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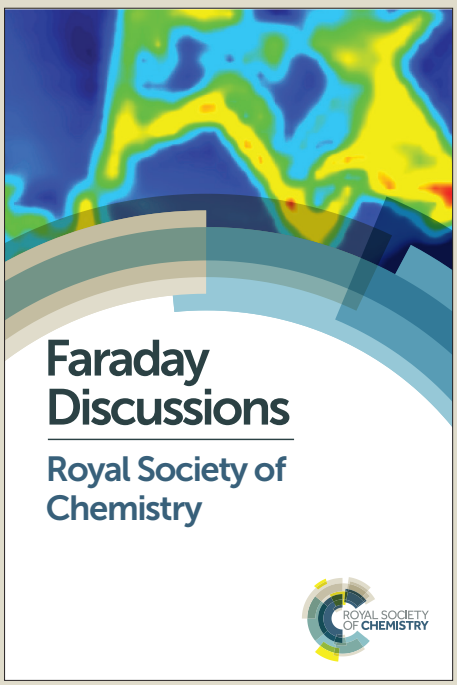

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ARTICLE

Enriched electroactive homoacetogenic biocathode improves the microbial electrosynthesis of acetate through carbon dioxide reduction

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In the direction of generating value added chemicals from CO_2 reduction through microbial electrosynthesis, considering the crucial impact of electrode material for the biofilm development and electron delivery, an attempt was made in this study to evaluate the efficiency of two different materials as biocathodes and their respective output in terms of electrosynthesis. Electrode material is a key component in the MES process. Several electrodes such as platinum, graphite foil, dimentionally stable anode (DSA) and graphite rod, VITO-CoRE™ electrodes were tested for their suitability for ideal electrodes combination in a three electrode cell setup. Bicarbonates (dissolved form of carbon dioxide) was reduced to acetate by selectively developed biocathode under mild applied cathodic potential of -400 mV (vs SHE) in 500 mL of single chamber MES cells operating for more than four months. Among the two electrode combinations evaluated, VITO-CoRE-PL (VC-IS, plastic inert support) as cathode and VITO-CoRE-SS (VC-SS, stainless steel metal support) as counter electrode showed higher production efficiencies (4127 mg/L) with volumetric production rate of 0.569 kg/m^3/d than graphite rod (1523 mg/L) with volumetric production rate of 0.206 kg/m^3/d. Contrary to the production efficiencies, the coulombic efficiency was higher with second electrodes combination (40.43%) than the first electrodes combination (29.91%). Carbon conversation efficiency to acetate showed higher for VC-IS (90.6%) than graphite rod (82.0%).

Introduction

Carbon dioxide (CO_2) reduction for the generation of renewable energy and value added chemicals is one of the thirst areas of present research developments. Microbial electrosynthesis (MES) is one of the recent applications of bioelectrochemical systems (BES), that involves the cathodic reduction reactions to generate biofuels and speciality chemicals[1-5]. The bacteria that metabolizes on the conductive electrode surface acts as the catalysts for the chemical conversions in BES and are known as electroactive biocatalyst. Other forms of BES are microbial fuel cells (MFC) and microbial desalination cells (MDC) where the biocatalysts functions on the anode[6-9] and in microbial electrolysis cells (MEC), biocatalyst functions on the cathode[10-12]. On the whole, the BES processes are considered as the biotechnology with electrodes in which solid state electrodes can provide a stable and sustainable solution[13]. Anode serves as terminal electron acceptor for the discharge of excess/surplus microbial reducing equivalents and cathode provides reducing equivalents required for biochemical processes. The applied electrode potential tunes the strength of the electron acceptor or donor. It results in electric current which determines the biocatalytic performance of electroactive biofilms[14-18].

CO_2 plays a key role in the eventual climate change due to its accumulation in the atmosphere. The control of CO_2 emission into atmosphere requires novel ideas that can assure the future generations with sustainable growth options. Considerable research is being carried out to convert CO_2 to various commercial products by thermochemical and electrochemical technologies, which need high energy input and but also generate a wide variety of products[19]. MES is a novel biocathode-driven production technology for the reduction of CO_2 to chemicals and biofuels. It is characterised as a promising way to sustainable development and new carbon-consuming technologies[3,20]. The specificity of the product formation from CO_2 is largely influenced by the bacteria and its metabolic functions and electrical potential that drives the process[2,12]. Acetate is the basic intermediary product compound that is formed by the photosynthesis process from CO_2 conversion and is the most important step to generate available carbon form for biological domain. Further, acetate can be used as the substrate for the various biological building blocks of life. Homo acetogenic bacteria are a specialized group of bacteria that are able to produce acetate from two molecules of carbon dioxide (CO_2) through the Wood-Ljungdahl pathway (WLP)[21-22]. Acetogens are facultative autotrophs that can grow by the consumption of hydrogen (H_2)

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or carbon monoxide (CO) that is usually coupled to the reduction of CO_2. Also, large variety of organic substrates, including hexoses, pentoses, alcohols, methyl groups and formic acid can be used as the substrates[21,23]. On the whole acetogens are present in 23 different bacterial genera. Most acetogens are found in phylum Firmicutes, which are Gram-positive bacteria with low GC content. The genera, *Clostridium* contains acetogenic as well as non-acetogenic species, whereas other genera such as *Acetobacterium* or *Sporomusa* contain only acetogens. Most known acetogens belongs to the *Clostridium* and *Acetobacterium* genera[21-22]. Among the large number of acetogens, three organisms have been studied quite extensively (*Moorella thermoacetica, Acetobacterium woodii and Clostridium ljungdahlii*). These bacteria were primarily studied for the biocathode driven CO_2 fixation. Another advantage of acetogens to use in MES process is their metabolic flexibility. Acetogens were ubiquitously distributed in alkaline, high-salt and hot environments, in deep subsurface samples, the termite hindgut and in the human intestines. They are also found in terrestrial soil, in freshwater and marine sediments[22]. This metabolic flexibility can be an advantage for the their application in MES which is hybrid system with biological electrochemical functions.

The proof of concept studies of MES process was reported by Nevin et al.,[17,24] and showed the production of acetate, butyrate, oxy-buterate and formate by the bioelectrochemical production by *Sporomusa ovata, Clostridium ljungdahlii, Clostridium aceticum, and Moorella thermoacetica* at an applied potential of -400 mV (SHE). Later Nie et al.,[25] achieved higher acetate production rates by *Sporomusa ovata.* Besides pure cultures, few researchers were able to produce acetate with mixed and adapted mixed cultures at various applied potentials ranging from the -400 mv to -950 mV[18,20,26-29]. Due to several obvious advantages of the process such as low energy input and CO_2 fixation, it is gaining much global attention. Along with whole cell biocatalyst, use of specific enzymes in bioelectrocatalysis for CO_2 sequestration is also finding possible application for the sustainable energy and chemicals generation[3,30].

In the present study, an attempt was made to generate sustainable homoacetogenic biocathode that reduce CO_2 to acetate under mild applied potential conditions where the hydrogen production can be completely limited. A detailed evaluation was also performed for the selection of electrodes combination for the potential window analysis where no hydrogen can be produced and to avoid the interference of fermentation process. Furthermore, a detailed discussion is made on the benchmarking microbial electrosynthesis process with respect to type of bacterial catalyst, energy efficiency and production rate.

2. Experimental

2.1 Homoacetogenic electroactive bacteria

Homoacetogenic bacteria that has specific metabolic competence to produce acetate from the inorganic carbon source (CO_2) was enriched from the granular activated sludge. The granular activated sludge was collected from the anaerobic digester that treating effluents of potato processing industry (Cargill Sas van, Ghent). It contains 6% of total solid (TS) and 75% of volatile solid (VS) content along with <1% of inorganic carbon. A four stage selective enrichment methodology was applied to enrich the electroactive homoacetogenic bacteria for biocathode development. During the first stage the sludge collected from anaerobic digester was subjected to heat treatment (90 °C for 1 hour) to eliminate the methanogenic bacteria and to retain the acetogenic bacterial spores[31]. In the second stage, the heat treated sludge was subjected to heterotrophic growth using glucose as substrate in nutrient broth medium at pH 6.0 under 35 °C to activate the metabolism of whole consortia. Heterotrophically growing active culture of acetogenic bacteria was shifted to autotrophic condition by providing the mixture of CO_2 and H_2 as substrate, where the only homoacetogenic bacteria were grown and they were enriched by repeated culturing on CO_2 and H_2 mixture for four cycles. The homoacetogenic activity was confirmed through the acetate production and inhibition of methane. The resultant consortia was used as inoculum for electroactive biofilm formation.

2.2 Bioelectrochemical systems

2.2.1 Design

MES reactor was designed with a three electrode set up (single chamber) and fabricated with glass bottle (total/working, 0.62/0.5 L) and included with suitable ports for the provision of electrodes and N_2 gas flushing, and was used in the present study (Fig 1). Initially, 6 different materials were screened to use as anode/counter electrode to select the most suitable one. As the BES is single chambered, if the counter electrode evolves oxygen, the biofilm on the working electrode cannot sustain its anaerobic properties to carry out the reduction reaction. Henceforth, different electrode materials, viz., platinum, dimensionally stable anode (DSA made of titanium coated with iridium mixed metal oxide, Magneto BV Netherlands), graphite foil, graphite rod, VITO-CoRE™ with stainless steel (VC-SS) and VITO-CoRE™ with inert plastic support (VC-IS) were chosen against VC-SS counter electrode in both abiotic and biotic (enriched bacteria in suspension) conditions under -400 mV of applied cathodic potential.

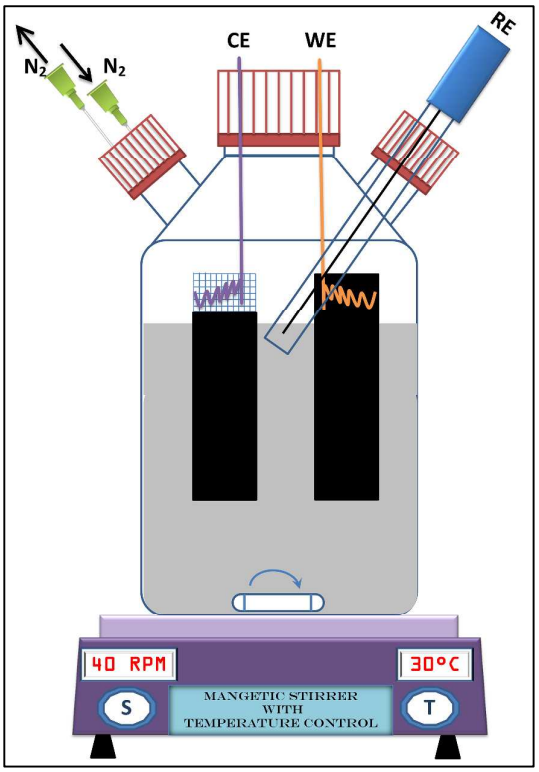

Fig 1: Schematic diagram of the single chamber microbial electrosynthesis cell. (CE, counter electrode; WE, working electrode and RE, reference electrode)

Performance of the electrodes was evaluated in terms of voltage reached and the electrode which showed lower potential than water electrolysis (1.23 Vs SHE). Two MES reactors (MES1 and MES2) of similar configuration, having VC-SS electrode[34] as anode (selected from screening experiment), were used in the current experiment. Two different cathode materials, viz., VC-IS in MES1 and graphite rod in MES2, were evaluated for their efficiency of biofilm growth as well as electron uptake for the acetate synthesis. The total surface area (37.5 cm^2) and the active surface area (the area that in contact with the electrolyte, 30 cm^2) of all the electrodes (anodes and cathodes) was maintained constant, except for DSA (6 × 2.5 cm) and platinum (surface area, 2.37 cm^2) due to the unavailability of sizes. A fine stainless steel wire, weaved through the electrode and extended through the airtight passage of the reactor cap, was used as current collector (Fig 1). No separate current collector was also used in case of platinum wire.

2.2.2 Operation

Both the MES were operated in batch mode at 30°C of temperature controlled oil bath. MES1 was operated for 127 days, while the MES2 was operated for only 47 days due to loosing biofilm on cathode and instability of output. The phosphate buffer media which was used during the enrichment of homoacetogenic bacteria was also used as media in the MES operation also. Phosphate buffer media containing NH_4Cl of 200 mg/L, $MgCl_2.6H_2O$ of 200 mg/L, Yeast Extract of 10 mg/L along with the trace elements solution (per litre, Nitrilotriacetic acid, 1.5 g; $MgSO_4$ x 7 H_2O, 3.0 g; $MnSO_4$ x H_2O, 0.5 g; NaCl, 1.0 g; $FeSO_4$ x 7 H_2O, 0.1 g; $CoSO_4$ x 7 H_2O, 0.18 g; $CaCl_2$ x 2 H_2O, 0.1 g; $ZnSO_4$ x 7 H_2O, 0.18 g; $CuSO_4$ x 5 H_2O, 0.01 g; $KAl(SO_4)_2$ x 12 H_2O, 0.02 g; H_3BO_3, 0.01 g; Na_2MoO_4 x 2 H_2O, 0.01 g; $NiCl_2$ x 6 H_2O, 0.03 g; Na_2SeO_3 x 5 H_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). Sodium bicarbonate was considered as substrate for BES at a rate of 3.44 g/L which is equivalent to 2.5 g/L of bicarbonates (HCO_3^-). Bromoethanesulfonic acid (BESA) was also added to the medium at a rate of 0.5 g/L to inhibit the possible methanogenic activity[31-33]. The final pH of the media was maintained at 7.0. During the start-up of reactor, 10% of the enriched inoculum (50 mL) added to MES along with 450 mL of media. Both, MES1 and MES2 were continuously poised at -600 mV Vs Ag/AgCl (-400 mV vs SHE) through chronoamperometry (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 (3.0M KCl) reference electrode. All the potentials mentioned further in the manuscript are vs SHE unless otherwise stated.

Consumption of bicarbonates in the electrolyte (less than 0.5 g HCO_3^-/L) was considered as time for feed change. To avoid sudden changes in the electrolyte concentration, only 60% of feed was replaced with fresh feed using siphon flow method. In the case of MES1, after start-up, the feed was changed on 21st, 28th, 35th, 43rd, 50th, 60th, and 70th day of operation. Later, a constant time interval of 7 days was maintained until 126th day of operation. In the case of MES2, feed replacement was done during 21st, 28th, 34th, 40th, 47th day of operation. Both the reactors were continuously flushed with the N_2 gas to maintain the anaerobic environment.

2.3 Analysis

Liquid samples from MES 1 and MES 2 were collected on every fourth day. During enrichment of homoacetogenic bacteria, along with liquid samples, the gaseous samples were also collected. The liquid samples were analysed for organic acids (formic acid, acetic acid, propionic acid and butyric acid), ethanol and pH, whereas the gaseous samples were analysed for H_2, CO_2, CH_4 and O_2. Prior to analysis through HPLC, the samples were filtered through 0.45 µm Acrodisc syringe filters. HPLC analyses was performed with an Agilent HPLC 1200 series equipped with a RID detector (Agilent 1260) set at a wavelength of 215 nm. The column used was a Agilent Hi-Plex column 8u (3000 mm X 7.7 mm) operated at 60 °C equipped with a guard column of the same material. Phosphoric acid (0.05% in isocratic gradient) was used as eluent at a flow rate of 1 mL/min. Injection volume of the samples and standards

was 20 µl. EZchrom software that provided by Agilent was used for HPLC data analysis.

Gaseous samples from the enrichment cultures and MES reactors were analysed with a Trace GC Ultra (Thermo Scientific) connected with 2 different thermal conductivity detectors. Carbosphere column (2m - 1/800SS) was used for hydrogen, whereas for carbon dioxide and methane, both Hayesep N column (1m – 1/800SS) and Molsive column A (1m - 1/800SS) were used at a constant temperature of 60 °C. pH was analysed with calibrated pH meter (WTW Multi 340i). The liquid samples from MES 1 and MES 2 reactors were also analyzed for bicarbonates through TOC analyzer (Multi N/C 3100 of Analytik Jena) with auto-sampler (APG 49 of Analytik Jena) according to the methodology developed by ISO 8245.

3. Results and discussion

3.1 Screening of electrodes for anode

Microbial electrosynthesis is a process that proceeds with the reduction of protons and electrons that are generated from the counter electrode or anode of the cell. As the present study was carried out in single chambered configuration, it was important to avoid the abiotic water electrolysis reaction. By maintaining cells with mild electrochemical activity, oxygen and hydrogen production can be controlled. These conditions helps to maintain the system anaerobic which is prerequisite for the biocatalyst activity. However, the net counter electrode (anode) potential that sustains in the cell also influences the cathode potential. Theoretically, efficient electrolysis process continues at the potential of 1.23 V (Vs SHE)[35,36]. However, it is also found to be influenced by the type of electrode materials and the surface area considered for the electrode. Maintaining lower potentials on MES system also warrants the direct electron transfer rather than H_2 mediated electron transfer for acetate production.

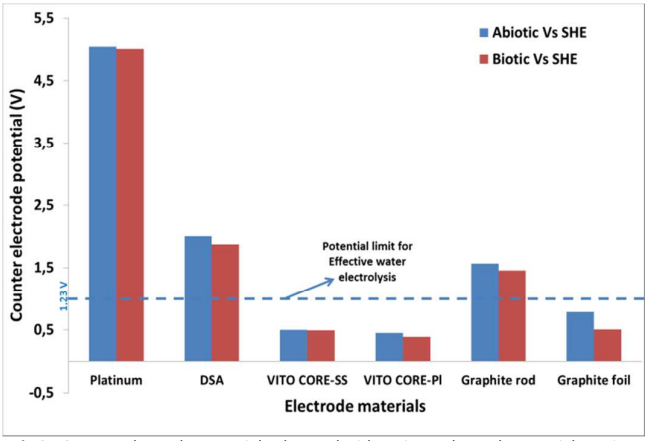

Fig 2: Counter electrode potentials observed with various electrode materials against VITO-CoRE electrode as working electrode at an applied potential of -600 mV. (Surface area of all the electrodes (except platinum) was equal. It is considered as 39 cm^2 and effective/reactive surface area is 30 cm^2). In case of platinum, as the economic and practical applicability of the platinum is expensive, a wire surface area consists of 2.37 cm^2 (effective/reactive surface area) was used.

To identify the efficient and suitable anode for MES, six different materials were screened under similar conditions of MES operation under both biotic and abiotic conditions (Fig 2). Among the six different electrodes used as the counter electrode, at -400 mV of working electrode potential, three electrodes viz., platinum, DSA and graphite rod, reached a potential higher than 1.23 V (Vs SHE), indicating the possibility of oxygen evolution. Platinum showed highest net counter electrode potential of 5.05 V under abiotic condition and 5.01 V under biotic conditions followed by DSA (abiotic, 2.01 V; biotic 1.87 V) and graphite rod (abiotic, 1.57 V; biotic 1.46 V). Even though graphite foil showed lower potential of 0.78 V under abiotic conditions, small rate of oxygen evolution was observed by the gases analysis from the reactor head space. In the case of biotic condition, 0.51 V of counter electrode potential was observed with no visible oxygen evolution. The two types of VITO-CoRE^{TM} electrodes (VC-SS and VC-IS) evidenced mild counter electrode potential (VC-SS, abiotic, 0.50 V and biotic 0.49 V; VC-IS, abiotic, 0.45 V and biotic 0.39 V) and no oxygen evolutions was observed under both abiotic and biotic conditions. The mild electrochemical activity and similar performance under both the biotic and abiotic conditions was indicated to select the VC-SS as the suitable counter electrode (anode) for further experimentation in MES towards acetate production.

3.2 Bioelectrosynthesis of acetate

Once the screening was done, two MESs were started up with selected VC-SS as anode and, VC-IS and graphite rod as cathodes (working electrode). During start-up stage (First phase, 50 days) of MES1, electroactive biofilm formation from the enriched homoacetogenic bacteria and subsequent stabilization of biofilm was observed at -400 mV with bicarbonates as the carbon source and electron acceptor for the production of acetate. As the inoculum was enriched with CO_2 and H_2 for acetate production, the initially MES1 showed acetate concentration of 101.42 mg/L which later decreased to 67.12 mg /L on 5th day with negligible current generation (Fig 3). From 8th day of operation, reduction of bicarbonates was confirmed with reduction current as well of metabolites analysis in electrolyte. By the end of 20th day, the acetate concentration reached to 755.67 mg/L (Fig 3a) with the reduction current density of -104 mA/m^2 (Fig 3b). During the second batch also the acetate production was found to increase gradually and reached to a concentration of 1231.43 mg acetate/L (30th day) with reduction current density of -133 mA/m^2. In the first phase, similar improvement in acetate production was observed with every feeding event and registered maximum at 35th day (4th cycle, 1972.86 mg/L; -165 mA/m^2). Further, this phase continued for another two batch cycles (until 50 days) to identify the optimum retention time for the 60% of feed replacement. During this period, the acetate production was found to be fluctuate between 1959.36 mg/L (38th day, -146 mA/m^2) and 1320.02 mg/L (50th day, -128 mA/m^2). After continuing for another two batch

cycling events, it was identified that the seven days as the optimum retention time for one batch of 60% feeding, and same retention time was respected till the end. During 51st day to 98th day of operation MES1 exhibited stable performance where the acetate production gradually increased from 1320.02 mg/L to a maximum concentration of 4127.01 mg/L (98th day, -142 mA/m^2) with the average reduction current density of -117 mA/m^2. Later both the acetate concentration and reduction current density was found to decrease until 127th day of operation and this period can be considered as the destabilization phase. Even after four feeding events, a decrease in acetate concentration was observed rather than improvement. By 126th day of operation acetate concentration was found to be decreased to 3270.26 mg/L. Similarly, reduction current density also found to decrease (126th day -67 mA/m^2).

The acetate production rate, the amount of acetate produced during a day (mg/L/d) was analysed to identify the specific bicarbonate reduction rate to acetate. Acetate production rates were also correlated well with start-up phase, stable operation and destabilization phase. During start-up phase the average acetate production rate was found to be 28.16 mg/L/d, which later increased to 59.94 mg/L/d (Fig 3b). During destabilization phase, the average acetate production rate registered negative value (-33.61 mg/L/d). The observed negative rate is due to the possible acetate oxidation at the anode of single chamber design. To support this phenomenon, a visible biofilm was observed on the anode electrode after 90 days of operation. When the oxidation of bio-electrochemically produced acetate at cathode is considered, the actual production rates of start-up and stabilization phases can be higher than visualized. As the media included was with the 0.5 g/L concentration of BESA, the conversion of acetate to methane is completely inhibited[31]. Along with acetate, negligible concentration of ethanol was also observed during start-up phase (10.05 mg/L, 35th day) and final phase (8.62 mg/L, 112th day). This can be due to the further bioelectrochemical reduction of acetate to ethanol. Besides ethanol production, the consumed acetate can be attributed to the biomass production of electroactive biofilm[12]. pH is also an important factor that influences the activity of the electroactive biofilm. Homoacetogenic bacteria favours mild acidic to neutral pHs. The initial pH of the present MES1 was maintained at pH 7.0. It was found to increase to alkaline pH with the time of operation. During stable phase of operation, catholyte pH was registered between 7.8 and 8.4. whereas in destabilization phase, it was found between 8.6 and 9.3. High alkaline pH, that is unfavourable for homoacetogenic bacteria prevailing in the cathodic chamber also one of the reason for the biofilm loss.

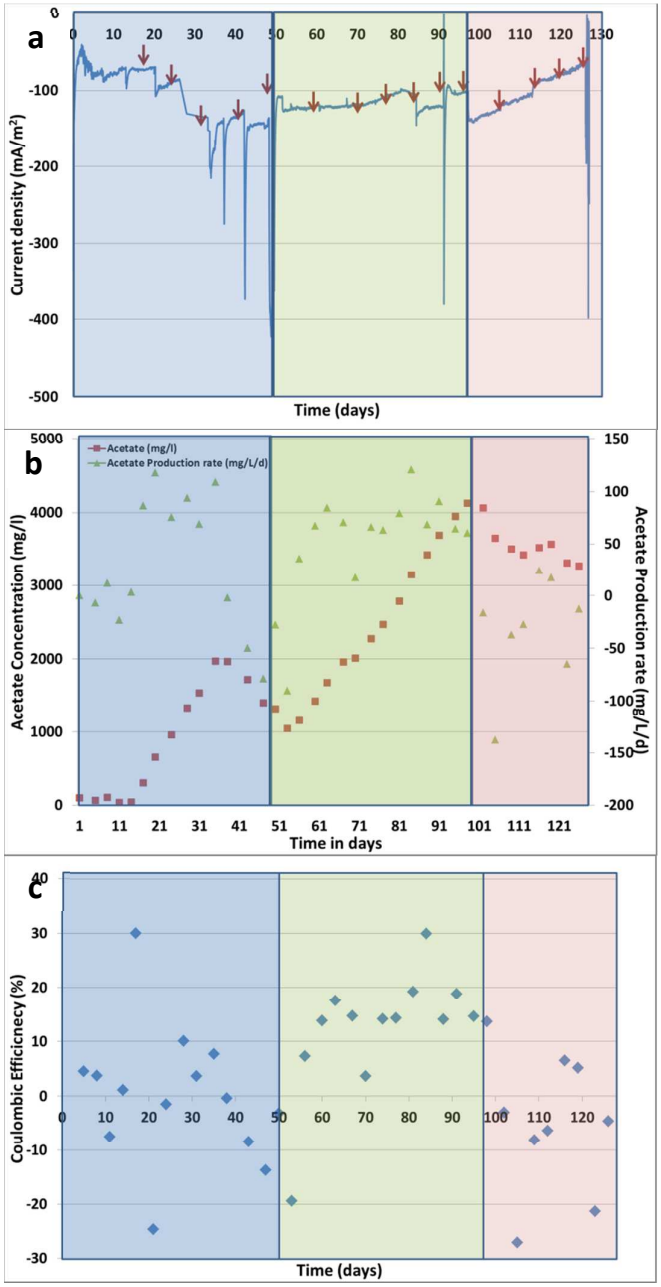

Fig 3: The performance of MES1 reactor during 127 days in batch mode operation with several feeding events (arrow mark depicts the feeding event). (a) Reduction current pattern during chronoamperometry (CA) used for the bicarbonates to acetates production; (b) acetate concentration and acetate production rate pattern during 127 days of operation; (c) coulombic efficiency during the total operation time.

In the case of MES2, initially, negligible reduction current was registered for 10 days (Fig 4a). The acetate transferred along with the enriched inoculum culture was (103.32 mg/L) observed degrading from the day 5 (84.22 mg/L) and it reached minimum concentration on 11th day (33 mg/L) (Fig 4b). Later, an increasing trend of acetate production associated with reduction current was observed. This depicts the electroactive biofilm formation and concomitant

conversion of bicarbonates to acetate. By the end of first feeding cycle, the acetate production reached to 206.49 mg/L with an average production rate of 5.15 mg/L/d. It gradually increased with every feeding event and reached to 821.25 mg/L (75.62 mg/L/d) and 1279.42 mg/L (76.33 mg/L/d) respectively by the end of second and third feeding cycles. The maximum concentration of acetate was observed on 40th day (4th cycle, 1523.2 mg/L; 40.67 mg/L/d) with the current density of -53 mA/m^2. Among the 47 days of operation, the reduction current density was significantly increased from 23rd day of operation and registered maximum on 39th day (-147 mA/m^2).

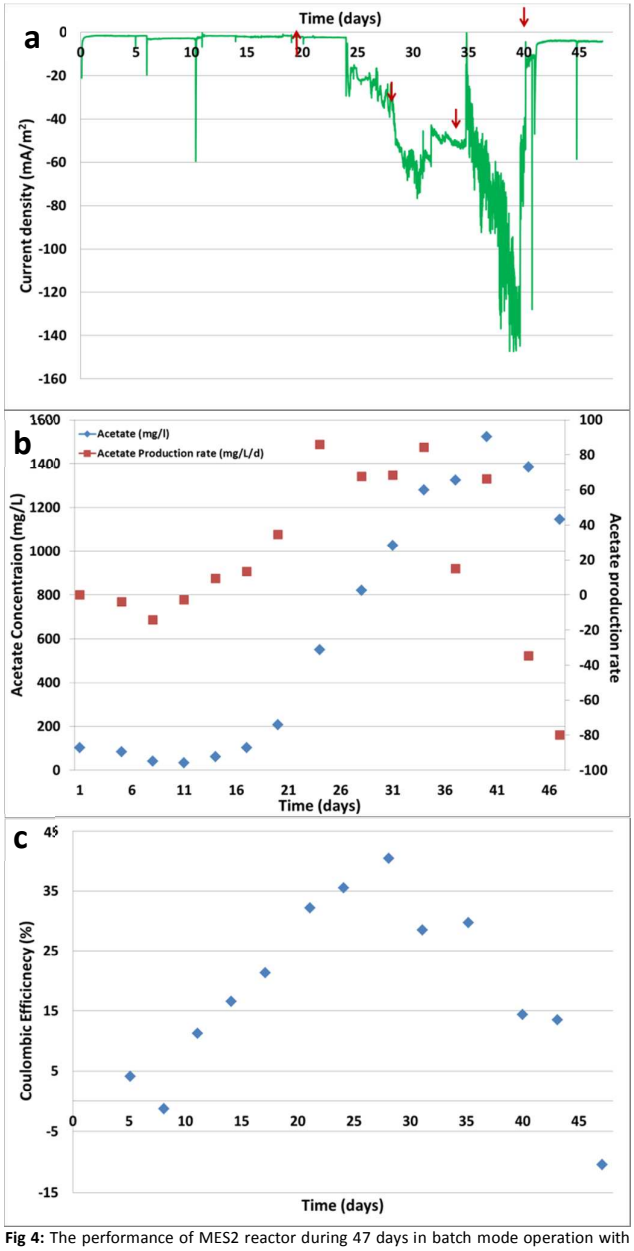

Fig 4: The performance of MES2 reactor during 47 days in batch mode operation with several feeding events (arrow mark depicts the feeding event). (a) Reduction current pattern during chronoamperometry (CA) used for the bicarbonates to acetates production; (b) acetate concentration and acetate production rate pattern during 127 days of operation; (c) coulombic efficiency during the total operation time.

During this operation, no ethanol production was observed. Later, visible detachment of biofilm was observed. It is also correlated well with the drop in reduction current and acetate concentration in the system. End of this cycle, the acetate concentration was decreased to 1145.25 mg/L with the acetate production rate of -54.20 mg/L/d. Even after biofilm loss also, the system was continued operation with regular feeding cycles for 5 consecutive cycles but no further acetate production and no reduction current were observed confirming the no biofilm formation. As MES2 was operated for lesser period than MES1 and the loss of biofilm occurred immediately after reaching the maximum acetate production and reduction current, no specific phases such as stable and final phases were observed. Similar to MES1, the catholyte pH of MES1 also found to increase to alkaline conditions. By the 30th day of operation (3rd feeding cycle), the pH was increased to 7.8 and later it was found to increase at rapid rate and reached 8.7 by 44th day of operation (5th cycle).

3.3 Coulombic efficiency

In microbial electrosynthesis, coulombic efficiency (CE%) is a relative expression that signifies the ratio between total charge (coulombs) that consumed in the reduction process and the actual charge contributed in the formation/production of desired product. It can be calculated with the formula CE(%) = C_P/C_T. where, C_P is the product of b (number of electrons consumed for the product, for acetate production it is 8), n (number of moles of product) and F (Faraday's constant (96,485 C/mol). C_T is total coulombs consumed that can be derived from integrating charge with the time[20]. Since ethanol was detected in negligible concentrations and the electron demand for acetate to ethanol conversion is lower (4 e^-) than acetate, considering it for CE calculation do not show significant difference on total CE. In the case of MES1, during stable phase of operation, maximum CE was registered as 29.91% and minimum as 3.65% with an average value of 23.52%. Whereas during start-up phase and destabilization phase, the maximum CE were registered as 18.38% and 6.54% respectively. Biofilm formation phase demands high amount of energy for the biomass formation and related metabolic activities. In the start-up phase, the electrons accepted by the cathode might be involved more in the metabolic functions to establish the biofilm on cathode surface. The acetate produced also participate in the production of various molecules which also decreases the observed acetate production in the electrolyte. So, the resultant CE is recorded lesser. A well-established electroactive biofilm requires less energy for the metabolic functions that reflected in the higher CE than start-up phase. The least CE values recorded in the destabilization phase might be attributed to two events. Firstly, in the destabilization phase, the biofilm observed on the anode electrode involves in the oxidation of acetate that produced due to cathodic reduction reaction. Second, due to the unfavourable conditions prevails in the system, the cathode electrode cannot efficiently participate in acetate production. In the case of MES2, CE increased gradually with the time of

operation and reached to maximum of 40.42% on 28th day (second cycle). Later a gradual drop was observed. During second and third cycle of operations, it was limited to 29.11% and 13.93% respectively. As the biofilm has detached and no acetate production observed in the fifth cycle of operation, negative value of CE was registered -10.43% (47th day). On the whole, MES2 registered an average CE value of 17.71%. The CE was found to be higher for the MES2 with graphite rod as cathode compared to MES1 with VC-IS. However, the formed biofilm was not sustained for long term operation.

Table 1: The Consolidated representation of calculated parameters from the basic results obtained in the present study.

Parameter	*Units*	MES1			MES2
		Start-up	Stable phase	Final phase @	
Maximum acetate concentration	*mg/L*	1972.86	4127.01	3568.41	1523.20
Production rate (Maximum/Average)	*mg/L/day*	117.96/26.97	120.90/56.94	24.37/--	85.96/20.68
Maximum current density	mA/m^2	-165	-142	-67	-147
Coulombic efficiency (CE)	*%*	29.97	29.91	6.54	40.43
Carbon conversion efficiency #	*%*	90.10	90.61	19.90	81.97
Volumetric production rate*	$kg/m^3/day$	0.269	0.569	--	0.206
Surface based production rate	$g/m^2/day$	4.497	9.490	--	3.447

* Calculated from the average performance during each phase
\# Calculated based on the feeding event
@ The values of average production rate, volumetric production rate and surface based production rates were negative. So the respective values were not presented in table

3.4 Carbon conversion efficiency

Carbon conversion efficiency is considered as one of the crucial parameter that shows effective conversion of substrate to product. The efficiency of bioelectrochemical reduction of bicarbonates (electron acceptors) to acetate can be found by calculating ratio between the carbon equivalents present in initial concentration of substrate and final product in each operating cycle (Table 1). It should be noted that the Carbon conversion efficiency calculation is considered only the products those observed in the electrolyte but the biomass produced for biofilm and suspension. In the case MES1, during the first cycle operation, it was registered as 47.08%. Gradually a steady increase in carbon conversion efficiency was observed with every feeding event and a maximum of 90.10% was registered on 35th day (start-up phase). During stable phase of operation it was registered as 90.61% on 84th day and with an average value of 62.38%. Due to the destabilization, in the final phase, carbon conversion efficiency was registered very low (19.90%). The trend observed for carbon conversion efficiency is found to be similar with CE. The carbon converted to acetate involves in the biomass production. If we consider the participation of carbon in biomass production, CCE may be higher than the reported. In the case of MES2, a different pattern of carbon conversion efficiency. In the first cycle only 8.25% of carbon was converted to acetate. Then a sharp increase in carbon conversion to acetate observed in the second cycle and registered 81.97%. It is the maximum efficiency for MES2. During third and fourth feeding cycles, the efficiency is limited to 61.09% and 32.50% respectively. Later due to destabilization, no significant efficiency of carbon was converted to acetate. Compared to MES2, MES1 showed higher bioelectrocatalytic conversion of carbon to acetate and the conversion process was sustained for longer period of time. On the whole, the registered average values in stable phase of MES1 are encouraging for the early stage research on bio-electrochemical reduction process of CO_2 to organic molecule production (Table 1). The difference between MES1 and MES2 is in the electrode material used for cathode (VITO CORE-PL in MES1 and graphite rod in MES). The better performance of MES1 could attributed to its high BET surface area of VITO CORE compared to graphite[34]. This also led to a better biofilm growth on MES1 which could be operated for 127 days while MES2 saw a loss of biofilm after 44 days and had to be stopped.

3.5 Discussion

The present study was performed with the single chambered system under mild operation conditions at 30 °C with the continuous applied potential of -400 mV. The thermodynamic potential required for the conversion of HCO_3^- to acetate is found to be -280 mV (SHE)[2] and the present study used still higher potential than the thermodynamic requirement. The potential losses related to the electrodes and microbial interactions, microbial electron transfer and electrolyte conductivity determines the required potential for the microbial electrosynthesis. Design of present study was based on characteristics that helps for the understanding of acetogenic biocathode production at lower cathode potential and the maintaining the biofilm for the long term use in single chamber that also helps for the upscaling aspects of the MES process. Compared to additional supplementation of H_2 in the reactor, direct feeding of electrons to acetogens through electrodes is likely to be practically more feasible. The lower energy input and renewable bacterial biocatalyst employment are advantageous aspects. Standard cathodic potential for bicarbonate/acetate redox couple is -280 mV[2]. A biofilm of *S. ovata* that was grown on H_2, when introduced into a cathodic chamber containing bicarbonate-based medium with no organic compounds except vitamin mixture, resulted in acetate production by reducing directly the bicarbonate. Under an applied potential of -400 mV (vs SHE) and in presence of a well-developed *S. ovata* biofilm, carbon dioxide or bicarbonate acted as the sole electron acceptor and got reduced to acetate[17,24]. Along with acetate, 2-oxobutyrate was also produced in smaller concentrations. Current consumption by *S. ovata* was found to be constant and correlated well with the acetate production with the coulombic efficiency of more than 86%. Production of both the compounds was stopped when current supply was stopped. Further, various acetogenic microorganisms such as *Sporomusa silvacetica, Sporomusa sphaeroides, Clostridium ljungdahlii, Clostridium aceticum* and *Moorella thermoacetica* as pure cultures showed their more

than 88% efficiency in converting CO_2 to acetate[24]. Under same applied potential of 0.4 V and S. ovata as biocatalyst, Nie et al., could achieve the higher acetate production rate of 1.13 mM/d[25]. Present study, MES1 showed 2.04 mM/d of maximum production rate (average, 0.97 mM/d) during stable operating phase.

The efficiency of the total carbon equivalents supplied to total carbon resulted in the product can be called as carbon conversion efficiency. MES1 exhibited maximum carbon conversion efficiency of 90.61% in stable phase followed by start-up phase. In the final phase, it was limited to the 19.90%, which might be due to the biofilm degeneration phase. In the case of MES2, 81.97% of carbon conversion efficiency was registered. Carbon conversion efficiency determines the carbon footprint of the system and practical applicability of the MES process on upscaling. The use of bicarbonates, instead of CO_2 is the major advantage to achieve higher conversion efficiencies. For direct CO_2 application, it is important to choose a system that solubilizes the CO_2 at higher rate or developing the electrolyte that favours both CO_2 solubility and biocathodic functions. Various reactor designs such as continuous stirred tank reactor, hallow fiber membrane reactor, bubble column, and airlift reactors used for the syngas fermentation can be applied in MES design [37-40]. As the biocatalyst of MES is electrode dwelling and the bioelectrochemical conversion of CO_2 to acetate occurs in the electrode vicinity designing hybrid reactors with BES and conventional reactor design can fetch these advantages. That in turn improve the bioelectrocatalytic reduction of CO_2 to products. Biological components such as using CO_2 solubilizing enzymes also can be considered for the higher carbon conversion efficiency. Carbonic anhydrase enzyme system to solubilize the CO_2 and further integrating the electrolyte with the acetogenic biocathode can be an interesting option for the improved productivities[3,41]. In the present study, higher carbon conversion efficiency can be majorly attributed to the use of bicarbonates in the system. It also suggests that an electroactive biocathode can effectively reduce the dissolved CO_2 to acetate. Evaluation of various approaches to improve the CO_2 solubility is also interesting to explore.

The electrodes used as cathode, their conductive properties and the surface coatings that are involved in the electron transfer mechanism also influences the production efficiency. A detailed study was done using various types of electrodes as cathodes that enhances the electrode and microbe-electron transfer rate for the CO_2 reduction using *S. ovata*[13]. Electron transfer rate enhancing strategies were validated by modifying the cathode surface properties which in turn resulted in improved acetate production from CO_2 reduction. Biofilms of *S. ovata* on carbon cloth that functionalized with positive-charged modifications such as chitosan, cyanuric chloride, 3-aminopropyltriethoxysilane, metals and (gold and palladium) nickel nanoparticles resulted in 3-7 folds improved acetate production than plain carbon cloth. However, other positively charged surfaces tested with melamine or ammonia gas did not stimulate acetate electrosynthesis[13]. The chitosan coated carbon cloth showed maximum surface based production rate (13.8 g/m^2/day), which is higher than the VITO electrodes (9.49 g/m^2/day). The VITO CoRE™ electrode manufactured with the activated carbon functioned efficiently for the CO_2 reduction and the coulombic efficiency can be compared to the other studies that employed similar mixed cultures and other economic electrode materials but at higher cathodic potentials than -400 mV (Fig 5a).

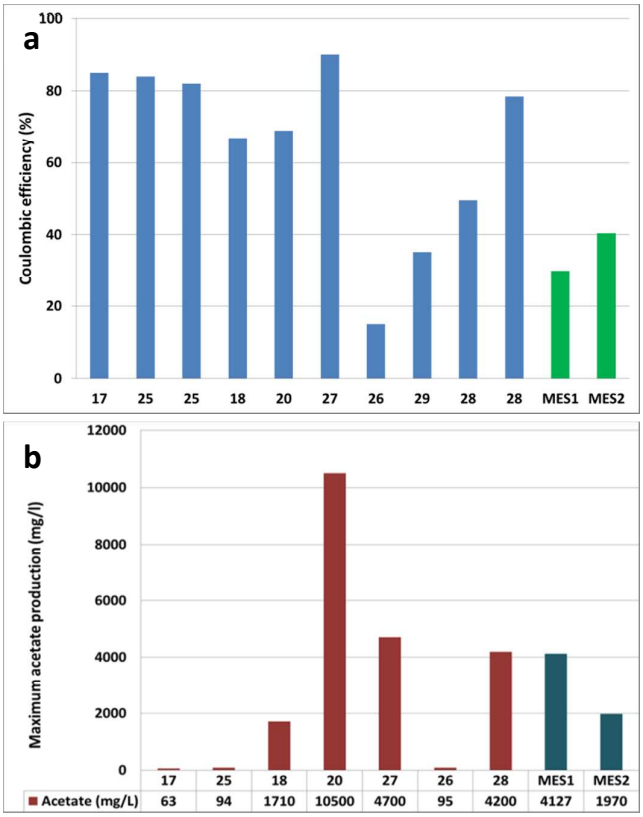

Fig 5: Comparative evaluation of the results with the state of the art microbial electrosynthesis of acetate from CO_2. (X-axis represents studies with the references and their respective production efficiencies)

The volume of the bioelectrochemical system is also important parameter that determines the efficiency and the losses associated to the input energy and process efficiency. In the case of MFCs, it was clearly observed that the reactor volume is inversely proportional to the electron transfer efficiency[9,42]. In MES also, maximum volume of the studies were limited to the 250 mL of cathodic volume in dual chambered configuration. For both MES1 and MES2, the electrolyte volume was considered as 500 mL with single chambered configuration. The maximum acetate production achieved with the mixed culture in dual chambered configuration at a poised potential of -590 mV, is 10.5 g/L [20] followed by Su et al.,[27] with 4.7 g/L at cathodic potential of -900 mV (Fig 5b). The present study achieved 4.13 g/L acetate with -400 mV which is almost equal to the concentration achieved by Jourdin et al.,[28] at -850 mV. In MES mechanism, the reaction is also influenced by the

electrode surface area or/and the biofilm covered on the electrode to the bulk liquid (electrolyte). So the projected surface area and surface based production rate ($g/m^2/d$) explains MES functionality. Jourdin et al.,[28] achieved highest surface based production rate of 37 $g/m^2/d$ with nanoweb reticulated vitreous carbon at -850 mV followed by Jiang et al.,[26] (19 $g/m^2/d$ at -800 mV) and Su et al.,[27] (10 $g/m^2/d$ at -700 mV) with carbon felt. VITO CoRE™ electrode registered 9.49 $g/m^2/d$ at -400 mV, where H_2 mediated electron transfer completely limited and only direct electron transfer is possible (Table 1). Maintaining higher cathodic potentials can also leads to the methane production[26]. The MFCs were proven to sustain the lower potentials for long term operation and the present study showed interesting results for acetate from CO_2 can warrant the direct integration of stable MFC for the acetate production through biocathodic reduction reaction.

4. Conclusions

Microbial electrosynthesis driven by the biocathode offers a wide range of chemicals from the inorganic carbon (CO_2). The selection of bacteria and the operating conditions influences the product and production efficiency. Formation of electroactive biofilm, stability and reproducibility of such biofilm delivers commercial application of this process. This is the first study that proved single chambered operation for bicarbonate reduction with enriched bacteria and was successfully operated for more than four months. VITO-CORE™ electrodes were found to be effective for CO_2 to acetate production. The biofilm became fragile at higher concentration of acetate and identifying optimum concentrations of the biofilm and design of required operational conditions to improve the stability of the microbial electrosynthesis process is necessary. On integration with other MES systems where the produced acetate can be used for the synthesis of higher organic compounds such as ethanol, butyrate, ethylene, etc., will provide the sustainable competition between global warming and energy needs.

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