ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2015, **51**, 3235

Received 18th December 2014,
Accepted 9th January 2015

DOI: 10.1039/c4cc10121a

www.rsc.org/chemcomm

Microbial electrosynthesis of butyrate from carbon dioxide†

R. Ganigué,‡ S. Puig,*‡ P. Batlle-Vilanova, M. D. Balaguer and J. Colprim

This work proves for the first time the bioelectrochemical production of butyrate from CO_2 as a sole carbon source. The highest concentration of butyrate achieved was 20.2 mMC, with a maximum butyrate production rate of 1.82 mMC d^{-1}. The electrochemical characterisation demonstrated that the CO_2 reduction to butyrate was hydrogen driven. Production of ethanol and butanol was also observed opening up the potential for biofuel production.

The depletion of fossil resources, their ever-increasing high price and the negative environmental impacts derived from their use are forcing the transition to more sustainable energy and chemical production models, based on renewable and carbon-neutral commodity chemicals and fuels. Carbon dioxide (CO_2) can be transformed into added-value products mainly by chemical transformations, photochemical, chemical and electrochemical reductions, biological conversions, reforming, and inorganic transformations.[1] Current CO_2 mitigation and conversion techniques require extremely large surface and volumes, energy intense processing steps and/or chemicals and expensive catalysts.[2] Microbial electrosynthesis has been recently postulated as a promising approach to transform CO_2 into value-added compounds.[3] In such systems, carboxydotrophic microorganisms are harnessed to fixate CO_2 into products *via* the Wood–Ljungdahl pathway, using electrical current as a driving force. This concept was first proven by Nevin *et al.* (2010), who transformed CO_2 into acetate using pure cultures, up to a concentration of 2 mM.[4] Two subsequent studies from Marshall and co-workers increased, using mixed cultures, the acetate titer to 28.5 mM and 175 mM, respectively.[5,6]

To date, acetate has been the sole product of CO_2 reduction in BioElectrochemical Systems (BESs). However, the autotrophic production of acetate is not very attractive from the economic standpoint due to its low market price. In this light, several authors have attempted to upgrade it to higher value products. Sharma and co-workers investigated the biocatalysed reduction of acetic and butyric acid to bioalcohols and mid-chain fatty acids *via* direct electron transfer at −0.65 V *vs.* standard hydrogen electrode (SHE). They observed the transformation of those into a mixture of products, including 0.8 mM of methanol, 0.2 mM of ethanol, 0.4 mM of propanol, 0.6 mM of butanol and 0.2 mM of acetone, as well as lower amounts of propionic and caproic acids.[7] In a similar way, Steinbusch *et al.* (2010) studied the bioelectrochemical ethanol production through mediated acetate reduction with methyl viologen. They reached 13.5 mM of alcohols as well as C4 compounds.[8] Finally, Eerten-Jansen *et al.* (2013) poised the cathode potential at −0.9 V *vs.* SHE to biologically reduce acetate, obtaining 6.8 mM of caproate and 3.0 mM of butyrate as main products.[9] The present work proves for the first time the bioelectrochemical concomitant production of acetate and butyrate from CO_2. Butyrate is an industrial feedstock with many applications in the pharmaceutical and chemical industries, and can be converted into fuels through esterification.[10]

Two experiments were performed to prove the bioelectrochemical production of butyrate from CO_2. These were conducted in 240 mL two-chambered H-type BES. A cathode made of commercial carbon cloth (NuVant's ELAT® LT2400W, FuelCellsEtc, USA), with an area of 9 cm^2 and an area to volume ratio of 0.075 cm^2 mL^{-1}, was used as a working electrode. An Ag/AgCl (+0.197 V *vs.* SHE, model RE-5B, BASI, United Kingdom) was also placed in the cathodic chamber as a reference electrode, whereas a titanium rod (Ti plus 50 g m^{-2} Pt, Magneto, The Netherlands) served as a counter electrode in the anodic compartment. Both chambers were filled with 120 mL of mineral medium similar to ATCC 1754 (Tanner *et al.*, 1993),[21] containing 2-bromoethanesulfonate to inhibit methanogenesis (see ESI,† Table S1). These compartments were separated by a cationic exchange membrane (CMI-7000, Membranes International Inc., USA) and stirred to avoid mass transfer limitations. The cathode compartment had two butyl-rubber sampling ports. Finally, the cells were wrapped with a coil of plastic tubing connected to a thermostatic bath to control the operational temperature. Temperatures for first and

LEQUIA, Institute of the Environment, University of Girona, Campus de Montilivi, Girona, Catalonia, E-17071, Spain. E-mail: sebastia@lequia.udg.cat

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c4cc10121a

‡ These authors contributed equally.

second experiments were 35.5 ± 2.5 °C and 33.9 ± 1.1 °C, respectively.

The biocathode was poised at a potential of −0.8 V *vs.* SHE using a potentiostat (BioLogic, Model SP50, France), based on a three-electrode configuration. Software from the same producer (EC-Lab v10.37) was used to run simultaneous multitechnique electrochemistry routines, which included cyclic voltammetry (CV) and chronoamperometry (CA). The parameters for CV are as follows: scan rate: 1 mV s^{-1}; E_i = −0.8 V *vs.* SHE; E_f = 0.0 V *vs.* SHE. For CA, the parameters were $E_{cathode}$ (E) = −0.8 V *vs.* SHE.

Initially, a control experiment was performed in 120 mL airtight serum bottles to prove that no bacterial growth or metabolite production occurred when reducing power was not provided. Bottles were filled with 100 mL of mineral medium and 5 mL of enriched carboxydotrophic mixed culture from a syngas (32% CO, 32% H_2, 8% CO_2 and 28% N_2) fermenting lab-scale reactor capable of producing two-carbon (C2) and four-carbon (C4) organic acids and alcohols.[11] The inoculum was dominated by species of the genus *Clostridium* (see ESI,† Table S2). Bottles were regularly sparged with CO_2 up to a pressure of 2 atm, and incubated at 37 °C for a period of 60 days. During that period, no increase in optical density or concentration of products was observed.

Subsequently, a BES H-type system was inoculated using 5 mL of the same enriched carboxydotrophic mixed culture from a syngas fermenting lab-scale reactor, and operated as described. Pure CO_2 (Praxair, Spain) was bubbled regularly every 2–3 days for a period of 5 minutes to ensure substrate availability (at 33.9 °C, concentration at saturation of 26.9 mM of carbon (mMC) as CO_2). Liquid samples were taken periodically from both anodes and cathodes for the monitoring of liquid products concentration and pH. The volume withdrawn was substituted with fresh mineral medium. The concentration of organic acids and alcohols was analysed using an Agilent 7890A gas chromatograph (Agilent, USA) equipped with a DB-FFAP column and a flame ionisation detector. Gas samples were periodically drawn for the analysis of hydrogen, carbon dioxide, methane, oxygen and nitrogen (H_2, CO_2, CH_4, O_2, N_2) in the biocathode headspace. Samples were analysed using a second channel of the GC equipped with an HP-Molesieve column and a thermal conductivity detector (TCD).

Next, Fig. 1 depicts the total accumulated concentration of products, coulombs supplied, together with the product speciation and pH at the cathode during a 19 days experiment. The demand of electrons increased linearly from day 0 until the end of the experiment. A total of 7000 coulombs were supplied to the system, with a current demand of around 6.3 A m^{-2} on day 17. At the end of the experiment the total concentration of products was 55 mMC. Microbial electrosynthesis commenced with the production of acetate, and its concentration increased almost linearly throughout the experiment, reaching a maximum concentration of 40 mMC. Butyrate was first observed on day 3, and during the following 9 days was produced at a rate of 1.49 mMC d^{-1}, up to a maximum concentration of 15 mMC. Butyrate is one of the end products of *Clostridium carboxidivorans*/*Clostridium ragsdalei*, bacteria present in the inoculum, and could have been directly produced from CO_2 *via* the Wood–Ljungdahl pathway coupled to acetyl-CoA reduction.[12] Under acidic conditions and excess of

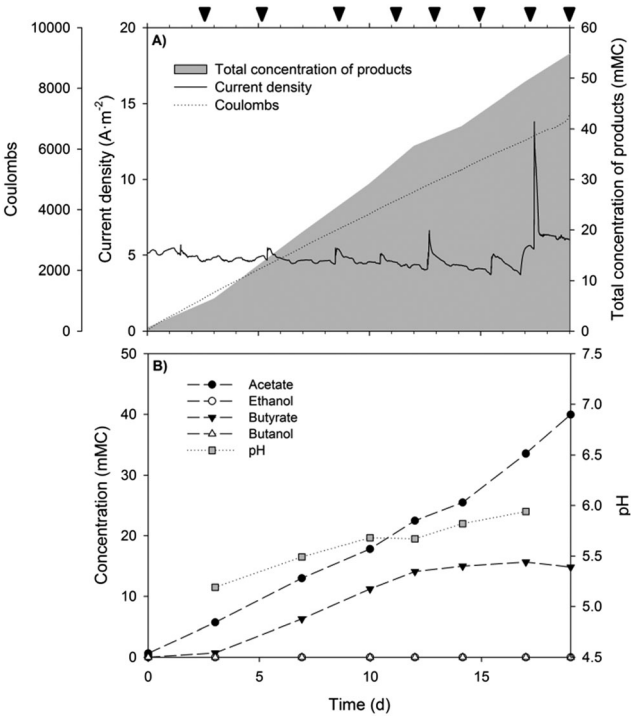

Fig. 1 (A) Current density, total Coulombs supplied and total accumulated concentration of products in the BES. (B) Accumulated concentration of the different products and pH in the biocathode. Black inverted triangles on the top part of the figure indicate sampling and subsequent flushing with CO_2.

reducing power, butyrate production may be favoured over acetate by acetogens because of a slightly higher pK_a (4.82 *vs.* 4.76).[13]

To validate that butyrate can be produced from CO_2 in a BES, a second experiment was conducted with the same set-up and conditions. Fig. 2 shows that current demand exponentially increased throughout the experiment, with current densities increasing from 3 to 20 A m^{-2}. The concentration of total added-value products increased in parallel, reaching 104 mMC on day 34. The rate of carbon fixation into products was linear from day 7 to day 27, around 4.1 mMC d^{-1}. Initially, acetate was the only compound produced, and its maximum concentration – around 55 mM – was reached on day 20. The evolution of pH in the biocathode was linked to the production of organic acids, and it decreased from pH 7 on day 7 to pH 6.35 on day 20. At that point, butyrate was detected, together with ethanol. The simultaneous presence of both compounds in the fermentation broth opens up a new potential route for butyrate production. *Clostridium kluyveri*, which had been detected in the parent syngas fermentation reactor,[11] can produce butyrate by the combination of acetate and ethanol, by the so-called chain elongation reactions.[14,15] In this light, it is unclear which is the true mechanism governing butyrate production, or whether both metabolic routes occurred in parallel.

The production of butyrate subsequently led to the decrease of the concentration of acetate and the pH, which reached values of pH 4.63 on day 34. In parallel, the concentration of ethanol and butanol increased to 30.8 mMC and 7.3 mMC, respectively. It is hypothesized that the decrease of pH, coupled

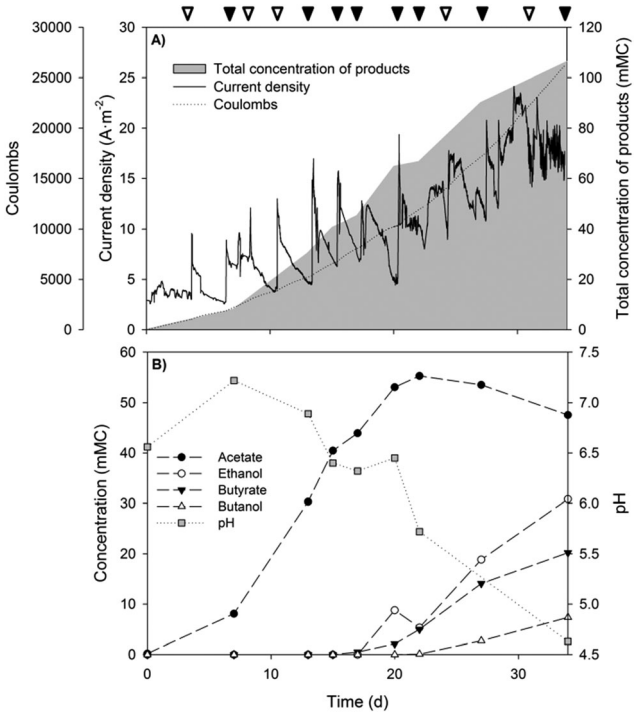

Fig. 2 (A) Current density, total Coulombs supplied and total accumulated concentration of products in the BES. (B) Accumulated concentration of the different products and pH in the biocathode. White inverted triangles on the top part of the figure indicate CO_2 flushing and black triangles sampling.

to the high concentrations of organic acids, and the increase of available reducing power (as will be later discussed) led to the re-assimilation of acetate and butyrate and their conversion into ethanol and butanol. Homoacetogens obtain their energy by proton-gradient driven phosphorylation. If high concentrations of unionised organic acids penetrate the bacterial membrane, they can cause the proton gradient between inside and outside to collapse. The conversion of acids to solvents is one of the mechanisms that *Clostridium* sp. utilise to prevent further pH decrease during fermentations. In this respect, ethanol production started when the concentration of acetate was 40 mMC, corresponding to an unionised acetic acid concentration of 0.91 mM. As a consequence, pH increased from pH 6.32 to 6.45 from days 17 to 20. Although higher acetic acid concentrations and similar pH values were reached by Marshall *et al.* (2013), no butyrate or ethanol production was reported.[6] This could be explained by two reasons: (i) the lower amount of reducing power supplied in that study, which could have limited solventogenesis; and (ii) differences in the bacterial community.

When comparing the performance of the BES in the present study, to the syngas fermentation experiments performed by Sánchez and co-workers using the same biocatalyst, overall production of C4 compounds was fairly similar (around 30% of the total products), although more alcohols were produced in the syngas study. Besides, rates of CO_2 transformation to products reached in the present study (2.9 mMC d^{-1} and 4.1 mMC d^{-1}, for the first and second experiment, respectively) were comparable to the maximum observed in the syngas fermentation study (2.4–4.9 mMC d^{-1}).[11] Given the high reducing power availability in the syngas – 32% CO and 32% H_2, it is likely that production rates were limited by the low solubility of CO and H_2, being the mass transfer of these compounds the key bottleneck of syngas fermentation technology. In this light, MES of butyrate in BES has the potential to circumvent this barrier by the *in situ* supply of reducing power, either in the form of electrons or H_2.

Turnover CVs were periodically carried out to characterise electrochemically both BESs. Fig. 3 presents two representative CVs performed under turnover conditions, one corresponding to the control (abiotic) test and the second one when the biofilm had fully developed, producing organic acids (acetate and butyrate) and bioalcohols (ethanol and butanol) from CO_2. A sudden increase of the current demand was observed on the abiotic test at a cathode potential of −0.65 V *vs.* SHE. This CV shape is typically linked to the catalytic production of H_2 using non-precious metals (*i.e.* carbon cloth) as electrodes.[16] During the production of organic acids and alcohols, hydrogen production started before the control CV, at −0.55 V *vs.* SHE. The production of H_2 at higher cathode potential and the higher current indicated that its production was partially biocatalysed, decreasing the important energy losses associated to purely electrolytic reduction.[16] In this respect, it is important to bear in mind that proton availability plays a critical role in H_2 production, and hence the reducing power availability for bacterial utilization. The lower the pH, the higher the H_2 production, and electron availability. This phenomenon explains the exponential increase of the coulombs supplied and current density during the second experiment, linked to the decrease in media pH. Besides, the higher reducing power availability likely favoured the production of more reduced end-products than acetate, such as butyrate, ethanol or butanol.

The coulombic efficiencies of the experiments were calculated based on the current demand and final concentration of products, and were 28% and 32%, respectively. Two main electron sinks of the system were hypothesized: (i) protons were reduced to hydrogen at the electrode surface, consuming electrons (Fig. 3). In this light, part of the electrons supplied may have been lost as un-utilized hydrogen, diffusing out of the cathode through the connectors and the membrane, or stripped during the periodic CO_2 flushing. These phenomena have been extensively described in the literature;[17] and

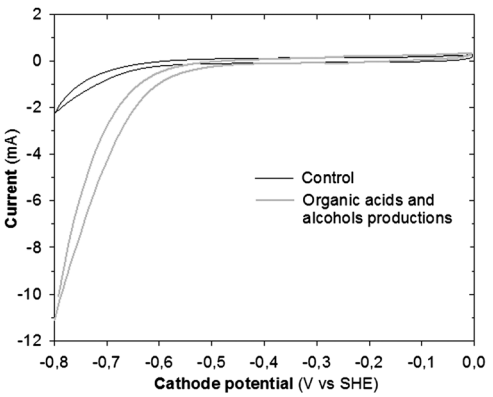

Fig. 3 Turnover cyclic voltammograms of an abiotic control (synthetic medium) (black) and organic acids and alcohols production biofilm (grey). The scan rate was 1 mV s^{-1}.

(ii) the oxidation of water was likely the main reaction in the anode, which led to the production of O_2. Oxygen can permeate to the cathode through the cationic exchange membrane, being subsequently reduced to water again or used for oxidizing organic compounds, with the associated consumption of electrons.[18] Gas sample analysis showed low H_2 concentrations in the headspace (0–1% v/v). On the contrary, high dissolved oxygen (DO) concentrations (8 $mgO_2\ L^{-1}$) were measured in the anode compartment at the end of the experiment, revealing this second potential sink as the main contributor to electron loses from the system.

Finally, it is important to consider that separation and recovery of fermentation products, even from highly specific pure cultures, can account for over 60% of the total production costs.[19] To date, a number of technologies have been developed for the separation of organic acids from fermentation broths (both online and offline), including liquid:liquid extraction and electrodialysis.[10] Recently, Andersen and co-workers developed a processing pipeline to transform carboxylates into fine chemicals by combining Membrane Electrolysis (ME) and Biphasic Esterification (BE).[20] This is of special interest because MES of butyrate from CO_2 could be coupled to this concept, with organic acids produced in the cathode being extracted and concentrated in the anode, prior to esterification.

This study demonstrates for the first time the bioelectrochemical CO_2 transformation to butyrate. The electrochemical characterisation demonstrated that the CO_2 reduction to butyrate was hydrogen driven. Production of ethanol and butanol was also observed at low pH values and high concentrations of undissociated organic acids, opening up the potential for the bioelectrochemical production of biofuels from CO_2 as a sole carbon source. Future work should aim to increase product titers and coulombic efficiency of the system by minimising anodic oxygen production and its diffusion to the cathode.

The authors would like to thank the Spanish Ministry (Best-Energy, CTQ2011-23632) for its financial support in this study. LEQUIA has been recognised as a consolidated research group by the Catalan Government (2014-SGR-1168). R.G. gratefully acknowledges support from Beatriu de Pinós fellowship (BP-2011-B) and FP7 Marie Curie Career Integration Grants (PCIG13-GA-2013-618593). P. B.-V is supported by a project grant from the Catalan Government (2014 FI-B1 00119).

Notes and references

1 M. Mikkelsen, M. Jørgensen and F. C. Krebs, *Energy Environ. Sci.*, 2010, **3**, 43–81.
2 R. S. Haszeldine, *Science*, 2009, **325**, 1647–1652.
3 K. Rabaey and R. A. Rozendal, *Nat. Rev. Microbiol.*, 2010, **8**, 706–716.
4 K. P. Nevin, T. L. Woodard, A. E. Franks, Z. M. Summers and D. R. Lovley, *mBio*, 2010, **1**, e00103.
5 C. W. Marshall, D. E. Ross, E. B. Fichot, R. S. Norman and H. D. May, *Appl. Environ. Microbiol.*, 2012, **78**, 8412–8420.
6 C. W. Marshall, D. E. Ross, E. B. Fichot, R. S. Norman and H. D. May, *Environ. Sci. Technol.*, 2013, **47**, 6023–6029.
7 M. Sharma, N. Aryal, P. M. Sarma, K. Vanbroekhoven, B. Lal, X. D. Benetton and D. Pant, *Chem. Commun.*, 2013, **49**, 6495–6497.
8 K. J. J. Steinbusch, H. V. M. Hamelers, J. D. Schaap, C. Kampman and C. J. N. Buisman, *Environ. Sci. Technol.*, 2010, **44**, 513–517.
9 M. C. A. A. Van Eerten-Jansen, A. Ter Heijne, T. I. M. Grootscholten, K. J. J. Steinbusch, T. H. J. A. Sleutels, H. V. M. Hamelers and C. J. N. Buisman, *ACS Sustainable Chem. Eng.*, 2013, **1**, 513–518.
10 M. Dwidar, J.-Y. Park, R. J. Mitchell and B.-I. Sang, *Sci. World J.*, 2012, 471417.
11 P. Sánchez, R. Ganigue, L. Bañeras and J. Colprim, *Chem. Eng. J.*, 2014, under review.
12 J. Daniell, M. Köpke and S. D. Simpson, *Energies*, 2012, **5**, 5372–5417.
13 A. S. Gössner, F. Picardal, R. S. Tanner and H. L. Drake, *FEMS Microbiol. Lett.*, 2008, **287**, 236–242.
14 R. K. Thauer, K. Jungermann, K. Decker and P. P. H. Pi, *Bacteriol. Rev.*, 1977, **41**, 809.
15 M. T. Agler, B. A. Wrenn, S. H. Zinder and L. T. Angenent, *Trends Biotechnol.*, 2011, **29**, 70–78.
16 P. Batlle-Vilanova, S. Puig, R. Gonzalez-Olmos, A. Vilajeliu-Pons, L. Bañeras, M. D. Balaguer and J. Colprim, *Int. J. Hydrogen Energy*, 2014, **39**, 1297–1305.
17 A. W. Jeremiasse, H. V. M. Hamelers and C. J. N. Buisman, *Bioelectrochemistry*, 2010, **78**, 39–43.
18 M. C. A. A. Van Eerten-jansen, A. Ter Heijne, C. J. N. Buisman and H. V. M. Hamelers, *Int. J. Energy Res.*, 2012, 809–819.
19 I. Bechthold, K. Bretz, S. Kabasci, R. Kopitzky and A. Springer, *Chem. Eng. Technol.*, 2008, **31**, 647–654.
20 S. J. Andersen, T. Hennebel, S. Gildemyn, M. Coma, J. Desloover, J. Berton, J. Tsukamoto, C. V. Stevens and K. Rabaey, *Environ. Sci. Technol.*, 2014, **48**, 7135–7142.
21 R. S. Tanner, L. M. Miller and D. Yang, *Int. J. Syst. Bacteriol.*, 1993, **43**, 232–236.

