Bioelectrochemistry 123 (2018) 150–155

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Electrotrophic activity and electrosynthetic acetate production by *Desulfobacterium autotrophicum* HRM2

Zehra Zaybak [a,b], Bruce E. Logan [a], John M. Pisciotta [a,b,*]

[a] Department of Civil and Environmental Engineering, The Pennsylvania State University, 212 Sackett Building, University Park, PA 16802, USA
[b] Department of Biology, West Chester University of Pennsylvania, West Chester, PA 19383, USA

ARTICLE INFO

Article history:
Received 23 February 2018
Received in revised form 26 April 2018
Accepted 29 April 2018
Available online 04 May 2018

Keywords:
Biocathode
Desulfobacterium autotrophicum HRM2
Bioelectrochemical system
Electrofuel
Electrotroph
Microbial electrosynthesis

ABSTRACT

Electroautotrophic microorganisms accept electrons from a cathode as source of reducing equivalents to drive CO_2 fixation by poorly understood mechanisms. Acetogenic bacteria were the first group found to possess the capability for electroautotrophic metabolism in pure culture with associated electrosynthesis of acetate as primary metabolite. Identification of additional electrotrophic species can contribute to our understanding of this unusual form of metabolism. Here, bioelectrochemical techniques, chemical analysis and microscopy were used to determine electrotrophic metabolism of *Desulfobacterium autotrophicum* HRM2. Chronoamperometry showed increasing current uptake over 21 days of incubation in duplicate bioelectrochemical system sets. Linear sweep voltammetry indicated peak current uptake at −243 mV. High performance liquid chromatography (HPLC) analysis quantified acetate accumulation in anaerobic minimal media containing inorganic carbon as sole carbon source, consistent with electrosynthesis. Scanning electron microscopy and live/dead staining by epifluorescence microscopy analysis indicated viable 1–2 μm cells after 76 days of cultivation under electroautotrophic conditions. The genome of *Db. autotrophicum* HRM2 is fully sequenced and, thus, could provide insight into the biochemical and physiological mechanisms by which electrotrophic cells utilize cathode-derived electrons. This research expands the diversity of facultative autotrophs capable of electrotrophic metabolism to include the sulfate-reducing marine bacterium *Db. autotrophicum* HRM2.

1. Introduction

Bioelectrochemical systems (BESs) utilize electrode redox reaction catalyzing microorganisms for diverse applications. These range from electrosynthesis of commodity chemicals from carbon dioxide to renewable energy storage, bioremediation and wastewater treatment-linked renewable electricity generation [1]. Initial BES research focused on identifying microbial species and physiological mechanisms involved in the donation of organically derived electrons to the BES anode. This was largely because heterotrophic anode biofilms are easily grown, even in pure culture, using model exoelectrogens like *Shewanella oneidensis* MR-1 or *Geobacter sulfurreducens*. Physiological, biophysical, and genotypic studies into different species of anode reducing bacteria revealed how cellular components, including cytochromes, soluble flavins and microbial nanowires, play specialized roles in transfer of electrons from bacteria through a relatively thick anode biofilm to a BES anode [2]. Recently, the clustered regularly interspaced short palindromic repeats interference (CRISPRi) technology was applied in combination with a small regulatory RNA (sRNA) for transcriptional and translational regulation of gene expression to achieve higher efficiencies of extracellular electron transfer in *Shewanella oneidensis* [3].

Biocathode research has lagged in contrast to bioanode research. This is largely attributable to the intrinsic difficulty of growing biocathodes, particularly as autotrophic pure cultures [4]. To gain insight into the physiological and metabolic factors governing biocathode colonization and electron uptake, sequenced, model electrotrophs capable of current-dependent metabolism on biocathodes are needed [5]. Cathodes are the source of reducing equivalents while inorganic carbon, primarily in the form of soluble bicarbonate or CO_2, is the sole carbon source. Previously described electrotrophic bacteria include acetogenic members of the genera *Clostridium*, *Moorella* and *Sporomusa* [6,7]. Acetogenic electrotrophs fix CO_2 through the Wood-Ljungdahl pathway, also known as the reductive acetyl-CoA pathway. This pathway is centered on the anaerobic Ni-Fe carbon monoxide dehydrogenase acetyl CoA synthase (CODH/ACS) enzyme complex [8–10]. Methanogenic archaea represent a second, unrelated group of prokaryotes that, interestingly, also possesses Ni-Fe CODH/ACS and the unusual ability to grow as a biocathode [11]. Electrotrophic methanogens employ cathode-

* Corresponding author at: Department of Biology, West Chester University of Pennsylvania, West Chester, PA 19383, USA.
E-mail address: jpisciotta@wcupa.edu (J.M. Pisciotta).

https://doi.org/10.1016/j.bioelechem.2018.04.019
1567-5394/

delivered electrons to reduce CO_2 into CH_4, either directly or by way of hydrogen, through a process called electromethanogenesis [11,12]. An electrotrophic methanogen possessing CODH has been cultivated under axenic conditions [13]. Iron-oxidizing bacteria that possess CODH have similarly been cultivated electroautotrophically [14,15]. Electromethanogenic archaea and the electroacetogenic bacteria form predominantly low molecular weight organic products (i.e. methane, acetate) from substrate CO_2. While polyhydroxyalkanoates have reportedly been formed by a mixed consortia [16], electrosynthesis of complex organic molecules has not been described in pure culture and little is known regarding other species of electroautotrophic microorganisms capable of electrosynthesis.

A fourth group of prokaryotes that encode the Ni-Fe CODH/ACS enzyme complex, apparently conserved in electroautotrophs, are the facultatively autotrophic sulfate reducing bacteria (SRBs). *Desulfobacterium autotrophicum* HRM2 is a metabolically well-characterized and fully sequenced representative of this group [17,18]. *Db. autotrophicum* HRM2 grows lithoautotrophically on CO_2 and H_2 in the presence of sulfate using a bidirectional Wood-Ljungdahl pathway centered on Ni-Fe CODH/ACS [19,20]. Heterotrophic SRBs of the *Desulfovibrio* genus do not encode Ni-Fe CODH/ACS and only oxidize organic matter down to the level of acetate. Heterotrophic SRBs have been studied in BESs and can reduce protons to H_2 using electrons delivered from a cathode, but do not carry out organic molecule electrosynthesis [21,22]. Heterotrophic SRBs appear to establish electrical connections with electrodes using hydrogenase enzymes [21]. The established role of SRBs in microbially induced corrosion (MIC) of metals supports this notion. Direct electron transfer has been described for lithoautotrophic SRB bearing closest homology to the genera *Desulfobacterium* [23–25]. A lithoautotrophic strain in the *Desulfopila* genus is similarly capable of direct electron uptake from cathodes [26]. Here we investigated the hypothesis that the fully sequenced sulfate-reducing marine bacterium *Desulfobacterium autotrophicum* HRM2 possesses electrotrophic and electrosynthetic activity. To test this, the microorganism was cultured in minimal media in a bioelectrochemical system with an applied cathode potential under anaerobic growth conditions with CO_2 as the sole carbon source. Electrochemical techniques, epifluorescent and scanning electron microscopy in conjunction with chemical analysis methods demonstrated sustained electrotrophic metabolism with acetate electrosynthesis in this model SRB.

2. Materials and methods

2.1. Microorganism source and bioelectrochemical system construction

An axenic culture of *Db. autotrophicum* HRM2 (ATCC® 43914) was obtained from the American Type Culture Collection (ATCC) and grown under anaerobic conditions at 26 °C for two weeks in ATCC medium #1627 containing 10 mM lactate and 1.25 g/L yeast extract. Cells were centrifuged and washed twice in anaerobic PBS to remove organic carbon, resuspended in 1 mL catholyte solution and immediately inoculated into sterile H-cell BES cathode chambers. The anolyte and catholyte solution consisted of autotrophic ATCC medium #1627 adjusted to pH 7.0 with omission of resazurin. Dual chamber, H-type BESs were constructed as previously described [27]. Anode and cathode chambers were separated by a pretreated cation exchange membrane (Nafion 117). Each chamber of the autoclaved BES contained 120 mL autotrophic ATCC medium #1627 and 70 mL headspace. Anodes and cathodes consisted of acid pretreated graphite plates (GraphiteStore, IL) prepared as described previously [27]. Heat sensitive reference electrodes (Ag/AgCl, RE-5B, Bioanalytical Systems, Inc.) were ethanol-sterilized, air dried in a laminar flow biosafety cabinet and installed with the working electrode in the cathode chamber. Chronoamperometric and chronocoulometric measurements were performed using a MPG-2 potentiostat (Bio-Logic Science Instrument).

2.2. Bioelectrochemical system operation

To first acclimate *Db. autotrophicum* HRM2 cells to chemolithoautotrophic conditions, cathode chambers were degassed with a H_2-CO_2 gas mix (80:20) for 15 min before startup of duplicate BES at −400 mV (versus SHE) for 10 days. After this acclimation phase, cathodes were transferred into new reactors (R1 and R2) in a COY anaerobic chamber. Into the original reactors, new sterile cathodes and reference electrodes were installed to examine cathode colonization by acclimated planktonic cells in one half volume (60 mL) of original catholyte (R3 and R4). Sixty milliliters of sterile anaerobic catholyte solution was added to bring up to a total working volume of 120 mL before degassing with N_2-CO_2-gas-mix (80:20) for 10 min. Reactors were operated at a set potential of −400 mV for 7 days and then the applied potential was decreased to −500 mV as the final setting for the remaining 76 days of operation.

2.3. Chemical analysis

Carbon dioxide consumption from cathode chamber headspace gas was analyzed weekly using a gas chromatograph (model 310; SRI Instruments, CA) equipped with a 1-m silica gel column (Restek Corporation, Bellefonte, PA) and a thermal conductivity detector (detection limit 0.01%). Soluble metabolites were quantified using a Shimadzu ultra-high-performance liquid chromatograph (5-μm column, Restek Corporation, Bellefonte, PA; 250 mm by 4.6 mm, Allure organic acids) using a phosphate buffer eluent (6.8 g/L KH_2PO_4, pH 2.3) and a photodiode array detector. Volatile fatty acids were examined by a GC (Shimadzu GC-2010 Plus) equipped with a Stabilwax-DA column (30 m × 0.32 mm × 0.5 μm, Restek, Bellefonte, PA) and a flame ionization detector as previously described [28].

2.4. Microscopic analysis

To determine if cells recovered from BESs were viable after prolonged cultivation under electroautotrophic conditions, examination of cell viability was carried out. Aseptically recovered biofilm material was obtained using a sterile razor blade in the anaerobic chamber from the cathode of BES R2 after 76 days of operation at −500 mV. Samples were immediately treated and examined using live/dead BacLight stain (ThermoFisher Scientific) following manufacturer's instructions. Photomicrographs were obtained using an Olympus BX61 epifluorescent microscope equipped with a DP72 digital camera.

The cathode surface of BES R4 was examined using a scanning electron microscope (SEM). After 76 days of operation at −500 mV, the cathode was prepared by fixing at 4 °C in 5 mL of phosphate solution (pH 7.2) containing 2.5% glutaraldehyde and 1.5% paraformaldehyde. The cathode was, then, sequentially dried in an ethanol-water series of 50%, 70%, 85%, 90%, and 100% ethanol and stored overnight in the dark at 4 °C in fresh 100% ethanol. This cathode was then critical point CO_2-dried and sputter coated. SEM analysis was carried out using an E-SEM (FEI Quanta 200 instrument, Hillsboro, OR) equipped with an electron dispersive X-ray spectroscopy (EDX).

3. Results and discussion

3.1. Current density and carbon dioxide consumption with acetate production

Db. autotrophicum HRM2 was cultured in two sets of duplicate bioelectrochemical systems (BESs). Set one consisted of two identical BESs, R1 and R2, that were initially sparged with a H_2-CO_2 gas mix (80:20) to establish biocathodes colonized with *Db. autotrophicum* HRM2. Set two consisted of duplicate BESs, R3 and R4, possessed new sterile graphite cathodes and were inoculated with established *Db. autotrophicum* biocatholyte solutions from R1 or R2 to determine the

capacity for active transfer and cathode colonization at −500 mV (versus SHE) using solely electrotrophic conditions. Both BES reactor sets showed uptake of cathode electrons, consumption of CO_2 and development of progressively greater current densities over time. This is consistent with sustained electroautotrophy by *Db. autotrophicum* HRM2 (Fig. 1a, b).

BESs inoculated with *Db. autotrophicum* HRM2 consumed electrons from negatively poised cathodes with coincident carbon dioxide removal and produced acetate which accumulated in catholyte solutions (Fig. 2). Sterile control showed minimal baseline current consumption, no significant change in headspace carbon dioxide and no detectable accumulation of acetate. For duplicate BESs R3 and R4, the recovery of electrons in acetate was 83 ± 6% of electrons consumed from the cathode (Supplemental Information). By mass balance analysis of the carbons in consumed carbon dioxide and in produced acetate, 89.7 ± 9.5% of consumed CO_2 was recovered in produced acetate (Fig. 2). These results indicated *Db. autotrophicum* HRM2 can utilize a cathode as an electron donor for CO_2 reduction to acetate through the process of microbial electrosynthesis. Results with R3 and R4 reveal that acclimated electrolyte cell suspensions from BESs with *Db. autotrophicum* HRM2 can be used for electrotrophic culture expansion into new BESs possessing sterile electrodes.

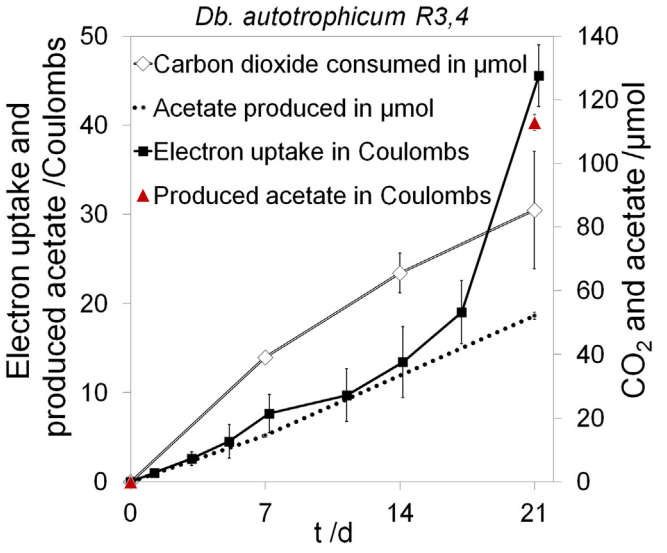

Fig. 2. Stoichiometric analysis of carbon dioxide consumption, acetate production, electron uptake and coulombs in produced acetate over time by *Db. autotrophicum* HRM2 with a poised cathode at −500 mV in duplicate BESs (average of R3 and R4).

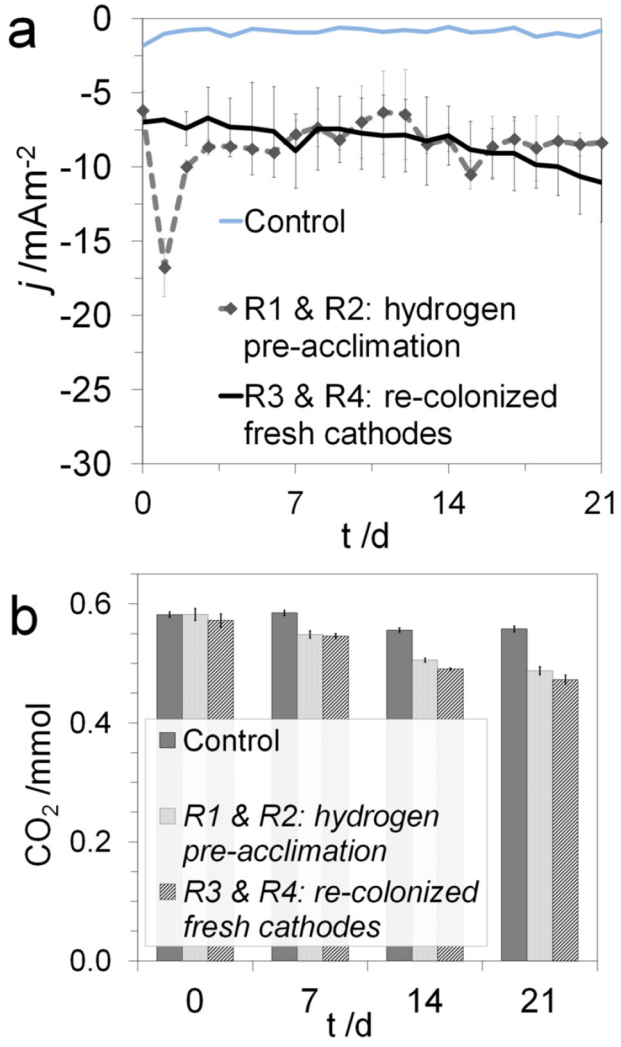

Fig. 1. (a) Current density consumption and (b) consumption of CO_2 over a period of 21 days by *Db. autotrophicum* HRM2 in two sets of duplicate BESs, R1 and R2 (pre-acclimated with H_2-CO_2-mix), and R3 and R4 (fresh cathodes re-colonized with established electrotrophic cells).

To determine if electrons supplied from cathodes were required for consumption of headspace CO_2, a subsequent 28-day cathode disconnection test was carried out. For this, BESs R1 (first replicate of initially H_2/CO_2 acclimated cathode) and R3 (first replicate of transfer colonized cathode) containing *Db. autotrophicum* HRM2 were disconnected from the potentiostat. This ensured that the cathode could not supply electrons as reducing equivalents to *Db. autotrophicum* HRM2 cells. Conversely, replicates R2 and R4 remained connected to the potentiostat with no change to the settings. For disconnected BESs R1 and R3, no significant decrease in headspace CO_2 was detected over the course of this trial, indicating *Db. autotrophicum* HRM2 cells ceased electrotrophically-driven metabolism of carbon dioxide (Fig. 3). In contrast, over the same 28-day test period, BESs R2 (replicate of initially H_2/CO_2 acclimated cathode) and R4 (replicate of transfer-colonized cathode never acclimated with H_2/CO_2) remained potentiostat-connected at −500 mV. These even numbered reactors exhibited sustained carbon dioxide uptake (Fig. 3). Electrosynthetic acetate production was evident by its accumulation in the culture media as detected by HPLC.

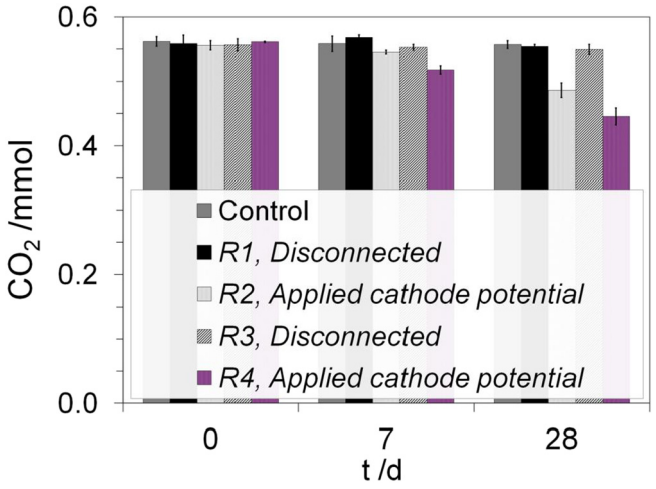

Fig. 3. Comparison of consumption of CO_2 over time by *Db. autotrophicum* HRM2 in BESs disconnected without applied cathode potential (R1 and R3) and with applied cathode potential (Control, R2 and R4). Disconnected BESs R1 and R3 exhibited no significant decrease in carbon dioxide over 28 days. Only connected BESs R2 and R4 with *Db. autotrophicum* HRM2 exhibited continuous consumption of CO_2 molecules.

Interestingly, the R4 biocathode consumed more CO_2 than the initially H_2/CO_2-established R2. Specifically, BES R4 resulted in 19 ± 2% decrease of CO_2 headspace gas relative to its disconnected control R3, whereas R2 showed a 12 ± 2% decrease relative to its disconnected control R1 on day 28. This could suggest adaptation and selection of an enriched electrotrophically active population of *Db. autotrophicum* HRM2 cells. In summary, these observations collectively confirm the electroautotrophic activity of *Db. autotrophicum* HRM2 regardless of whether the culture is derived from an acclimated cathode source or a cathode colonized by adapted planktonic cells.

3.2. Linear sweep voltammetry and cyclic voltammetry analysis

A peak current uptake at −243 mV for BESs R1 and R2 was revealed during linear sweep voltammetry (LSV) analysis. These biotic reactors had on average a 2.4 ± 0.3 times greater current uptake capacity with an average current density of −14.2 ± 1.5 mA/m^2 at −243 mV compared to the control. In addition, towards more negative potentials of −600 mV, biotic BES exhibited a substantially higher ability to uptake electrons. On average, this was 3.7 ± 0.4 times greater with an average current density of −12.4 ± 1.2 mA/m^2 at −400 mV, 5.7 ± 0.9 greater with an average current density of −25.8 ± 4.1 mA/m^2 at −500 mV and 4.5 ± 1.0 greater with an average current density of −68.5 ± 15.4 mA/m^2 at −600 mV compared to the LSV profile of the sterile control which stayed relatively flat at increasingly negative potentials (Fig. 4).

Cyclic voltammetry (CV) further suggested electrocatalytic activity of *Db. autotrophicum* HRM2 by revealing a reversible redox couple with midpoint potential at −226 mV for biotic BESs (R1 and R2) (see Fig. 4, inset panel). Multi-heme (class III) c-type cytochromes are active around this midpoint potential and exist in the periplasm of various SRBs, including *Db. autotrophicum* HRM2, where they facilitate rapid, reversible electron transfer reactions [29–31]. In the cyclic voltammograms of biotic BESs R1 and R2, an anodic peak appeared at −433 mV. This might be attributable to the oxidation of [Ni/Fe/Se] hydrogenase (HysAB). This enzyme is known to be expressed under chemoautotrophic growth conditions in *Db. autotrophicum* HRM2 [29]. Future transcriptomic and proteomic studies could investigate the relative expression levels of such possible physiological mediators of electroautotrophy over a range of applied potentials.

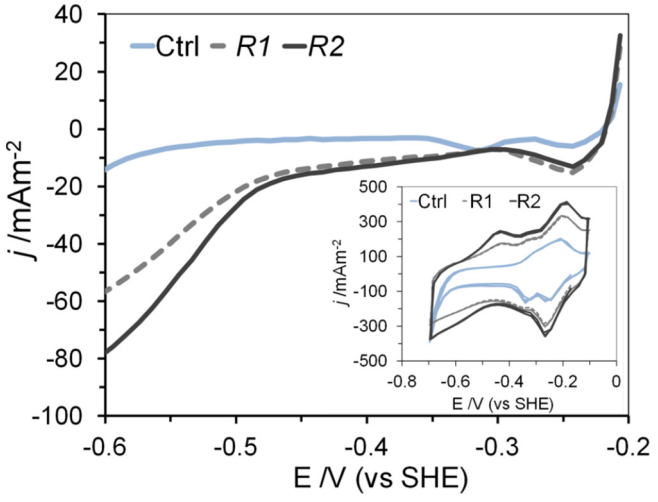

Fig. 4. Linear sweep voltammograms (LSVs) with scan rate dE/dt = 0.5 mV/s and (inset panel) cyclic voltammograms (CV) with scan rate dE/dt = 10 mV/s for sterile BES (control) and duplicate biotic BESs, R1 and R2, inoculated with *Db. autotrophicum* HRM2.

3.3. Microscopic analysis of viable cells and cathode surface

At the conclusion of BESs operation, the cathode surface of R4 was examined using a scanning electron microscope. Possible scattered colonies of *Db. autotrophicum* HRM2 were visible on the cathode surface suggesting direct electron transfer to electroautotrophically grown cells (Fig. 5a and Fig. S1). To determine if cells recovered from BESs were viable after prolonged cultivation under electroautotrophic conditions, examination of cell viability was carried out at the end of the experiment using LIVE/DEAD BacLight stain. Despite the physically harsh biofilm removal process, the majority of the 1–2 μm cells stained green. This indicates *Db. autotrophicum* HRM2 cells on the cathode surface survived and possibly replicated during extended BES operation at applied potential of −500 mV for two and one half months (76 days) (Fig. 5b). Consistent with the cathode scrapings, viable cells (green) and few dead (red) planktonic cells were observed in the electrolyte solution recovered from the biocathode chamber via sterile syringe (Fig. 5c).

4. Conclusions

Electrotrophic microbes catalyze reduction reactions on cathodes and are promising candidates for microbial electrosynthesis of organic molecules such as acetate from carbon dioxide [32]. The physiological mechanisms diverse electrotrophs use to uptake electrons from cathodes are not fully understood [33,34]. Prior studies into biocathodes and electron uptake by cells have often utilized mixed culture enrichments with metagenomic identification of constituent populations [35–37]. Genetically identified organisms often cannot be isolated or lack electron uptake. Identification of axenically culturable electroautotrophs is desirable. Improved insight into the microbial diversity of electrotrophic species and the physiological and biochemical electron transfer mechanisms at work at the microbe-cathode interface can facilitate scale-up and commercialization of bioelectrochemical systems. Such systems can be utilized for storage of renewable energy and the electrosynthesis of diverse commodity chemicals.

Results of this study demonstrate that *Db. autotrophicum* HRM2 is capable of sustained electrotrophic metabolism with electrosynthesis of acetate under electroautotrophic culture conditions. Carbon dioxide was only consumed from the headspace of electrically-connected BES reactors inoculated with *Db. autotrophicum* HRM2. Acetate electrosynthesis was evident by its accumulation in culture media of connected BESs. Viable, 1–2 μm cells consistent with the cell morphology of *Db. autotrophicum* HRM2 under chemolithoautotrophic growth conditions were recovered at the end of the experiment demonstrating cells survived extended electroautotrophic culture conditions of up to 76 days [38]. SEM analysis of the biocathode surface indicated adherent biofilm. Cyclic voltammetry suggested multi-heme cytochromes and or hydrogenases could be responsible for the observed pattern of electron uptake. Two major mechanisms of electron uptake from cathodes to cells have been characterized previously: direct and indirect [39].

Indirect electron uptake is associated with hydrogenases whereas direct uptake of electrons from cathodes is associated with surface and periplasmic cytochromes [26]. In *Desulfovibrio*, surface expression of multi-heme cytochromes can change in response to extracellular electron availability [40]. The surface cytochromes of *Desulfovibrio* species mediate cathode binding via bonding between cytochrome amide groups and cathode surface carboxyl groups [41]. *Db. autotrophicum* HRM2 utilizes the reductive Wood–Ljungdahl pathway centered on CODH/ACS for CO_2 fixation when grown chemolithoautotrophically on H_2 as its electron donor [17,19]. Hydrogenases, namely the [Ni/Fe/Se]-containing hydrogenase HysAB, catalyze oxidation of H_2 for electron delivery into the cell [29]. The genome of *Db. autotrophicum* HRM2 encodes multiple hydrogenases, both soluble and membrane-associated [17]. It was shown that free hydrogenases released from cells can associate with cathodes to catalyze reduction of protons to form hydrogen

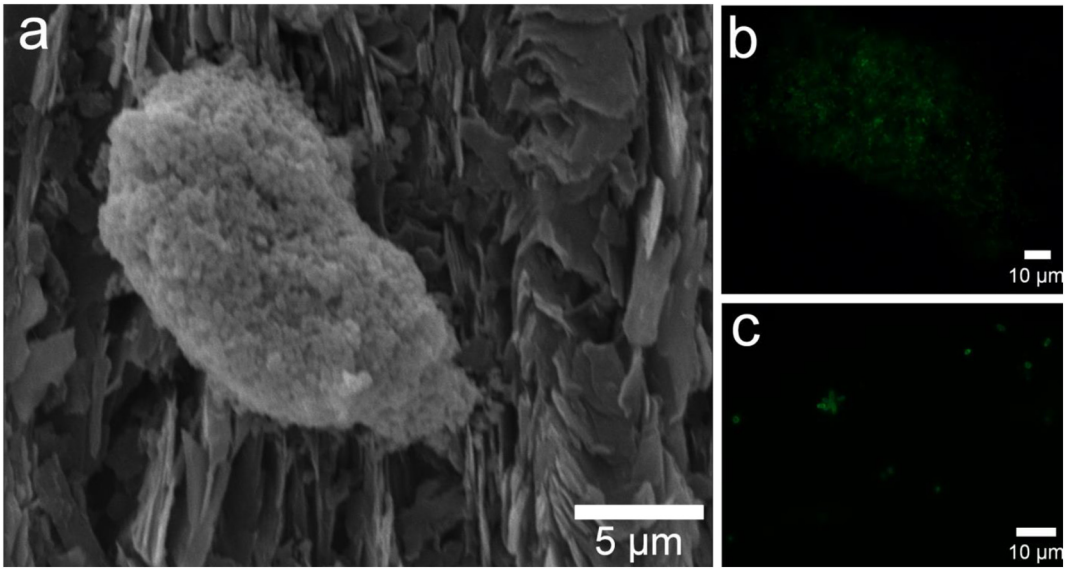

Fig. 5. (a) Scanning electron microscopic image of putative cell colony of *Db. autotrophicum* HRM2 attached on a graphite cathode surface in R4. (b) *Db. autotrophicum* HRM2 biofilm material scraped from graphite cathode surface and (c) planktonic *Db. autotrophicum* HRM2 cells in catholyte solution stained with live/dead BacLight viability stain. Live cells stain green.

gas [33]. Further research will be required to conclusively determine if either or both of the mechanisms suggested by our CV results and micrographs are utilized by *Db. autotrophicum* HRM2 during cultivation under electroautotrophic conditions.

As a fully sequenced, metabolically well-characterized SRB, *Db. autotrophicum* HRM2 can serve as a model organism for basic research into electrotrophic and electrosynthetic metabolism, cellular electron transfer mechanisms and microbial electrosynthesis. Pure culture, omic studies using the model electrotroph *Db. autotrophicum* HRM2 can identify genes upregulated under electroautotrophic conditions for identification of the core physiological mechanistic components at work during electron uptake on biocathodes. This is currently a major question in the field of electromicrobiology. Examination of the electrotrophic components at work in the autotrophic SRB *Db. autotrophicum* HRM2 compared to those present in evolutionarily divergent groups, including electrotrophic methanogens and acetogens, can help to identify conserved factors required for electrotrophic metabolism. Improved understanding into the basic biology of electrotrophic metabolism can contribute towards improved electrosynthetic energy storage systems, production of electrofuels and the synthesis of novel bioproducts.

Acknowledgements

The authors would like to thank John Cantolina in the Penn State Huck Institutes of the Life Sciences for his assistance with SEM imaging. This research was funded by an award from the King Abdullah University of Science and Technology (KUS-I1-003-13).

Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2018.04.019.

References

[1] S. Bajracharya, M. Sharma, G. Mohanakrishna, X. Dominguez Benneton, D.P.B.T.B. Strik, P.M. Sarma, D. Pant, An overview on emerging bioelectrochemical systems (BESs): technology for sustainable electricity, waste remediation, resource recovery, chemical production and beyond, Renew. Energy 98 (2016) 153–170.
[2] F. Kracke, B. Lai, S. Yu, J.O. Krömer, Balancing cellular redox metabolism in microbial electrosynthesis and electro fermentation – a chance for metabolic engineering, Metab. Eng. 45 (2018) 109–120.
[3] Y. Cao, X. Li, F. Li, H. Song, CRISPRi–sRNA: transcriptional–translational regulation of extracellular electron transfer in *Shewanella oneidensis*, ACS Synth. Biol. 6 (2017) 1679–1690.
[4] B.J. Eddie, Z. Wang, A.P. Malanoski, R.J. Hall, S.D. Oh, C. Heiner, B. Lin, S.M. Strycharz-Glaven, Candidatus Tenderia electrophaga, an uncultivated electroautotroph from a biocathode enrichment, Int. J. Syst. Evol. Microbiol. 66 (2016) 2178–2185.
[5] A.P. Malanoski, B. Lin, B.J. Eddie, Z. Wang, W.J. Hervey, S.M. Glaven, Relative abundance of 'Candidatus Tenderia electrophaga' is linked to cathodic current in an aerobic biocathode community, Microb. Biotechnol. 11 (2018) 98–111.
[6] K.P. Nevin, T.L. Woodard, A.E. Franks, Z.M. Summers, D.R. Lovley, Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds, MBio 1 (2010).
[7] K.P. Nevin, S.A. Hensley, A.E. Franks, Z.M. Summers, J.H. Ou, T.L. Woodard, O.L. Snoeyenbos-West, D.R. Lovley, Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms, Appl. Environ. Microbiol. 77 (2011) 2882–2886.
[8] H.G. Wood, S.W. Ragsdale, E. Pezacka, The acetyl-CoA pathway: a newly discovered pathway of autotrophic growth, Trends Biochem. Sci. 11 (1986) 14–18.
[9] M.J. Russell, W. Martin, The rocky roots of the acetyl-CoA pathway, Trends Biochem. Sci. 29 (2004) 358–363.
[10] S.W. Ragsdale, E. Pierce, Acetogenesis and the Wood-Ljungdahl pathway of CO (2) fixation, Biochim. Biophys. Acta (12) (2008) 27.
[11] S. Cheng, D. Xing, D.F. Call, B.E. Logan, Direct biological conversion of electrical current into methane by electromethanogenesis, Environ. Sci. Technol. 43 (2009) 3953–3958.
[12] M. Villano, L. De Bonis, S. Rossetti, F. Aulenta, M. Majone, Bioelectrochemical hydrogen production with hydrogenophilic dechlorinating bacteria as electrocatalytic agents, Bioresour. Technol. 102 (2011) 3193–3199.
[13] P.F. Beese-Vasbender, J.-P. Grote, J. Garrelfs, M. Stratmann, K.J.J. Mayrhofer, Selective microbial electrosynthesis of methane by a pure culture of a marine lithoautotrophic archaeon, Bioelectrochemistry 102 (2015) 50–55.
[14] A. Bose, E.J. Gardel, C. Vidoudez, E.A. Parra, P.R. Girguis, Electron uptake by iron-oxidizing phototrophic bacteria, Nat. Commun. 5 (2014).
[15] Z.M. Summers, J.A. Gralnick, D.R. Bond, Cultivation of an obligate Fe(II)-oxidizing lithoautotrophic bacterium using electrodes, MBio 4 (2013).
[16] S. Srikanth, M. Venkateswar Reddy, S. Venkata Mohan, Microaerophilic microenvironment at biocathode enhances electrogenesis with simultaneous synthesis of polyhydroxyalkanoates (PHA) in bioelectrochemical system (BES), Bioresour. Technol. 125 (2012) 291–299.
[17] A.W. Strittmatter, H. Liesegang, R. Rabus, I. Decker, J. Amann, S. Andres, A. Henne, W.F. Fricke, R. Martinez-Arias, D. Bartels, A. Goesmann, L. Krause, A. Pühler, H.-P. Klenk, M. Richter, M. Schüler, F.O. Glöckner, A. Meyerdierks, G. Gottschalk, R. Amann, Genome sequence of *Desulfobacterium autotrophicum* HRM2, a marine sulfate reducer oxidizing organic carbon completely to carbon dioxide, Environ. Microbiol. 11 (2009) 1038–1055.
[18] J. Amann, D. Lange, M. Schuler, R. Rabus, Substrate-dependent regulation of carbon catabolism in marine sulfate-reducing *Desulfobacterium autotrophicum* HRM2, J. Mol. Microbiol. Biotechnol. 18 (2010) 74–84.
[19] S. Länge, R. Scholtz, G. Fuchs, Oxidative and reductive acetyl CoA/carbon monoxide dehydrogenase pathway in *Desulfobacterium autotrophicum*, Arch. Microbiol. 151 (1988) 77–83.

[20] R. Schauder, A. Preuß, M. Jetten, G. Fuchs, Oxidative and reductive acetyl CoA/carbon monoxide dehydrogenase pathway in *Desulfobacterium autotrophicum*, Arch. Microbiol. 151 (1989) 84–89.
[21] E. Croese, M. Pereira, G.-J. Euverink, A. Stams, J. Geelhoed, Analysis of the microbial community of the biocathode of a hydrogen-producing microbial electrolysis cell, Appl. Microbiol. Biotechnol. 92 (2011) 1083–1093.
[22] F. Aulenta, L. Catapano, L. Snip, M. Villano, M. Majone, Linking bacterial metabolism to graphite cathodes: electrochemical insights into the H2-producing capability of *Desulfovibrio* sp, ChemSusChem 5 (2012) 1080–1085.
[23] H.T. Dinh, J. Kuever, M. Muszmann, A.W. Hassel, M. Stratmann, F. Widdel, Iron corrosion by novel anaerobic microorganisms, Nature 427 (2004) 829–832.
[24] D. Enning, J. Garrelfs, Corrosion of Iron by sulfate-reducing Bacteria: new views of an old problem, Appl. Environ. Microbiol. 80 (2014) 1226–1236.
[25] H. Venzlaff, D. Enning, J. Srinivasan, K.J.J. Mayrhofer, A.W. Hassel, F. Widdel, M. Stratmann, Accelerated cathodic reaction in microbial corrosion of iron due to direct electron uptake by sulfate-reducing bacteria, Corros. Sci. 66 (2013) 88–96.
[26] P.F. Beese-Vasbender, S. Nayak, A. Erbe, M. Stratmann, K.J.J. Mayrhofer, Electrochemical characterization of direct electron uptake in electrical microbially influenced corrosion of iron by the lithoautotrophic SRB *Desulfopila corrodens* strain IS4, Electrochim. Acta 167 (2015) 321–329.
[27] J.M. Pisciotta, Z. Zaybak, D.F. Call, J.-Y. Nam, B.E. Logan, Enrichment of microbial electrolysis cell (MEC) biocathodes from sediment microbial fuel cell (sMFC) bioanodes, Appl. Environ. Microbiol. 78 (2012) 5212–5219.
[28] Z. Zaybak, J.M. Pisciotta, J.C. Tokash, B.E. Logan, Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems, J. Biotechnol. 168 (2013) 478–485.
[29] M. Dörries, L. Wöhlbrand, R. Rabus, Differential proteomic analysis of the metabolic network of the marine sulfate-reducer *Desulfobacterium autotrophicum* HRM2, Proteomics 16 (2016) 2878–2893.
[30] M. Rosenbaum, F. Aulenta, M. Villano, L.T. Angenent, Cathodes as electron donors for microbial metabolism: which extracellular electron transfer mechanisms are involved? Bioresour. Technol. 102 (2011) 324–333.
[31] J. Haladjian, P. Bianco, F. Guerlesquin, M. Bruschi, Electrochemical study of the electron exchange between cytochrome C3 and hydrogenase from *Desulfovibrio desulfuricans* Norway, Biochem. Biophys. Res. Commun. 147 (1987) 1289–1294.
[32] L. Jourdin, S. Freguia, V. Flexer, J. Keller, Bringing high-rate, CO_2-based microbial electrosynthesis closer to practical implementation through improved electrode design and operating conditions, Environ. Sci. Technol. 50 (2016) 1982–1989.
[33] J.S. Deutzmann, M. Sahin, A.M. Spormann, Extracellular enzymes facilitate electron uptake in biocorrosion and bioelectrosynthesis, MBio 6 (2015).
[34] S.T. Lohner, J.S. Deutzmann, B.E. Logan, J. Leigh, A.M. Spormann, Hydrogenase-independent uptake and metabolism of electrons by the archaeon *Methanococcus maripaludis*, ISME J. 8 (2014) 1673–1681.
[35] L. Yu, J. Duan, X. Du, Y. Huang, B. Hou, Accelerated anaerobic corrosion of electroactive sulfate-reducing bacteria by electrochemical impedance spectroscopy and chronoamperometry, Electrochem. Commun. 26 (2013) 101–104.
[36] M. Sharma, N. Aryal, P.M. Sarma, K. Vanbroekhoven, B. Lal, X.D. Benetton, D. Pant, Bioelectrocatalyzed reduction of acetic and butyric acids via direct electron transfer using a mixed culture of sulfate-reducers drives electrosynthesis of alcohols and acetone, Chem. Commun. 49 (2013) 6495–6497.
[37] Y. Xiang, G. Liu, R. Zhang, Y. Lu, H. Luo, Acetate production and electron utilization facilitated by sulfate-reducing bacteria in a microbial electrosynthesis system, Bioresour. Technol. 241 (2017) 821–829.
[38] K. Brysch, C. Schneider, G. Fuchs, F. Widdel, Lithoautotrophic growth of sulfate-reducing bacteria, and description of *Desulfobacterium autotrophicum* gen. nov., sp. nov, Arch. Microbiol. 148 (1987) 264–274.
[39] K. Igarashi, S. Kato, Extracellular electron transfer in acetogenic bacteria and its application for conversion of carbon dioxide into organic compounds, Appl. Microbiol. Biotechnol. 101 (2017) 6301–6307.
[40] X. Deng, N. Dohmae, K.H. Nealson, K. Hashimoto, A. Okamoto, Multi-heme cytochromes provide a pathway for survival in energy-limited environments, Sci. Adv. 4 (2018).
[41] C.S. Kang, N. Eaktasang, D.-Y. Kwon, H.S. Kim, Enhanced current production by *Desulfovibrio desulfuricans* biofilm in a mediator-less microbial fuel cell, Bioresour. Technol. 165 (2014) 27–30.