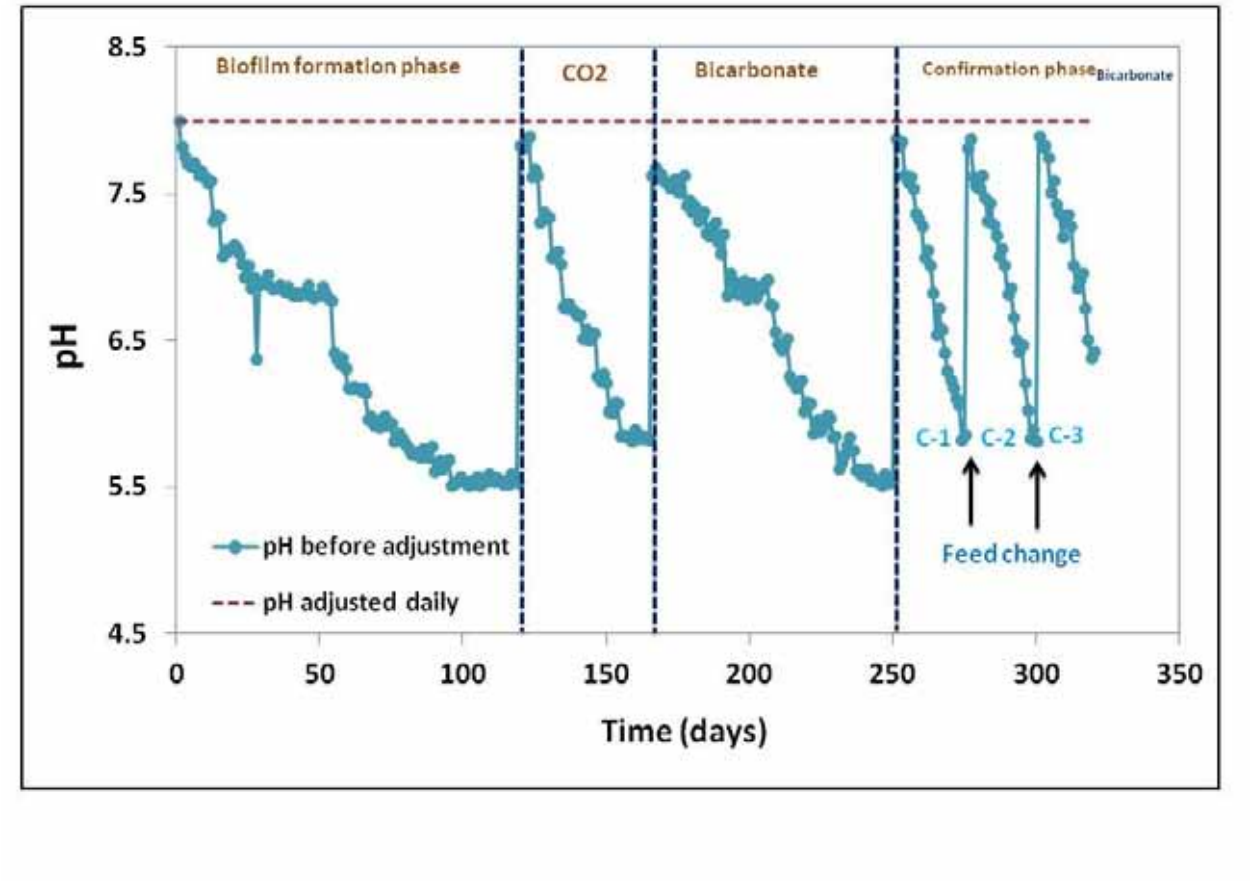

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