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

Mohita Sharma¹,²,³#, Jhansi L Varanasi²#, Pratiksha Jain¹,²#, Prem Dureja², Banwari Lal², Xochitl Dominguez-Benetton³, Deepak Pant³, Priyangshu M Sarma¹,²*

¹ TERI University, 10 Institutional Area, Vasant Kunj, New Delhi-110070, India
² TERI, Darbari Seth Block, India Habitat Centre, New Delhi-110003, India
³ Separations and Conversion Technologies, VITO- Flemish Institute for Technological Research, Boeretang 200, 2400 Mol, Belgium

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 (3.5M 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,

# These authors contributed equally to this manuscript and should be considered as co-first authors.

Corresponding author: Tel: +0091 11 24682100, Fax: +0091 11 24682144, E-mail: priyanms@teri.res.in
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.

Keywords
Microbial electrosynthesis (MES); biocathode; direct electron transfer (DET); activated carbon fabric (ACF); sulphate reducing bacteria (SRB)

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 (eg. CO₂) or organic chemicals (eg.
volatile fatty acids) into extracellular organic compounds (Soussan et al., 2013; Schröder., 2011; Rabaey and Rozendal., 2010a). 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 by generation of reduced NADH within the cell through microbial electrocatalytic 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 H₂ (Rozendal et al., 2009; Sleutels et al., 2013), caustic soda (Rabaey et al., 2010b), hydrogen peroxide (Rozendal et al., 2009), methane (Wagner et al., 2009; Villano et al., 2010; Cheng et al., 2009), caproate, caprylate (Van Eerten-Janse 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 H₂ as energy carrier. Steinbusch et al. (2008) proved that increasing H₂ 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. Materials and 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$_4$Cl, 416 mg KH$_2$PO$_4$, 8 mg CaCl$_2$, 96 mg MgCl$_2$·6H$_2$O, 1.98 mg FeCl$_2$·4H$_2$O, 2.37 mg CoCl$_2$·6H$_2$O, 0.59 mg MnCl$_2$·4H$_2$O, 0.034 CuCl$_2$·2H$_2$O, 0.062 mg H$_3$BO$_3$, 0.073 mg Na$_2$MoO$_4$·2H$_2$O, 0.069 mg Na$_2$SeO$_3$, 0.095 mg NiCl$_2$·6H$_2$O, 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. 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 ºC for a maintenance period of 15 minutes 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 butyric rubber stoppers. The headspace of the Cg was initially sparged with N\textsubscript{2} gas and then sealed using silicon and aluminium caps after the addition of substrate and electrolyte. In the second case, a continuously N\textsubscript{2}-sparged reactor (Cn) was set, where an aseptic needle was inserted in the liquid phase of the reactor for spraying. After 24 hours 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\textsubscript{2}-purged syringe. Subsequently, the samples were centrifuged at 10,000 rpm for 10 minutes 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 -0.85 V (vs. Ag/AgCl/3.5 M KCl) recording chronoamperometry (CA). Cyclic voltammetry (CV) measurements were evaluated over a range of -0.9 V to 0.2 V (vs. Ag/AgCl/ 3.5 M KCl) at a scan rate of 1 mVs\(^{-1}\).
2.5 Calculations

Total coulombs consumed by bioelectrochemical reduction of acetic and butyric acids were calculated as described by Equation (Eq.) 1. (Liu and Logan 2004), for each substrate:

\[ Q_i = \frac{F b_i S_i V}{M_i} \times 1000 \]  

Eq. 1

Where \( F \) is Faraday’s constant (96,485 Cmol\(^{-1}\)), \( b_i \) is the number of moles of electrons consumed per mole of substrate (\( b_a = 8, b_b = 20 \)), \( S_i \) is the substrate consumed, \( V \) is the working volume of the reactor and \( M_i \) is the molar mass of the corresponding substrate. In the same way, coulombs available at a certain point of experimentation can be calculated knowing the punctual concentration of the substrates. The amount of coulombs (\( C_p \)) distributed in the moles of individual products (\( n_i \)) bioelectrochemically synthesized during the process was calculated using Eq. 2 (Marshall et al., 2012):

\[ C_p = F b_i n_i \]  

Eq. 2

Capacitance is the ability of a cell to hold electrical charge and is a measure of the amount of electrical energy stored at an applied electric potential. Energy stored in a capacitor is equal to the work done to charge it (Raghavulu et al., 2012). Both capacitance and energy calculations were done using data obtained by CV analysis using Eq. 3 and Eq.4,

\[ C = \frac{Q}{V} \]  

Eq. 3

\[ E = \frac{Q^2}{2C} = \frac{CV^2}{2} \]  

Eq. 4

Where, \( E \) is the energy (J), \( C \) is the capacitance (F), \( Q \) is the amount of charge obtained (C) and \( V \) is the maximum applied potential (V) (Raghavulu et al., 2012).
3. Result and discussion

One of the major bottlenecks for conversion of electrical energy into organic chemicals via microbial electrocatalytic systems has been the unavailability of biocathodes that can achieve selected transformations at relevant kinetic rates. In our recent study (Sharma et al., 2013a), the development of SRB biocathode was achieved. The same inoculum (TERI-MS-003) was used here to develop a biocathode in a low-cost, easy to assemble, single chambered reactor as shown in the schematic (ES 1), operating in a continuous stirring mode to reduce mass transfer limitations. The sole electron donor available is the cathodic electrode surface whereas the electron acceptor is the VFAs (acetic and butyric acid) present in the medium, which get further reduced down to alcohols, acetone, elongated VFAs and other energy carriers.

The biocathode made from TERI-MS-003 inoculum is capable of performing bioelectrosynthesis. In our previous study, it was observed that glycerol was one of the primary products detected in Cg reactor while in the case of reactor where N₂ was continuously sparged (Cn), propionate, propanol, acetone, ethanol were predominantly detected (Sharma et al., 2013a). In order to further elucidate product profiles and elaborate on the metabolic routes, in the present study we followed two experimental protocols in batch mode of operation. In the first case, a glass reactor was completely made anaerobic and air tight (Cg), and was poised at −0.85 V vs. Ag/AgCl/3.5 M KCl. This facilitated accumulation of H₂ in the headspace presumably form electrochemical origin after water electrolysis. The product profile represented here was drawn after consideration of the triplicate experiments, where the results obtained were within the limits of experimental
The headspace gas composition of Cg reactor after one day of reactor operation was 15 mM H$_2$ and 5 mM CO$_2$, on day 9 the maximum concentration of H$_2$ (39 mM) and CO$_2$ (19 mM) were reached and by end of the experiment H$_2$ and CO$_2$ concentration were respectively 33 mM and 11 mM. Product profile from the Cg reactor is shown in Fig. 1. Glycerol, succinate, ethanol, hydrogen and propionate were the products detected. Steinbusch et al. (2010) studied ethanol and butyrate production from VFAs by microbial reactors as inoculum, using methyl viologen as mediator where concentrations up to 83 mg L$^{-1}$ ethanol and 53 mg L$^{-1}$ butyrate were obtained respectively. A more recent study by Van Eerten-Jansen et al. (2013) using mixed cultures, with Clostridium kluyveri as the predominant microbe, produced up to 739 mg L$^{-1}$ caproate, 263 mg L$^{-1}$ butyrate, 36 mg L$^{-1}$ caprylate, and 27 mg L$^{-1}$ ethanol, without using redox mediators. In the latter study, in-situ electrogenerated H$_2$ was presumptively the electron donor for the bioelectrochemical reduction of acetate. Both studies involved accumulation of H$_2$ in the headspace of the reactor. Comparatively, the Cg reactor case of this study generated a maximum of about 296 mg L$^{-1}$ ethanol, being significantly higher than both preceding studies. With respect to classic fermentation, concentrations of propionate comparative to the investigations by Arslan et al. (2012) were obtained. In the later study H$_2$, CO$_2$ and substrate concentrations were controlled in mixed culture fermentations using different waste streams, obtaining product profiles with C2, C3, C4 and C6 compounds in varying concentrations. Particularly, the highest concentrations there obtained of propionate in the presence of H$_2$ in the headspace were in the range of 55 to 217 mg L$^{-1}$. Here we report maximal propionate concentrations of about 467 mg L$^{-1}$ by days 8-9 of operation (Fig. 1), bringing microbial
electrosynthesis systems to a promising perspective. Maximum concentration of succinate produced was 105 mg L\(^{-1}\), whereas glycerol was 26 mg L\(^{-1}\). From Fig. 1, \(\text{H}_2\) accumulation can also be appreciated for the Cg bioelectrochemical reactor. By the end of the experiment, acetic and butyric substrates were almost fully utilized. Additionally, variation in pH, redox potential and \(\text{H}_2\) plays a major role in the overall reactor dynamics. Using the protons available, the most readily formed products from acetate and butyrate are propionate and ethanol (Agler et. al., 2011). The predominance of ethanol and propionate in this Cg system can be attributed to lower pH (more protons) and presence of hydrogen in the reactor environment. Since ethanol accumulation renders biological reduction reactions thermodynamically unfeasible, more reduced products were not detected in Cg system where accumulation of ethanol was seen as the experiment progressed (Sharma et. al., 2013a). It is likely that some microbes with a characteristic metabolism present in this mixed culture can readily use the electrons from the electrode or the hydrogen gas in their pathways, in a differentiated mode creating variation in the product profile.

In the second protocol, \(\text{N}_2\) was continuously flushed in a similar reactor set up (Cn) in order to replace \(\text{H}_2\) and possibly other gases accumulated in the headspace, that are formed either biologically or (bio)-electrochemically. The major products detected for Cn were acetone, isopropanol, propanol, propionate, isobutyrate, isovalerate, valerate and heptanoate. This indicates a clear deviation in product profile from the previous Cg reactor. As in the case of propionate, the presence of \(\text{H}_2\) decreases from 467 mg L\(^{-1}\) in the Cg reactor to 170 mg L\(^{-1}\) in the Cn reactor. Other major changes were the production of additional alcohols such as
propanol/isopropanol and longer chain carbon compounds like valerate and heptanoate in
the Cn reactor.

Hence, ethanol produced in the Cn reactor may get converted or incorporated into longer
chain carbon products, which is in good concordance to our earlier reported study (Sharma
et al., 2013a). Due to continuous flushing of N₂ gas, H₂ partial pressure becomes negligible;
however there would still be a possibility that some amount of cathodic H₂ may be
entrapped in the form of adsorbed hydrogen, at the electrode surface (Jitaru, 2007) and
therefore integrated into some of the organics formed. Such adsorbed intermediated H₂
would be consistent with the adsorption-like behavior earlier detected by electrochemical
impedance spectroscopy (Sharma et al. 2013a), which was absent in the Cg reactors.

Compared to previously reported bioelectrochemical conversion products as enlisted in
Table 1 (ES 2), most of the conversions reported in this study are in concordance with the
previously reported cases. As seen from Fig. 1, most of the substrate concentration fed to
the batch reactors was used up in both cases. Besides variability in electrolyte pH was
negligible due to the self-buffering capacity of the system (Fig. 2). In the case of Cg
reactor, pH started to decrease by the end of the operation, which may be attributed to
accumulated headspace HPP, making the electrolyte slightly more acidic. The control
experiments were set-up as described in section 2.2. The concentration of acetate and
butyrate added as substrate in these reactors remained relatively unchanged even by the end
of the experimental operation of 15 days. There was also no significant current or
production of biochemicals observed.

**Fig. 1 and Fig. 2**
3.1 Effect of presence of hydrogen in the headspace

In general $\text{H}_2$ is known to play an important role in the metabolism of SRB where it can be both consumed and produced by acting as reducing agent or by its production in the absence of sulphate respectively. The $\text{H}_2$ produced by electrocatalysis can be further utilized by SRB hydrogenases (and cytochromes) in the classic catalytic reaction. The reducing equivalents generated in the process are then incorporated directly into the organic products through SRB bioelectrocatalysis (Aubert et al., 2000; Beech and Sunner, 2004). As a consequence, the presence of $\text{H}_2$ in Cg reactor directs the SRB metabolic pathways such that a characteristic product profile is established. Such profile drastically diverted from the one achieved under Cn environment (Fig. 1). Thus, product formation is possible in the presence and accumulation of electrogenerated $\text{H}_2$ as electron donor, but HPP is clearly an essential driver for product selectivity and bioelectrochemical conversions achieved by SRB. $\text{H}_2$ evolution is also the major competing reaction for conversion of organics to further reduced bioelectrochemical products (Jataru, 2007). However in the case of Cg reactor, since the maximum $\text{H}_2$ production throughout the operation was never over 40 mM, it could not act as an impediment in bioelectrochemical driven product conversions but it certainly influenced the range of products derived from the two reactors. It was also observed that all the coulombs consumed from the substrate were not in proportion to the number of coulombs converted into products (Fig. 3, ES3). One of the postulates for this discrepancy would be the limitations of the analytical approach employed in the study. By the end of operation of both Cn and Cg reactors, almost all the equivalent coulombs provided via the substrate were found to be exhausted (Fig. 3).
Potential reactor leakages, conversion into biomass and bubbles trapped in electrode have been described as alternative reasons (Marshall et al., 2012). In the present study, it was observed that the unclosed coloumbic balance was not diverted to biomass production as confirmed by the absence of significant increase in turbidity in the reactor. This was in agreement with the observations from previous studies (Nevin, et al. 2010 and Strycharz et al., 2010) which clearly demonstrates that limited amount of electrons get diverted to biomass production and majorly contribute only for bioelectrochemical conversions. Such discrepancy could also be potentially explained by the lack of ion exchange separator between working and counter electrode, leading to (bio) electrochemical oxidation to CO$_2$ of part of the organics formed at the biocathode; however, from previous experiments (Sharma et al., 2013a) and the results obtained here, very negligible amount of the accumulated headspace gases in Cg was composed of CO$_2$ (1–8%) and in either case, no methane was noticeable. In summary, enriched or declined H$_2$ partial pressure in reactor headspace during SRB cathodic electrosynthesis re-routes organic production towards assortments of diverse chemicals, respectively.

**Fig. 3**

**3.2 Voltammetric evaluation**

Voltammetric techniques help to demonstrate the ability of the electrode surfaces in driving reduction reactions by the biocatalysts (Sharma et al., 2013b). In this study, cyclic voltammetry (CV) was used to understand the biocatalytic phenomenon of charge transfer occurring during microbial electroreduction reactions.
CV profiles indicated alteration in electrochemical behavior with the change in headspace composition. Catalytic currents were almost similar for both the cases (Fig. 4), however there was significant variation in charge distribution for both the experiments. Charge is an indicator of the number of electrons present at an instant during an electrochemical reaction (Raghavulu et al., 2012). The voltammetric profiles showed higher charge for Cg (14 C) than for Cn (9.45 C, Fig. 4), which suggests that the availability of electrons on the electrode was higher when the system was hermetically closed. This is also evident from the coloumbic distribution of products calculated (Fig. 3) wherein the total transferred electrons were found to be distributed into a larger diversity of products in Cn than in Cg, thus limiting the available electrons on the electrode.

On the other hand, capacitance is a measure of amount of ion storage at an applied electric potential. Similar to the charge, higher capacitance was observed during Cg operation (Cg: 17.55 F; Cn: 11.12 F, Fig. 4) thus indicating efficient ion holding capacity for Cg system. Ions stored in a capacitive electrode are proportional to the work done to migrate them towards the electrode (Raghavulu et al., 2012). The energy (ion storage) levels for Cg were slightly higher than Cn (Cg: 6.34 J; Cn: 4.02 J, Fig. 4).

For both experiments (Cg and Cn) the nature and size of the electrode material, the electrolyte used, and the initial substrate were same thus any change in charge distribution during CV indicates variations of SRB metabolism under the influence of headspace gas composition which also result in differentiated thermodynamic constraints.

Fig. 4
3.3 Proposed metabolic routes

The metabolic routes are influenced by the amount and composition of gases present in the headspace as can be seen in the product profile corresponding to the different reactors. Considering the products obtained and earlier reported bioelectrochemical conversions as enlisted in ES2, an overall route has been proposed for this community of EA-SRB microbes (ES 4). For both sets of reaction (Cg and Cn), Acetyl-CoA is an important biochemical intermediate. Moreover, since most anaerobic bacteria cannot oxidize acetyl-CoA to CO\(_2\) via the citric acid cycle, it is also their most frequently used source of high-energy phosphate (Thauer et al., 1977).

In the Cg reactor, succinate, glycerol and propionate are the major products, all of which can be formed with acetyl-CoA as the intermediate. From acetyl-CoA, through electron transport phosphorylation, oxaloacetate, malate, fumarate and succinate are formed (Thauer et al., 1977). Further, reduction of succinate to glycerol has been demonstrated by Soussan et al. (2013). Propionate can be formed from pyruvate (Thauer et al., 1977).

In the continuously sparged set, a number of products were detected mainly due to the diversion of electron donating reactions. Acetate reduction has been previously reported to produce ethanol (Agler et al., 2011). Further, ethanol and acetate can combine to give either butyrate or caproate (Thauer, 1977) depending upon the stoichiometry of ethanol, which might also explain the absence/consumption of ethanol during the later stages. This behaviour was also reported previously (Sharma et al., 2013a). Acetate in combination with acetyl-CoA forms acetaldehyde, which in turn may form acetone and isopropanol. Acetate and propionate may fuse to give n-valerate (Thauer, 1977). Isopropanol along with the
propionate can form isovalerate. Similarly, butyrate and propanol can react to form heptanoate. Caproate may be formed by combination of acetate with either ethanol or butyrate (Steinbusch et al., 2011; Thauer et al., 1977). Also, carboxylic acids may reduce to form their respective alcohols (propionate to propanol and butyrate to butanol) (Agler et al., 2011).

Main driver of these conversions under Cn conditions is assumed to be the direct electron transfer (DET) from the electrode to the EA-SRB, present in the mixed consortium TERI-MS-003 that was used as an inoculum. It has been reported in few studies earlier that SRB can directly exchange electrons with electrode surfaces (Sharma et al., 2013a; Venzlaff et al., 2013; Wan et al., 2012). Besides it is also capable of forming good EA biofilms on cathodic surfaces (Cordas et al., 2008; Wan et al., 2011). As previously studied, EA biofilms in general are known to influence the rate of electron transfer besides the applied cathodic potential (Villano et al., 2010). In Cg conditions, H₂ acts as an important energy carrier and reducing equivalent for the bioelectrochemical reduction transformations.

3.4 Some practical considerations for scale up studies

Prior to system scale up, optimization of a wide variety of operational parameters is necessary, ranging from set potential to temperature, pH, concentration of substrates, etc. Besides, tight anoxicity and hermeticity in the reactor is critical when products characteristic of Cg conditions are prospected since, the appropriate partial pressure of H₂ is needed to achieve the desired conversions. On the other hand, as reported previously by Perez et al. (2013) gas stripping can be a major impediment for the recovery of products in
continuously N\textsubscript{2} sparged type of reactors because highly volatile products like alcohols
(which have higher vapor pressure and less solubility in water) will be the first to be lost
due to evaporation and continuous gas sparging. This also brings in difficulties to anticipate
if low columbic conversions are due to inadequacy of the biocathode or the loss of products
due to vaporization as a result of gas stripping.

Nonetheless, the use of mixed culture provides additional benefits of working under not so
sterile conditions and adoption of the technology in-situ for simultaneous treatments of
waste streams and energy recovery, reducing the overall cost of implementing the
technology. Product market value was also assessed in this study in relation to the used
substrate compounds i. e. acetate and butyrate. A comparative cost analysis was performed
for all the products detected in both Cg and Cn experiments. The retail cost of purchasing
these chemicals (per Litre) from a major supplier in India (HiMedia) was considered. As
can be seen in ES 5, in hermetically closed setup, the most profitable product that can be
targeted for mass production includes isovaleric acid and butanol. Besides the market value,
other important factors that govern selectivity are the stability and energy density of the
product, down streaming cost involved in processing these products to high grade
chemicals and the flexibility of directly integrating it with the existing infrastructure. For
instance, butanol is considered a beneficial product as it can be effortlessly stored,
distributed and transported via the existing pipelines and used with gasoline, highlighting
the benefits of eco-friendly microbial electrosynthesis method over other conventional
biomass based production methods (Lovley and Nevin., 2011).
4. Conclusions

Electroactive SRB biocathodes serve as efficient biocatalysts and the metabolic routes
shifts with alteration of headspace environment. Though most of such metabolic
interventions are generally carried out though metabolic engineering, this study
demonstrates that a set of economically desirable (bio) chemicals can be
bioelectrochemically synthesized without any genetic manipulations in the biocatalyst.
Other bigger challenges like effective separation of these microbial electrosynthesized
chemicals, pathway diversion towards single product recovery, downstream processing and
development of rapid and efficient in-situ chromatography methods will be subject of
future research in this realm of microbial electrosynthesis.

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<tr>
<th>Parameter</th>
<th>Cg</th>
<th>Cn</th>
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<td>Total Q (C)</td>
<td>14.92</td>
<td>9.45</td>
</tr>
<tr>
<td>Capacitance (F)</td>
<td>17.55</td>
<td>11.12</td>
</tr>
<tr>
<td>Energy (J)</td>
<td>6.34</td>
<td>4.02</td>
</tr>
</tbody>
</table>

E/V vs. Ag/AgCl/3.5 M KCl
Graphical Abstract (for review)
Highlights

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