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Influence of headspace composition on product diversity by sulphate reducing bacteria biocathode

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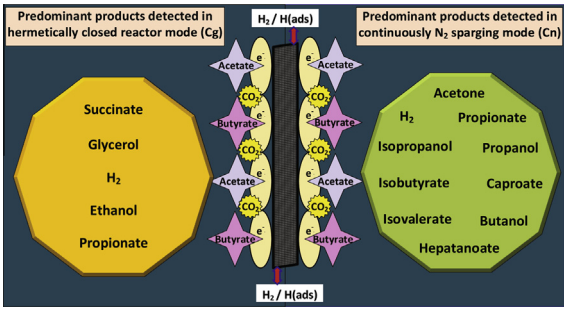
HIGHLIGHTS

- (Bio)electro/biochemically produced hydrogen determine the final product profile.
- Several valuable chemicals were microbially electrosynthesized.
- Headspace environment should be regulated to achieve the desired bioelectrochemical conversions.

GRAPHICAL ABSTRACT



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ABSTRACT

Mixed culture of sulphate reducing bacteria named TERI-MS-003 was used for development of biocathode on activated carbon fabric fastened to stainless steel mesh for conversion of volatile fatty acids to reduced organic compounds under chronoamperometric conditions of −0.85 V vs. Ag/AgCl (3.5 M KCl). A range of chemicals were bioelectrosynthesized, however the gases present in headspace environment of the bioelectrochemical reactor governed the product profile. Succinate, ethanol, hydrogen, glycerol and propionate were observed to be the predominant products when the reactor was hermetically sealed. On the other hand, acetone, propionate, isopropanol, propanol, isobutyrate, isovalerate and heptanoate were the predominant products when the reactor was continuously sparged with nitrogen. This study highlights the importance of head space composition in order to manoeuvre the final product profile desired during a microbial electro-synthesis operation and the need for simultaneously developing effective separation and recovery strategies from an economical and practical standpoint.

1. Introduction

Recently there has been an emerging class of study on microbes which are capable of taking up electrons from cathodic surfaces and utilizing them for a series of electrochemical transformations through which they reduce inorganic (e.g. CO_2) or organic chemicals (e.g. volatile fatty acids) into extracellular organic compounds (Soussan et al., 2013; Schröder, 2011; Rabaey and Rozendal, 2010). Microbial electrosynthesis requires some external electrical input to drive the conversions and overcome cathodic over-potentials, since many of the coupled electrochemical reactions are usually not thermodynamically feasible (Harnisch and Schröder, 2010). This electrical enhancement manipulates the redox metabolism

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by generation of reduced NADH within the cell through microbial electrodic interface reactions (Pandit and Mahadevan, 2011). More recently, major advances have been made in this realm of microbial electrosynthesis signifying the urgent need of research in this sector for production of value added chemicals. The successful demonstration of directly feeding electrons to acetogens with electrodes and the concept of integration of photovoltaics with electricity driven microbial reduction to organics was pitched by Nevin et al. (2010). Besides, there have been reports where this process is used for the production of H_2 (Rozendal et al., 2009; Sleutels et al., 2013), caustic soda (Rabaey et al., 2010), hydrogen peroxide (Rozendal et al., 2009), methane (Wagner et al., 2009; Villano et al., 2010; Cheng et al., 2009), caproate, caprylate (Van Eerten-Jansen et al., 2013) and combination of one or more of the above mentioned chemicals (Lovley and Nevin, 2013; Marshall et al., 2012; Angenent and Rosenbaum, 2013).

In our previous study, we reported the possibility of bioelectrochemically reducing acetic and butyric acids to a number of organic products such as alcohols and acetone by a mixed electroactive (EA) sulphate reducing bacteria (SRB, now designated as TERI-MS-003) based biocathode (Sharma et al., 2013a). Electrons used for such conversions are derived mainly from direct electron transfer (DET). Yet a minor role was attributed to H_2 as energy carrier. Steinbusch et al. (2008) proved that increasing H_2 partial pressure (HPP) by accumulation in the headspace would result in a metabolic shift from acidogenesis to alcohol production. Villano et al. (2010) showed that the product profile can be influenced by the gases present in the headspace mainly by hydrogen generation along with bioelectrochemical conversion of carbon dioxide to methane when cathode potential was poised more negative than −0.7 V vs. Ag/AgCl. However in our study, methane production was not observed, presumably due to high salinity and acidic pH of the electrolyte.

Following our earlier results and the rationale of such above mentioned citations, the effect of HPP is investigated here as a step further to elucidate the mechanistic features involved in SRB electrosynthesis in Bioelectrochemical systems (BES). This overall research aims to culminate in practical application to recycle and subsequently divert energy in the form of biochemicals, particularly from low grade organic carbon present in wastewaters like fermentation effluents.

2. Methods

2.1. Inoculum and electrolyte

Inoculum of a mixed EA-SRB, TERI-MS-003 consortium was taken from a previously running bioelectrochemical reactor (Sharma et al., 2013a).The inoculum (10% v/v) was added to the electrolyte used for reactor operation, that consisted of a synthetic feed composed of 572 mg NH_4Cl, 416 mg KH_2PO_4, 8 mg $CaCl_2$, 96 mg $MgCl_2{\cdot}6H_2O$, 1.98 mg $FeCl_2{\cdot}4H_2O$, 2.37 mg $CoCl_2{\cdot}6H_2O$, 0.59 mg $MnCl_2{\cdot}4H_2O$, 0.034 $CuCl_2{\cdot}2H_2O$, 0.062 mg H_3BO_3, 0.073 mg $Na_2MoO_4{\cdot}2H_2O$, 0.069 mg Na_2SeO_3, 0.095 mg $NiCl_2{\cdot}6H_2O$, 0.055 mg $ZnCl_2$ and 10 g NaCl per liter of demineralized water. The substrate used in the electrochemical cell was 0.1 M each of acetic and butyric acid. The pH of the feed was adjusted to 5 using NaOH at the start of the experiment.

2.2. Reactor set up

Reactors consisted of a single chamber glass set up with a total volume of 0.525 L out of which 0.475 L was used as a working volume (ES1). Activated carbon fabric (ACF) (HEG Ltd, India) of $6 \times 8 \times 0.27$ cm^3 fastened to a stainless steel (SS) mesh (316 grade) of the same projected surface area was used as working electrode material for development of the bio cathode. Other properties of the ACF electrode material have been described previously (Sharma et al., 2013b). Platinum rod (Metrohm, Netherlands) was used as a counter electrode. All materials were rinsed with demineralized water and properly sterilized at 121 °C for a maintenance period of 15 min prior to experimentation. The reactors were continuously magnetically stirred and maintained at ambient laboratory temperature of 24 ± 1 °C and pH was monitored throughout the experiment. Two types of reactor configurations were arranged to perform the experiments in triplicate. In the first case, hermetically closed reactor (Cg) was set using butyl rubber stoppers. The headspace of the Cg was initially sparged with N_2 gas and then sealed using silicon and aluminium caps after the addition of substrate and electrolyte. In the second case, a continuously N_2-sparged reactor (Cn) was set, where an aseptic needle was inserted in the liquid phase of the reactor for spraging. After 24 h of poising the cell in abiotic conditions, the TERI-MS-003 (10% v/v) was used to inoculate the reactor. Ag/AgCl (3.5 M KCl) from Metrohm (Netherlands) was used as a reference electrode and kept at a distance of 1.3 cm from the working electrode (cathode), out of the projected path between the working and the counter electrodes. Two more set of reactors were also set up, as controls. In the first type, the conditions were exactly similar to the experimental Cg and Cn set ups with the omission of inoculum. In the second type of control experiment, the reactors were inoculated but not poised by the potentiostat (open circuit conditions).

2.3. Analytical methods

For analysis, 5 mL of samples were collected from the sampling port of the reactors using sterile and N_2-purged syringe. Subsequently, the samples were centrifuged at 10,000 rpm for 10 min and supernatant was filtered with 0.44 μm pore diameter Whatman® filter paper. pH was measured using a pH meter with relevant probes (Mettler Toledo 7 multi, India).Volatile Fatty acids (VFA) in the liquid phase were analyzed using Gas Chromatograph (GC) 7890N (Agilent, USA) equipped with flame ionizer detector and DB-WAXetr high polarity column (30 m × 530 μm; id 1 μm). The oven temperature was programmed from 140 °C with ramping of 1 °C per min up to 158 °C. The injector and detector temperatures were 220 °C and 230 °C, respectively. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. Other organic products such as succinate, formate, malate, acetone, glycerol etc. were analyzed using HPLC 1100 (Agilent, USA) with Aminex 87H (Bio-Rad, USA) column. Head space gas analysis was performed by using GC 7890N (Agilent, USA) fitted with NUCON SS packed column (length 2 m, id 2 mm) and thermal conductivity detector. Helium was used as carrier gas, at a flow rate of 6 mL min^{-1}. The operating temperatures of injector, oven and detector were 50, 100 and 150 °C respectively. Sampling was performed using a sterile gas lock syringe (Agilent, USA) after purging it with inert gas (N_2). Samples were analyzed immediately after collection. The analytical systems (GC and HPLC) were calibrated with standard chemicals from Sigma Aldrich in the range of concentration of the samples analyzed.

2.4. Electrochemical measurements

All electrochemical measurements were performed using a potentiostat (Autolab-PGSTAT 101, Metrohm, The Netherlands). The biocathode was employed as working electrode, the platinum rod (Metrohm, Netherlands) as the counter electrode and Ag/AgCl (3.5 M KCl) as reference electrode for all the set-ups studied. The working electrodes of the BES reactors were potentiostatically poised throughout the experiment at a cathodic potential of

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