Natural solar intermittent-powered electromethanogenesis towards green carbon reduction

Wang, Bo; Liu, Wenzong; Zhang, Yifeng; Wang, Aijie

Published in:
Chemical Engineering Journal

Link to article, DOI:
10.1016/j.cej.2021.134369

Publication date:
2022

Document Version
Peer reviewed version

Journal Pre-proofs

Natural solar intermittent-powered electromethanogenesis towards green carbon reduction

Bo Wang, Wenzong Liu, Yifeng Zhang, Aijie Wang

PII: S1385-8947(21)05941-6
DOI: https://doi.org/10.1016/j.cej.2021.134369
Reference: CEJ 134369

To appear in: *Chemical Engineering Journal*

Received Date: 19 September 2021
Revised Date: 25 November 2021
Accepted Date: 23 December 2021

Please cite this article as: B. Wang, W. Liu, Y. Zhang, A. Wang, Natural solar intermittent-powered electromethanogenesis towards green carbon reduction, *Chemical Engineering Journal* (2021), doi: https://doi.org/10.1016/j.cej.2021.134369

Natural solar intermittent-powered electromethanogenesis towards green carbon reduction

Bo Wang [a, b, c], Wenzong Liu [a] *, Yifeng Zhang [c], Aijie Wang [a]

a State Key Lab of Urban Water Resource and Environment, School of Civil and Environmental Engineering, Harbin Institute of Technology Shenzhen, 518055 Shenzhen, China

b Center for Electromicrobiology, Section of Microbiology, Department of Biology, Aarhus University, 8000 Aarhus C, Denmark

c Department of Environmental Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

*Corresponding author:

Wenzong Liu

State Key Lab of Urban Water Resource and Environment, School of Civil and Environmental Engineering, Harbin Institute of Technology Shenzhen, Xili, Nanshan District, Shenzhen, 518055, China

Email: liuwenzong@hit.edu.cn

Abstract

Microbial electromethanogenesis (EM), as a sustainable bioderived carbon-neutrality catalyzing platform, can be accelerated and regulated by weak power input for carbon fixation into value-added bioenergy. Solar electricity as a day-night intermittent renewable resource has been verified to effectively directly drive microbes to capture CO_2. However, understanding the influence mechanisms of higher CO_2 loading on EM is of intrinsic significance yet lacking. Herein, natural solar-powered bioelectrocatalytic CO_2 reduction to CH_4 under increasing bicarbonate concentrations was investigated. CH_4 recovery for the long-term measurement showed that CH_4 production rate positively responded to improved bicarbonate concentrations from 2.5 to 10 g $HCO_3^-{\cdot}L^{-1}$, exhibiting a robust and potent competence in CH_4 yield compared to reported EM. Whereas exceed bicarbonate mainly contributed to raised pH in the solution resulting in the proton limitation despite the intermittent driven-mode could mitigate pH shock. Electrochemistry results demonstrated that higher bicarbonate concentrations promoted the redox activity of electrode biofilm and lowered the system resistances, especially the charge transfer resistance. Adequately improving CO_2 loading can dynamically optimize the structure of anodic electroactive microorganisms and facilitate electron transfer. Furthermore, more functional cathodic *mcrA* genes were upregulated with elevated bicarbonates and the species of basophilic *Methanobacterium alcaliphilum* occupied predominated at the cathode. These findings open up a perspective avenue to carbon reduction using natural solar intermittent-powered EM.

Keywords: Solar energy; Microbial electromethanogenesis; Carbon reduction; Power-to-gas; Functional community evolution

1. Introduction

Towards carbon capture, utilization, and storage (CCUS), a set of natural processes, like green plants, algae, and bacteria, give clues to capture carbon dioxide (CO_2) into value-added products [1]. In principle, efficient CO_2 absorption and reduction entail external energy input [2]. Green plants absorb CO_2 from the atmosphere and produce carbon-containing sugars, which is called photosynthesis [3]. Similarly, algae consumes CO_2 in their part of the photosynthesis process [4]. Some bacteria in water or soil can utilize CO_2 under the requirement of chemical respiration for biomass growth or biogas production without direct solar power injection, albeit external energy can impel expeditious CO_2 capture [5].

Bioderived CO_2-reducing electrocatalytic systems for biomethane production, incorporating waste/wastewater treatment with energy recovery, driven by externally applied power, currently prospects much huger privileges in the progress of cutting edge carbon capture strategies [6], which may avail a carbon-neutral society [7]. This process also named electromethanogenesis (EM), employs electromethanogens with the ability of electron utilization via direct interspecies electron transfer, demonstrating advantages in terms of microbial growth rate and electron uptake compared with methyl oxidation methanogens (Methyl-ScoM + CoBSH $\rightarrow$ CH_4 + CoBS-SCoM, $\Delta G^{0'}_{CH_4} = -30\ kJ/mol$) [8]. Recently, natural sunlight-electricity-powered electromethanogenesis has been proposed to successfully increase the efficiencies of both electron utilization and CH_4 recovery by considering the properties of day-night intermittent inherence [9,10]. Such a solar-driven system upregulates capacitive storage

behavior preponderantly involved in the charge carrier of electro-transfer proteins, which unfolds promising perspectives for boosting microbial bioelectrocatalysis more practicably and sustainably. On another side for CO_2-reducing methanogenesis, CO_2 as the prominent media grips the bioelectrocatalytic rate. In a variety of CO_2-containing media, bicarbonate, the aqueous form of CO_2, represents an exciting alternative to supply electrons acceptors for EM, instead of gaseous CO_2 in consideration of its sparing diffusion in water-based electrolytes [11]. Also, the application of bicarbonate can accelerate more carbon fixation in such microbial electrocatalytic niches. Furthermore, the membrane is commonly used in EM apparatus for CO_2 reduction due to the requirement of H^+ generated via water electrolysis. However, a lower transfer of mass or nutrient could not be evaded, especially for the EM system with membrane. Although the autotrophic process is the main contributor to carbon reduction, yet mixotrophic conditions would benefit from the growth rate especially for the mutual functional microbial communities to promote carbon reduction [12].

Foregoing studies have unearthed that periodic polarization, viz., intermittent electro field, could ameliorate the pH disturbance [13]. However, scarce knowledge, for example, if higher bicarbonate concentrations or CO_2 loading without meticulous protection (i.e., all operations are close to real scenarios) could be beneficial for EM with the aid of real natural solar-intermittent driven power, and the effect of long-term shock of higher bicarbonates on the evolution of functional microbial fluids are not well dissected. In this study, depth-annotated long-term investigations (circa one year) of higher bicarbonate concentrations on stimulating microbial electrodes for carbon

capture are urgent to be well untangled under the natural solar-driven force. More importantly, the responses sourced from electrochemical properties of biofilms and shifty structures of functional floras and collaboration mechanisms, spotlighting on the biocathode evolution would be efficiently explained.

2. Materials and methods

2.1 Solar-powered bioelectrochemical reactors setup and operation

Twelve parallel single-chamber without membrane bioelectrochemical reactors (made of perspex) with 0.142 ± 0.008 L effective working volume were driven by real solar power, which converted natural solar light into direct current by the solar panel inserted with the voltage stabilizing converter, as previously reported [10]. The power capability of the solar panel was not strictly susceptible to strong or weak sunlight in the daytime, which can output a steady 1.0 V of applied voltage. A data acquisition card was employed to detect the cell voltage across the external resistor of 10 Ω (Keithley model 2700, Tektronix, Inc, Beaverton, OR, USA), and then was automatically recorded through a computer. A multi-layer foil sampling gasbag was connected to the needle glued on the top of the reactor to collect gases (100 mL capacity; LB-301-0.1, Dalian Delin Gas Packing Co., Ltd., China). The anode constituted carbon brush, composed of carbon fiber (fiber type: PANEX 33 160 K, radius 10.0 μm, Zoltek, St. Louis, MO, USA) with a 5.0 cm radius, 20 cm length, and 0.24 $m^2 \cdot g^{-1}$ surface area. The cathode consisted of carbon cloth with a 0.1 cm thickness and 7 $cm^2 \cdot g^{-1}$ surface area.

The incipient inoculum consisted of a mixture of biomass from the effluent of single-chambered microbial electrolysis reactors mainly producing CH_4, that contained

a majority of functional planktonic cells [10]. The influent recipe comprised (per L) 1.55 g $NaH_2PO_4 \cdot 12H_2O$, 2.77 g $Na_2HPO_4 \cdot 2H_2O$, 0.31 g NH_4Cl, 0.13 g KCl. The feedstock wastewater basically composed of small-molecule organic acids and equivalent chemical oxygen demand (COD) of 1.0 ± 0.1 $g \cdot L^{-1}$ was used as the organic carbon source, and different CO_2 concentrations in the form of sodium bicarbonate were utilized as the inorganic feedstock (Table S1). The abovementioned 12 bioreactors were subdivided into four groups, each of which was operated in triplicate and fed varying bicarbonate concentrations. All bioelectrochemical reactors were replenished for a fresh medium once the current dropped below 1 mA [14]. All bioreactors were put in a room with a steady temperature of 22 ± 2 °C (to be closer to the practical application) and ran for approximately ten months.

2.2. Measurements and calculations

After each fed-batch experiment termination, liquid samples were collected and preserved in different temperatures. One part stored in the 4 ℃ freezer was assigned to the chemical oxygen demand (COD) reagent tube with 2 mL for COD detection after filtering through a 0.22 μm membrane filter and heated by a heater (DRB 200, HACH, USA) for 120 min, which was then absorbency quantified by a spectrophotometer (DR6000, HACH, USA) [15]. The other part stored in the - 20 ℃ freezer was assigned to TOC (total organic carbon) tubes with the total volume of 20 mL for the total carbon testing (including total organic and inorganic carbon, TC), which was quantitatively measured through a TC-2000 (Shimadzu, Kyoto, Japan). The biogas samples (mainly contained H_2, CH_4, and CO_2) were also obtained and then were tested via a gas

chromatograph (Agilent 7892, USA) equipped with a thermal conductivity detector (TCD). pH and conductivity of the influent and effluent of the bioreactors were measured using a FiveEasy Plus pH meter (FE20, Mettler-Toledo International Inc., Shanghai, China) and a WTW Multimeter (MultiLine® Multi 3620 IDS) with IDS digital conductivity sensor (TetraCon® 925), respectively. ATP, protein, and cytochrome c of electrode biofilms were measured according to the precedent research [9].

The circuit current density was calculated according to Ohm's law normalized to the cathode surface area ($A \cdot m^{-2}$) or the reactor volume ($A \cdot m^{-3}$). The daily volumetric CH_4 production rate, (Y_{CH_4}) was calculated by the produced CH_4 yield (mol) dividing for each cycle after being normalized to the effective working liquid volume (0.142 ± 0.008 L) of the reactor per day (mol $CH_4 \cdot m^{-3}$ reactor$\cdot d^{-1}$). The contribution ratio of CH_4 generated by current also called the efficiency of electrons uptake in the product of CH_4 (the current to CH_4, mol $CH_4 \cdot m^{-3}$ reactor$\cdot d^{-1}$): $\eta_{CH_4} = \frac{8Fn_{CH_4}}{\int_0^t Idt}$. And the energy recovery efficiency, referring to the external electricity ending up in CH_4: $\eta_{energy} = \frac{-n_{CH_4}\Delta G_{CH_4}}{E_{ap}\int_0^t Idt}$. Here, n_{CH_4} is the moles of CH_4 produced for one cycle; 8 is the numbers of electrons generated 1 mol CH_4 from CO_2 reduction; ΔG_{CH_4} is the value of Gibb's free energy of CH_4 oxidation (-890.4 $kJ \cdot mol^{-1}$); E_{ap} is applied voltage (V); F is Faraday constant (96485 $C \cdot mol^{-1}$) and I is current (A or 1 $C \cdot s^{-1}$) [16].

2.3. Electrochemistry analysis

To precisely understand the electrochemical activities of biofilms fed by different bicarbonate concentrations, cyclic voltammetry (CV) and electrochemical impedance

spectroscopy (EIS) assays were executed at the end of effectuating a repeatable and stable system's performances for long-term operation by virtue of an electrochemical workstation (CHI660D, Chenhua Instruments Co., Ltd., China; EC-Lab V10.02 software). The CV was performed using a three-electrode configuration under a turnover condition with a scanning rate of 1 $mV \cdot s^{-1}$. While the EIS was in-situ conducted under the operational potential using a two-electrode configuration for a whole-cell and a three-electrode mode for the cathode bioelectrode with the frequency range of 100 kHz - 0.01 Hz and a 1 mV amplitude of the sinusoidal perturbation [9].

2.4. Microbial community analyses

Microbial DNA was extracted from electrode biofilms of all reactors at the end of the operation with steady CH_4 generation, using FastDNA™ Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) according to manufacturer's protocols. To avoid the distraction of the biomass floating in the reactors on biofilm communities, the electrodes were gently soaked with 50 mM PBS buffering for ca. 1 min before sampling after cutting pieces of the electrodes with a sterilized scissor [9]. The terminal DNA concentration and purification were measured by NanoDrop 2000 UV-visible spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V4 hypervariable regions of the bacteria 16S rRNA gene for bioanodes and biocathodes were amplified with primers: Forward 515F (5'-GTGCCAGCMGCCGCGG-3') and Reverse 806R (5'-GGACTACHVGGGTWTCTAAT-3'), which can identify all bacterial and most archaeal 16S sequences. Meanwhile, to reimburse low taxonomic resolution and

authenticity in 16S sequencing, and deliver precise infos about methanogens' activities and species diversity [17], the amplicon for *mcrA* genes of biocathodes with primers: Forward Mlf (5'- GGTGGTGTMGGATTCACACARTAYGCWACAGC -3') and Reverse Mlr (5'- TTCATTGCRTAGTTWGGRTAGTT -3'), was also executed. All amplicons were sequenced by using an Illumina MiSeq platform (Illumina, San Diego, USA), and sequencing analyses were accomplished as previously described [10]. Besides, to minimize the interference of random sequencing errors, low-quality sequences were eliminated, particularly those lacking a precise match with the forward primer, a recognizable reverse primer, and the length shorter than 200 nucleotides, and any ambiguous base calls. Quantitative PCR (qPCR) was further conducted to quantify *mcrA* genes of the cathode biofilm with identical primers [18]. The raw sequencing data have been submitted to National Center for Biotechnology Information Sequence Read Archive assigned accession numbers PRJNA737604 and PRJNA737612.

2.5. Cathode biofilm visual identification

To in-situ probing microbial distribution of the cathode biofilm with long-term higher CO_2 loading stimulation driven by real natural solar light in-depth, based on the characteristics of the cathode functional microorganisms, four diverse microbial levels with their representative primers: the domain of *Bacteria*, the phylum of *Euryarchaeota*, the family of *Methanobacteriaceae*, and the genus of *Methanosarcina* were marked via the fluorescence in situ hybridization (FISH), respectively. The probe sequencings of *Bacteria*, *Euryarchaeota*, *Methanobacteriacea*, *Methanosarcina* are 5'-GCTGCCTCCCGTAGGAGT -3' (5'6-FAM fluorophores), 5'-

CACAGCGTTTACACCTAG -3' (5'Cy3 fluorophores), 5'- TACCGTCGTCCACTC CTTCCTC-3' (5'Cy5 fluorophores), and 5'- GACCCAATAATCACGATCAC -3' (5'AQUA fluorophores), respectively. The samples of cathode biofilms were obtained using sterilized scissors when terminating reactors. And approximately 0.5 cm × 0.5 cm electrode biofilms were firstly pretreated to immobilize microorganisms via 4% paraformaldehyde (wt/wt) and 5% glutaraldehyde (v/v); then were further hybridized with specific fluorescence probes (abovementioned single probe was homogeneously mixed); subsequently, hybridized samples were further washed with 10× washing buffer to remove excess probes and dried; finally, hybridized-washed-dried samples with the addition of anti-fluorescent quencher were visually observed though the Laser Scanning Confocal Microscopy (CLSM, Leica SP8, ZEISS, Germany).

3. Results and discussion

3.1 Electron transfer and methanization feedbacks on bicarbonate concentrations

To evaluate the capability of higher CO_2 capture in solar-powered bioderived CO_2-reducing systems, all bioreactors were run circa one month after inoculation and CH_4 recovery was measured over nine month's fed-batch cycles when achieving consistent reactor performances. The current and CH_4 yield were observed to increase with the increase in bicarbonate concentrations, accompanied by expedited reaction time (Fig. 1a – b, Fig. S1a). The average time of each cycle varied from 6.28 days to 8.53 days at 2.5 and 15 g $HCO_3^{-}\cdot L^{-1}$, in which the shortest time was 4.51 ± 0.29 days ("±" means standard deviation, same below) at 10 g $HCO_3^{-}\cdot L^{-1}$ (Fig. S1b). The peak current density ranged from 12.00 ± 0.29 $A\cdot m^{-2}$ (59.16 ± 1.43 $A\cdot m^{-3}$) at 2.5 g $HCO_3^{-}\cdot L^{-1}$ to 17.36 ±

0.38 $A \cdot m^{-2}$ (85.59 ± 1.87 $A \cdot m^{-3}$) at 10.0 g $HCO_3^- \cdot L^{-1}$ (Fig. S1c), indicated sufficient electron donors could motivate the occurring of faster redox reaction. The highest CH_4 yield of 3.95 ± 0.21 mmol was obtained at 10.0 g $HCO_3^- \cdot L^{-1}$, giving rise to an average CH_4 production rate of 5.47 ± 0.29 mol $CH_4 \cdot m^{-3}$ reactor$\cdot d^{-1}$ (Fig. 1b). However, the percentage of produced H_2 and released CO_2 were attenuated with the increased bicarbonate concentrations, and the complete CH_4 content (100%) also displayed the natural solar day-night driven mode can be a perspective avenue to upgrade biogas.

The efficiency of current to CH_4 (η_{CH_4}) was also evaluated and the highest η_{CH_4} was 211 % for bioreactors with 10.0 g $HCO_3^- \cdot L^{-1}$ (Fig. 1c). These results suggested that the system efficiencies were escalated with the bicarbonate concentrations within a certain range, but higher bicarbonate concentrations would deteriorate CH_4 yield. The n_{CH_4} represents the moles of CH_4 produced for one cycle, which mostly consists of electron-derived CH_4 and suspended biomass-derived CH_4 [10]. In fact, the efficiency would be not over 100% when CH_4 was just regarded to be produced from biocathode reactions. However, for the single-chamber membrane-less bioelectrocatalytic system, the CH_4 was the sum of the amounts, which were from the biocathode recovery and suspended substrate conversion. Similar results also can be found in the study [19]. Furthermore, if we want to know the specific contribution from these two sources, the double-chamber system will be a good option; nevertheless, the expense and fouling of the membrane will bring other concerns, which will deteriorate the fancy of this technology in the future large-scale use.

Comprehensively compared to previously published research on

electromethanogenesis using a biocathode to convert CO_2 to CH_4, it could be easily concluded that the presence of membrane has a prevalence in such a system and higher applied voltages are required due to the occurrence of water splitting at the abiotic anode rather than the oxidation of organic matters at the biotic anode (more details could be found in Table S2). In addition, there was the out of tune between the efficiency of current (i.e., coulombs) to the carbon-containing product (i.e., CH_4) and the production rate of reductive product (Fig. 1d), implying energy investment did not completely be converted into CH_4 and thus causing another nominal squandering of resources. Further considering other exterior operation parameters control, for example, electrode modification [14], appropriate temperature and constant pH control [19,22], etc., admittedly, the natural solar-powered electromethanogenesis would still exhibit a considerably competitive competence in bioenergy recovery via green intermittent driven force than continuous direct electricity. Thus, natural solar-powered bioelectrocatalysis is expected to bring more novel research as well as launch further applications toward more high-value-added commodities and efficient carbon capture when coupling of electrode modification, the assistance of separators, and nanotechnology, etc.

The optimal pH scopes for most mesophilic methanogens vary from 6.8 to 8. Thus, when the system's pH expanded with the concentrations of bicarbonate without buffering regulation, and pH even reached over 8 at the bicarbonate concentration of 15 $g \cdot L^{-1}$ (Fig. 1e), the activity of methanogens may be inhibited, even including some functional bacteria, resulting in low CH_4 production. In addition, pH values are directly

connected to proton concentration, and proton would be a restricted factor for electron transfer in electrode biofilms. Luck et al. revealed that the electron transfer process of anodic biofilm depended on proton-transport limitation, which led to a formal redox potential shift with 48 ± 7 mV/pH unit under a minimal proton gradient [22]. Prior to our studies, it has been illustrated that there was a significant pH improvement ranging from 6.9 to 10.2 surrounding the 2 mm region of the cathode biofilm [23]. And altered pH caused the changes of standard Gibbs energy for thermodynamic methanogenesis ($CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$, $\Delta G^{0'}_{CH_4} = 23.53\ kJ/e^- eq$), varying from -33.89 $kJ \cdot mol^{-1}$ (at pH 6.9), to -21.47 $kJ \cdot mol^{-1}$ (at pH 9.1 with a 1 mm distance from the cathode biofilm), finally declined to -15.32 $kJ \cdot mol^{-1}$ (at pH 10.2). Thus, evaluated bicarbonates changed the proton transportation and subsequently affected the electrical communication between bioelectrodes. In addition, CO_2 capture via electromethanogenesis has been reported in a flurry of studies [24,25], however, most efforts have been dedicated to separating anode and cathode chambers with pH adjustment to optimize CH_4 production [20,26]. Indeed, the presence of a membrane would induce construction costs and entangle operations. Although the membrane-less apparatus is perceived as a preferable avenue to eliminate pH variation than a separator, since methanogenesis most presumably occurs in the membrane-less status, even in successive systems with acidification and carbonate limitation [9,27], pH polarization around a biocathode is still a possible agent as an obstacle for microbes in the biofilm due to higher pH values when no buffering regulation. The conductivity of the solution did not significantly change (Fig. 1e). Total carbon in the influent and effluent was

estimated, respectively (Fig. 1f). The results indicated that adequate higher concentrations of bicarbonate were constructive in the reduction of carbon emission, whereas extortionate loading received opposite consequences due to the evolution of microbial consortia.

Fig. 1 is here.

3.2. Electrochemical property analysis of electrode biofilms

The electrochemical profiles of mature biofilms can be a valid tool to reflect changes under the condition of long-term electrical stimulation (on/off) and sufficient electron acceptors and reveal electrochemistry characteristics. Anodic CV outlines depicted that the evaluated gradients of bicarbonate concentrations could benefit anode biofilms from achieving much higher redox current densities (the current was normalized to the surface area of electrodes unless otherwise specified), and when biofilms encountered the shock of much higher concentrations of bicarbonate for the long term, the catalytic activity of biofilms was significantly lowered (Fig. 2a). The peak current density increased at the similar potential of ca. -0.15 V with increased bicarbonate concentrations for bioanodes. When CV was conducted at the modest scan speed of 1 $mV \cdot s^{-1}$, the steady catalytic characteristics with a high signal-to-noise ratio were noticed. The electron transfer rate increased at a potential of ca. -0.30 V, especially at the concentration of 10.0 g $HCO_3 \cdot L^{-1}$, suggesting microbial metabolism catalyzing electron transport from the substrate to the electrode was continuously regenerated [28]. In addition, an obvious oxidative peak basically appeared above a potential of ca. -0.15 V, which was more than likely linked to H_2 production, as the peak vanished after

restricting the forward scan's reductive potential to -0.40 V [29]. Nevertheless, no H_2 was measured in the headspaces of all bioreactors except for that fed with 15.0 g $HCO_3 \cdot L^{-1}$. It might be explained by the fast consumption of H_2 as an energy intermediate by hydrogenotrophic methanogenesis, and the activity of methanogens would be limited under a beyond higher CO_2 loading environment. In addition, the absorption peak of heme of cytochrome c of anodic biofilms, as an indicator to assess the capacity of electron transfer, also supported this claim (Fig. S2a). By contrast, there were no obvious variations in cathodic voltammograms (Fig. 2b), which was more likely due to sufficient electron donors towards the cathode assembly when organic carbons became a restricted aspect. The ATP activity of cathode biofilm had no significant discrepancy (Table S4). The membrane-less single chamber system necessitates the input of organic carbon sources to sustain current generation from the redox of organics, which also chiefly differs from a double-chamber system only demanding inorganic carbon sources, that is attributed to water splitting occurring in the anode [30].

EIS was used to further distinguish the electron transfer and transfer resistance in the whole system and particularly underscore the biocathode under the long-term feedback (ca. 12 months) of high bicarbonates. Both of the resistances consisted of ohmic resistance (R_s), charge transfer resistance (R_{ct}), and diffusion resistance (R_d), which were fitted to the identical equivalent circuit at the two-constant model (Fig. S2b). For the whole cell, the system's total resistance decreased with the increased bicarbonate concentrations, however, the resistance of the system with 15.0 g $HCO_3^- \cdot L^-$

[1] shifted, and increased to 7 times (from ca. 300 Ω to over than 2000 Ω), compared with the lowest total resistance in the system with 10.0 g $HCO_3 \cdot L^{-1}$ (Fig. 2c)), which was also further supported the results of CV, that the redox activity of the anode was gliding in much higher concentrations. Two apparent semicircles were composed of the cathode Nyquist spectra, and most probably represented the ohmic resistance and the charge transfer resistance, respectively, and the latter generally determined the electron transfer rate [31]. It was in fact observed that the transportation limitation played a pivotal role in the bioderived CO_2-reducing system. The charge transfer resistance of bioreactors with 10.0 g $HCO_3^- \cdot L^{-1}$ (ca. 50 Ω) was almost 3.6 folds lower than that of 2.5 g $HCO_3^- \cdot L^{-1}$ (ca. 180 Ω) and far below than that of 15.0 g $HCO_3^- \cdot L^{-1}$ (Fig. 3d). The higher R_{ct} also suggested higher bicarbonate shock would restrain electron transfer in the biocathode, sequentially resulting in a low CH_4 yield. This result was also confirmed in the study of Izadi et al., [29] an obvious reduction in the charge transfer resistance could be achieved in the CO_2-reducing biofilm (principally converted CO_2 into acetate).

Together, appropriate bicarbonate concentrations can effectively promote the electron transfer of electroactive microorganisms (EAMs), but if beyond a certain threshold, it would lead to a decrease in the electrical activity of the anodic biofilm and a decrease in the redox capacity, and then affect the rate of electron transfer and proton diffusion, eventually bring about the low yield of targeted products.

Fig. 2 is here.

3.3. Responses of microbial community evolution on carbon capture

Biofilm, as a sort of dynamically well-balanced microbial assembly adhering on

the electrodes (i.e., a biocarrier of biopacking), in which plentiful microbes constitute a population community, displays unambiguous and synergetic relationships, and universally holds the prime jurisdiction for deposing wastes/pollutants and further converting them into bioenergy/fuel (and thus CH_4). Moreover, the composition, morphology, thickness, and other physicochemical characteristics of biofilm act as an integral function in recovering value-added products.

The inclusive identifications of both anodes and cathodes with specific functional species at the genus level were illustrated to compare the differences among the structures and compositions (Fig. 3). In general, more CO_2 pumped into the bioelectrochemical system can have a positive influence on the enrichment of cathodic methanogens and improve the capability of carbon capture (Fig. 3a). Biocathode, as the core biofilm, holds the pivotal responsibility for electromethanogenesis. More specifically, hydrogenotrophic methanogens (mainly composed of the genus of *Methanobacterium*) were further improved with the rise of bicarbonate concentrations (Fig. 3a), increased from the relative abundance of 86.28% (at the bicarbonate concentrations of 2.5 $g \cdot L^{-1}$) to that of 91.24% (at 10.0 g $HCO_3^- \cdot L^{-1}$). Nonetheless, the acetotrophic methanogenesis pathway ($CH_3COOH \rightarrow CH_4 + CO_2$) at the cathode was distinctly declined when improved bicarbonate concentrations (Fig. 3a), decreased from the relative abundance of 6.50% (2.5 g $HCO_3^- \cdot L^{-1}$) to that of 0.88% (15.0 g $HCO_3^- \cdot L^{-1}$).

Some disparate results showed the community evolution of the anodes (Fig. 3b). It has been reported that *Geobacter* as the dominant EAMs for electron transfer [32],

whose relative abundance increased with the bicarbonate concentrations, improved from 1.84% (2.5 g $HCO_3^-·L^{-1}$) to 5.33% (10.0 g $HCO_3^-·L^{-1}$) and then decreased to 2.80% (15.0 g $HCO_3^-·L^{-1}$), when encountered the shock of more bicarbonates. It was in accordance with the trend of the current density as well (Fig. S1c). Engrossingly, other representative EAMs, such as *Rhodopseudomonas*, *Comamonas*, *Pseudomonas*, decreased in concordance with evaluated bicarbonate concentrations. It has been corroborated that these EAMs proposed diverse electron transfer aptitudes or productivity [32,33], and adequately improving CO_2 loading can efficaciously optimize the structure of EAMs and upregulate electron transfer rates, and ultimately taking advantage of the amelioration of system performances. Intriguingly, when supplementing more bicarbonates, the relative abundances of hydrogenotrophic methanogens were increased from 20.21% (2.5 g $HCO_3^-·L^{-1}$) to 50.47% (10.0 g $HCO_3^-·L^{-1}$), representing a factor of ca. 2.50-fold difference, which implied that electrons in-situ generated at the anode were likewise utilized by anodic hydrogenotrophic methanogens. Furthermore, it was demonstrated that more COD can be expended within a much shorter cycle time (Fig. S1b) when intensified the bicarbonate concentrations, and it was beheld that the organic substrate consumption rate and Coulombic efficiency (CE) were evidently increased (Fig. S3a-b) when supplied enough CO_2. CO_2 reduction happening to either at the anode or cathode could availably work in parallel, which was also a conceivable explanation that higher current density could be achieved in higher concentrations of bicarbonate (Fig. S1c).

Due to the absence of the membrane in the single-chambered bioelectrochemical

system, electrons generated from the anode not only flowed to the cathode via an external circuit driven by renewable electricity, reducing CO_2 into CH_4 but also were synchronously consumed by anodic methanogens dominated by hydrogenotrophic methanogens to form CH_4. Of note, there was a similar trend of methanogens structure at both electrodes, owing to the absence of a membrane to allow microbes to freely reach and colonize from one electrode to the other electrode [26]. Strictly speaking, hydrogenotrophic methanogens were attached to the anode instead of competition with these on the cathode for electrons. It is conceivably attributed to the inherence of natural intermittence of solar power, scilicet power-on would facilitate the cathode taking in electrons whereas anodic microbes would use electrons in the period of power-off, which has been proved in previous studies [9,10]. EAMs performed as the pseudocapacitor accompanied by the occurring of the faradic process, when power was "cut-off" (i.e., without sunlight), superabundant electrons stored in EAMs would be released, and then further utilized by methanogens stuck to the anode, subsequently producing CH_4 [9,34]. This phenomenon has certainly occurred in earlier studies, but it has often been overlooked [13]. This could be an effective strategy to improve CH_4 production in the future and simultaneously trim the cost of infrastructure.

When it occurs to the shift in functional species beyond the bicarbonate concentration of 10 $g \cdot L^{-1}$, surprisingly, exceeding higher bicarbonate concentration (15.0 $g \cdot L^{-1}$) would restrain the growth of EAMs adhered on the anode, decreased to 4.07%, and especially *Geobacter* also descended to 2.80% (Fig. 3b). And this also would further instigate the scarcity of electrons sources, and subsequently cause the

damping of methanation activity, in particular dominated by the pathway of hydrogenotrophic methanogenesis. The lower generation of electrons, the lower production of CH_4. Meanwhile, more CO_2 invaded into the system could expedite the enhancement of acetogenins attached either on the cathode or the anode, increased to 0.13% and 3.07%, respectively, which was much higher than the relative abundance of acetogens fed by other concentrations. It might be hinted that an excess of CO_2 loading would be favorable for acetate production other than CH_4 (namely more electrons would flow to produce acetate). Moreover, this outcome opens up another perspective for future study when a proton or cationic exchange membrane is employed to compartmentalize the anode and cathode compartments and then advance the bicarbonate concentration. Presumably, the system would be anticipated to transform into an acetic acid-producing system powered by natural solar light, which means a higher concentration of CO_2 is conducive to the formation of other high value-added products except for CH_4.

Fig. 3 is here.

3.4. Cathodic species identification via *mcrA* gene sequencing and in-situ visualization of cathode biofilm

Since the alpha subunit of methyl-coenzyme M reductase, which is encoded by *mcrA*, has been reported to be responsible and highly expressed in all methanogens for the terminal step of CH_4 generation [8]. Thereby, to deeply unveil circumstantial variations of *mcrA* genes and sufficient real-time information for ascertainment at the species level, coupling of qPCR and mcrA sequencing is used to quantify *mcrA* genes

of archaeal methanogens and untangle the specific methanogenic species and activity. The total number of *mcrA* genes copies recovered in cathode samples increased with higher bicarbonate concentrations (Fig. 4a), increased from $3.77 \pm 0.47 \times 10^{13}$ copies·g^{-1}·m^{-2} (2.5 g HCO_3^-·L^{-1}) to $7.16 \pm 0.90 \times 10^{13}$ copies·g^{-1}·m^{-2} (10.0 g HCO_3^-·L^{-1}) and slightly decreased to $5.21 \pm 0.40 \times 10^{13}$ copies·g^{-1}·m^{-2} (15.0 g HCO_3^-·L^{-1}), which was consistent with those results of the relative abundances by 16S rRNA pyrosequencing, indicating that adequate CO_2 input can effectively inflame methanogens activity. At the species level, *Methanobacterium alcaliphilum* and *Methanosarcina horonobensis* as the predominant hydrogenotrophic and acetoclastic methanogens, respectively, were enriched at all cathodes (Fig. 4b). Basophilic M. *alcaliphilum* can thrive in a higher pH environment which can reach 8.3 - 9.9. And *Methanobacterium subterraneum* as the inferior abundance of hydrogenotrophic methanogen also can tolerate an alkaline environment whose pH varies from 7.8 to 8.8 [35]. These results implied that the pathway of current to CH_4 production primarily arose under alkaline conditions and thus granted more potentialities for CO_2 capture due to hydrogenotrophic EM making the cardinal contribution for CH_4 yield. Despite most acetotrophic methanogens, e.g., *Methanosarcina*, and *Methanosaeta*, prefer to grow in neutral conditions and adhere to the exterior of the cathodic biofilm, and they rely on direct interspecies electron transfer with cocultured microbes *Geobacter* [36].

Furthermore, coupling of FISH images could intuitively visualize the functional structures of cathodic biofilms (Fig. 4c). First, *Bacteria*, *Euryarchaeota*, *Methanobacteriacea*, *Methanosarcina* have marked the colors green, red, pink, and

blue with specific probes, respectively, in the field view. It could be found that the family of *Methanobacteriacea* occupied the major majority of the whole community, which is consistent with the sequencing results. The fluorescence intensity of a representative acetotrophic methanogen *Methanosarcina* also declined to some extent stimulated by higher concentrations of bicarbonate. The CLSM micrographs also revealed that moderately coincident distribution occurred between bacteria and archaea. Besides, it was noted that biofilm thickness increased with bicarbonate concentrations however, decreased at much higher loading (15.0 g $HCO_3^{-}\cdot L^{-1}$), suggesting excessive bicarbonate load is not conducive to the formation of homogeneous biofilms (Fig. S4a). Moreover, the protein content of cathode biofilms also displayed similar trends, indicating higher CO_2 loading could significantly improve biomass of the cathodes, especially as the biofilm matrix (Fig. S4b).

Fig. 4 is here.

3.5. Future directions on natural solar intermittent-powered electromethanogenesis

To mitigate the effects of increasing CO_2 levels on global climate, not only it is urgent to reduce the reliance on fossil fuels, but also explore an innovative eco-friendly route to absorb and utilize CO_2 from the atmosphere accompanied by reasonable energy investment and minimal environmental disturbance, which is fundamental to sustainable development and green inhabitation for human beings. CO_2 reduction via the electrocatalysis, photocatalysis, and biocatalysis technique is particularly appealing among various strategies since the required energy input might be invested from

sustainable sources such as solar energy. However, the low turnover, poor selectivity (as indicated by Faradaic efficiency), and exorbitant expenses of the sacrificial reductants are still the key challenges that need to be addressed (Table S5). Additionally, most semiconductors as the core of photocatalytic CO_2 reduction succumb to insufficient photovoltage or ineligible band edge positions to overcome CO_2 reduction's thermodynamic and kinetic hurdles. Compared abiotic catalysis approach, microbial biocatalytic CO_2 reduction employs solid electrodes as electron donors to provide electromicrobiology (as the biocatalysts) with infinite reducing power and ATP for their metabolism, and then these living microorganisms perform the conversion of exhausted gases into value-added chemical compounds. In addition, bacteria can self-repair and renew, resulting in a more extended operational lifespan. Since functional microorganisms are widely used and can be cheaply obtained and cultivated, they compensate for inexpensive catalysts. Furthermore, chemical catalysts are frequently limited to producing small molecule compounds, whereas microbes can create a broader spectrum of products with a greater end value. Finally, bacteria as living entities, are susceptible to evolution, thus they may be able to adapt to changes in the system's environment, such as feedstock compositions, gas pressure, temperature fluctuations.

Natural solar-driven microbial electromethanogenesis can effectively convert CO_2 into CH_4 under higher concentrations of bicarbonate (the schematic of the hybrid system shown in Fig. S5). Further developing it into a versatile microbial bioelectrocatalytic platform is feasible to be envisaged to realize sustainable water-carbon-energy nexus. First, the generated high-pure CH_4 would be injected into the gas

grid and then would be transferred into all energy storage. For example, CH_4 can be used for the feedstock of methane-oxidizing bacteria to produce single-cell protein as food [37]. CH_4 also could become biofuels to power vehicles, electricity, or heat for supporting households, or incorporated with the existing infrastructure. Furthermore, CO_2 emission from vehicles combustion can be fixed via plants and crops. Crops are then provided to households (animal feed and human dairy), and the generation of food waste would be fed into anaerobic digesters to produce biogas. Crude raw biogas also could be further upgraded via this technology (Fig. 5).

However, there are still some underlying yet substantial scientific questions to be addressed before wider application. First, it is still obscure that microbes are inclined to utilize what kind of forms of CO_2 are in the solution, as well as the complete mechanism of microbes' uptake of CO_2. Recently, Steffens et al. found that the tricarboxylic acid cycle, especially the plausibly irreversible citrate synthase reaction, can be reversed in anaerobic microorganisms and microbes could further fix CO_2 in the hydrothermal environment of rich CO_2 [38]. This process was also titled the reversed oxidative tricarboxylic acid cycle. It ingeniously uncovered that microbes might be constantly tweaking the levels of key enzymes in an unexpected way, thence they can assimilate CO_2 before encountering high loading of CO_2. This triggers an elegant harmony between microbial metabolism and their environment. In addition, Li et al. developed a mathematical model and systematically revealed that the diffusion (regarding the Fick's law) and interconversion of various CO_2 forms in the aquatic environment in EM, for example, hydrated $CO_{2(gas)}$, $CO_{2(aq)}$, $H_2CO_{3(aq)}$, $HCO_3^-{}_{(aq)}$ and

$CO_3^{-}{}_{(aq)}$, can influence the current generation as well as biocathode CH_4 production [39]. Intriguingly, the results illustrated that CO_2, $H_2CO_{3(aq)}$, and $HCO_3^{-}{}_{(aq)}$ were the main carbon source for CH_4 production when considering the possible reaction of $CO_{2(aq)} + OH^- \leftrightarrow HCO_3^{-}{}_{(aq)}$. Second, more data are urgently required to validate the duration of solar light and the influences of different regions on the technology. The manufacturing technology of solar panels is currently very mature and solar panels for sale on the market can stably output applied voltages and are not susceptible to strong or weak sunlight. More importantly, microbial bioelectrocatalysis is not keen on high voltages [40], despite solar panels can generate higher voltages. Last but not least, more rigorous operational parameters on the solar intermittent-driving microbial bioelectrocatalytic platform also need to be thoroughly estimated, such as electrode materials (with a high specific surface area), temperature, continuous feedback, hydraulic retention time, membrane, gas partial pressure, etc. Additionally, CH_4 has versatile applications, spanning from household utilization (fuel) to industrial manufacture (H_2 energy carrier and chemical precursors), however, it still is a greenhouse gas, which needs to be rigorous supply chain management.

Overall, the microbial bioelectrocatalytic system currently emerges widespread applications for CO_2 capture and conversion (reduction) in alternative higher-value chemicals, such as formate [41], acetate [42], alcohols [43], as well as complicated building blocks for pharmaceuticals or as biomolecules for diagnostic. Thus, inspired by this study, incorporated natural solar-intermittent power into bioderived CO_2-reducing electrocatalytic systems, and further combining with disciplines of

microbiology (biology), electrochemistry (biochemistry), and material (nanotechnology) science, carbon emission can be effectively alleviated.

Fig. 5 is here.

4. Conclusions

This work coupling of real solar light directly powering microbes and coarse operations for higher loading of carbon capture into CH_4 advances a fundamental for the water-carbon-energy nexus. In comparison to reported EM, CH_4 recovery for the long-term measurement indicated that CH_4 generation rate increased in response to increased bicarbonate concentrations from 2.5 to 10 g $HCO_3^{-}{\cdot}L^{-1}$, demonstrating a powerful competence in CH_4 yield. Higher bicarbonate concentrations improved the redox activity of electrode biofilms and reduced system resistances, particularly the charge transfer resistance. The structure of anodic electroactive microorganisms may be dynamically optimized and electron transport can be facilitated by increasing CO_2 input. With increased bicarbonates, more functional cathodic *mcrA* genes were upregulated, and the basophilic hydrogenotrophic methanogens *M. alcaliphilum* predominated at the cathode. These findings open up an innovative and simple avenue to the popularization of productive, remunerative, and effective microbial biotechnology for high-value-added biofuels and chemicals synthesis.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to express their gratitude to Dr. Xiaolan Zhang from the Institute of Microbiology, Chinese Academy of Sciences for helping with the biofilm analysis. The authors would also like to thank Prof. Dr. Barth F Smets, Dr. Marlene Mark Jensen, and Dr. Arnaud Dechesne from the Department of Environmental Engineering, Technical University of Denmark, for valuable comments on the data analysis and preliminary version of the manuscript. This research was financially supported by the Natural Science Foundation of Guangdong Province for Distinguished Young Scientists (No. 2021B1515020084), the National Natural Science Foundation of China (No. 51778607), and the Shenzhen Science and Technology Innovation Program (No. KQTD20190929172630447).

Appendix A. Supplementary material

Supplementary material to this article can be found online.

References

[1] C. Hepburn, E. Adlen, J. Beddington, E.A. Carter, S. Fuss, N. Mac Dowell, J.C. Minx, P. Smith, C.K. Williams, The technological and economic prospects for CO2 utilization and removal, Nature. 575 (2019) 87–97. https://doi.org/10.1038/s41586-019-1681-6.

[2] T.P. Senftle, E.A. Carter, The holy grail: Chemistry enabling an economically viable CO2 capture, utilization, and storage strategy, Acc. Chem. Res. 50 (2017) 472–475. https://doi.org/10.1021/acs.accounts.6b00479.

[3] S. Atsumi, W. Higashide, J.C. Liao, Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde, Nat. Biotechnol. 27 (2009) 1177–1180. https://doi.org/10.1038/nbt.1586.

[4] M. Kanno, A.L. Carroll, S. Atsumi, Global metabolic rewiring for improved CO2 fixation and chemical production in cyanobacteria, Nat. Commun. 8 (2017) 14724. https://doi.org/10.1038/ncomms14724.

[5] N. Kato, S. Mizuno, M. Shiozawa, N. Nojiri, Y. Kawai, K. Fukumoto, T. Morikawa, Y. Takeda, A large-sized cell for solar-driven CO2 conversion with a solar-to-formate conversion efficiency of 7.2%, Joule. 5 (2021) 687–705. https://doi.org/10.1016/j.joule.2021.01.002.

[6] F. Geppert, D. Liu, M. van Eerten-Jansen, E. Weidner, C. Buisman, A. ter Heijne, Bioelectrochemical Power-to-Gas: State of the Art and Future Perspectives, Trends Biotechnol.

34 (2016) 879–894. https://doi.org/10.1016/j.tibtech.2016.08.010.

[7] A.J. Ragauskas, C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick, J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer, T. Tschaplinski, The path forward for biofuels and biomaterials, Science (80-.). 311 (2006) 484–489. https://doi.org/10.1126/science.1114736.

[8] T. Wongnate, D. Sliwa, B. Ginovska, D. Smith, M.W. Wolf, N. Lehnert, S. Raugei, S.W. Ragsdale, The radical mechanism of biological methane synthesis by methylcoenzyme M reductase, Science (80-.). 352 (2016) 953–958. https://doi.org/10.1126/science.aaf0616.

[9] B. Wang, W. Liu, Y. Zhang, A. Wang, Bioenergy recovery from wastewater accelerated by solar power: Intermittent electro-driving regulation and capacitive storage in biomass, Water Res. 175 (2020) 115696. https://doi.org/10.1016/j.watres.2020.115696.

[10] B. Wang, W. Liu, Y. Zhang, A. Wang, Intermittent electro field regulated mutualistic interspecies electron transfer away from the electrodes for bioenergy recovery from wastewater, Water Res. 185 (2020) 116238. https://doi.org/10.1016/j.watres.2020.116238.

[11] G. Mohanakrishna, I.M. Abu Reesh, K. Vanbroekhoven, D. Pant, Microbial electrosynthesis feasibility evaluation at high bicarbonate concentrations with enriched homoacetogenic biocathode, Sci. Total Environ. 715 (2020) 137003. https://doi.org/10.1016/j.scitotenv.2020.137003.

[12] C.M. Dykstra, C. Cheng, S.G. Pavlostathis, Comparison of Carbon Dioxide with Anaerobic Digester Biogas as a Methanogenic Biocathode Feedstock, Environ. Sci. Technol. 54 (2020) 8949–8957. https://doi.org/10.1021/acs.est.9b07438.

[13] X. Zhang, A. Prévoteau, R.O. Louro, C.M. Paquete, K. Rabaey, Periodic polarization of electroactive biofilms increases current density and charge carriers concentration while modifying biofilm structure, Biosens. Bioelectron. 121 (2018) 183–191. https://doi.org/10.1016/j.bios.2018.08.045.

[14] B. Wang, W. Liu, W. Cai, J. Li, L. Yang, X. Li, H. Wang, T. Zhu, A. Wang, Reinjection oilfield wastewater treatment using bioelectrochemical system and consequent corrosive community evolution on pipe material, J. Biosci. Bioeng. 129 (2020) 199–205. https://doi.org/10.1016/j.jbiosc.2019.09.001.

[15] B. Wang, W. Liu, C. Varrone, Z. Yu, A. Wang, Hydrogen and Methane Generation from Biowaste: Enhancement and Upgrading via Bioelectrochemical Systems, in: P. Kumar, C. Kuppam (Eds.), Bioelectrochemical Syst., Springer, Singapore, Singapore, 2020: pp. 83–130. https://doi.org/10.1007/978-981-15-6868-8_5.

[16] D. Liu, T. Zheng, C. Buisman, A. Ter Heijne, Heat-Treated Stainless Steel Felt as a New Cathode Material in a Methane-Producing Bioelectrochemical System, ACS Sustain. Chem. Eng. 5 (2017) 11346–11353. https://doi.org/10.1021/acssuschemeng.7b02367.

[17] D. Wilkins, X.Y. Lu, Z. Shen, J. Chen, P.K.H. Lee, Pyrosequencing of mcrA and archaeal 16s rRNA genes reveals diversity and substrate preferences of methanogen communities in anaerobic digesters, Appl. Environ. Microbiol. 81 (2015) 604–613. https://doi.org/10.1128/AEM.02566-14.

[18] K. Takai, K. Horikoshi, Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes, Appl. Environ. Microbiol. 66 (2000) 5066–5072. https://doi.org/10.1128/AEM.66.11.5066-5072.2000.

[19] G. Zhen, X. Lu, T. Kobayashi, G. Kumar, K. Xu, Promoted electromethanosynthesis in a two-chamber microbial electrolysis cells (MECs) containing a hybrid biocathode covered with

graphite felt (GF), Chem. Eng. J. 284 (2016) 1146–1155. https://doi.org/10.1016/j.cej.2015.09.071.

[20] Q. Fu, Y. Kuramochi, N. Fukushima, H. Maeda, K. Sato, H. Kobayashi, Bioelectrochemical analyses of the development of a thermophilic biocathode catalyzing electromethanogenesis, Environ. Sci. Technol. 49 (2015) 1225–1232. https://doi.org/10.1021/es5052233.

[21] H.Y. Yang, Y.X. Wang, C.S. He, Y. Qin, W.Q. Li, W.H. Li, Y. Mu, Redox mediator-modified biocathode enables highly efficient microbial electro-synthesis of methane from carbon dioxide, Appl. Energy. 274 (2020) 115292. https://doi.org/10.1016/j.apenergy.2020.115292.

[22] B.G. Lusk, I. Peraza, G. Albal, A.K. Marcus, S.C. Popat, C.I. Torres, PH Dependency in Anode Biofilms of Thermincola ferriacetica Suggests a Proton-Dependent Electrochemical Response, J. Am. Chem. Soc. 140 (2018) 5527–5534. https://doi.org/10.1021/jacs.8b01734.

[23] W. Cai, W. Liu, B. Wang, H. Yao, A. Guadie, A. Wang, Semiquantitative detection of hydrogen-associated or hydrogen-free electron transfer within methanogenic biofilm of microbial electrosynthesis, Appl. Environ. Microbiol. 86 (2020) e01056-20. https://doi.org/10.1128/AEM.01056-20.

[24] C.M. Dykstra, S.G. Pavlostathis, Methanogenic Biocathode Microbial Community Development and the Role of Bacteria, Environ. Sci. Technol. 51 (2017) 5306–5316. https://doi.org/10.1021/acs.est.6b04112.

[25] J. Zabranska, D. Pokorna, Bioconversion of carbon dioxide to methane using hydrogen and hydrogenotrophic methanogens, Biotechnol. Adv. 36 (2018) 707–720. https://doi.org/10.1016/j.biotechadv.2017.12.003.

[26] M. Siegert, M.D. Yates, A.M. Spormann, B.E. Logan, Methanobacterium Dominates Biocathodic Archaeal Communities in Methanogenic Microbial Electrolysis Cells, ACS Sustain. Chem. Eng. 3 (2015) 1668–1676. https://doi.org/10.1021/acssuschemeng.5b00367.

[27] P. Clauwaert, W. Verstraete, Methanogenesis in membraneless microbial electrolysis cells, Appl. Microbiol. Biotechnol. 82 (2009) 829–836. https://doi.org/10.1007/s00253-008-1796-4.

[28] M. Enrico, R.J. B., B.D. B., H.R. M., B.D. R., Microbial Biofilm Voltammetry: Direct Electrochemical Characterization of Catalytic Electrode-Attached Biofilms, Appl. Environ. Microbiol. 74 (2008) 7329–7337. https://doi.org/10.1128/AEM.00177-08.

[29] P. Izadi, J.M. Fontmorin, A. Godain, E.H. Yu, I.M. Head, Parameters influencing the development of highly conductive and efficient biofilm during microbial electrosynthesis: the importance of applied potential and inorganic carbon source, Npj Biofilms Microbiomes. 6 (2020) 1–15. https://doi.org/10.1038/s41522-020-00151-x.

[30] C. Liu, B.C. Colón, M. Ziesack, P.A. Silver, D.G. Nocera, Water splitting-biosynthetic system with CO2 reduction efficiencies exceeding photosynthesis, Science (80-.). 352 (2016) 1210–1213. https://doi.org/10.1126/science.aaf5039.

[31] A.P. Borole, D. Aaron, C.Y. Hamilton, C. Tsouris, Understanding long-term changes in microbial fuel cell performance using electrochemical impedance spectroscopy, Environ. Sci. Technol. 44, (2010) 2740–2745. https://doi.org/10.1021/es9032937.

[32] B.E. Logan, R. Rossi, A. Ragab, P.E. Saikaly, Electroactive microorganisms in bioelectrochemical systems, Nat. Rev. Microbiol. 17 (2019) 307–319. https://doi.org/10.1038/s41579-019-0173-x.

[33] D. Xing, Y. Zuo, S. Cheng, J.M. Regan, B.E. Logan, Electricity generation by Rhodopseudomonas palustris DX-1, Environ. Sci. Technol. 42 (2008) 4146–4151.

https://doi.org/10.1021/es800312v.

[34] S. Fleischmann, J.B. Mitchell, R. Wang, C. Zhan, D.E. Jiang, V. Presser, V. Augustyn, Pseudocapacitance: From Fundamental Understanding to High Power Energy Storage Materials, Chem. Rev. 120 (2020) 6738–6782. https://doi.org/10.1021/acs.chemrev.0c00170.

[35] W. Cai, W. Liu, Z. Zhang, K. Feng, G. Ren, C. Pu, H. Sun, J. Li, Y. Deng, A. Wang, mcrA sequencing reveals the role of basophilic methanogens in a cathodic methanogenic community, Water Res. 136 (2018) 192–199. https://doi.org/10.1016/j.watres.2018.02.062.

[36] A.E. Rotaru, P.M. Shrestha, F. Liu, M. Shrestha, D. Shrestha, M. Embree, K. Zengler, C. Wardman, K.P. Nevin, D.R. Lovley, A new model for electron flow during anaerobic digestion: Direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane, Energy Environ. Sci. 7 (2014) 408–415. https://doi.org/10.1039/c3ee42189a.

[37] P.J. Strong, S. Xie, W.P. Clarke, Methane as a resource: Can the methanotrophs add value?, Environ. Sci. Technol. 49 (2015) 4001–4018. https://doi.org/10.1021/es504242n.

[38] L. Steffens, E. Pettinato, T.M. Steiner, A. Mall, S. König, W. Eisenreich, I.A. Berg, High CO2 levels drive the TCA cycle backwards towards autotrophy., Nature. 592 (2021) 784–788. https://doi.org/10.1038/s41586-021-03456-9.

[39] Z. Li, Q. Fu, H. Chen, S. Xiao, J. Li, Q. Liao, X. Zhu, A mathematical model for CO2 conversion of CH4-producing biocathodes in microbial electrosynthesis systems, Renew. Energy. 183 (2022) 719–728. https://doi.org/https://doi.org/10.1016/j.renene.2021.11.050.

[40] K. Rabaey, R.A. Rozendal, Microbial electrosynthesis - Revisiting the electrical route for microbial production, Nat. Rev. Microbiol. 8 (2010) 706–716. https://doi.org/10.1038/nrmicro2422.

[41] K.L. Dubrawski, X. Shao, R.D. Milton, J.S. Deutzmann, A.M. Spormann, C.S. Criddle, Microbial Battery Powered Enzymatic Electrosynthesis for Carbon Capture and Generation of Hydrogen and Formate from Dilute Organics, ACS Energy Lett. 4 (2019) 2929–2936. https://doi.org/10.1021/acsenergylett.9b02203.

[42] 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) e00103-10. https://doi.org/10.1128/mBio.00103-10.

[43] E. V. Labelle, C.W. Marshall, H.D. May, Microbiome for the Electrosynthesis of Chemicals from Carbon Dioxide, Acc. Chem. Res. 53 (2020) 62–71. https://doi.org/10.1021/acs.accounts.9b00522.