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

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ABSTRACT

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

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1. Introduction

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

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

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

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

2. Materials and methods

2.1. Design of dual chamber MES reactor

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

2.2. Operation

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

2.2.1. Bicarbonate as inorganic carbon source

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

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

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

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