

Evaluating propionate production in *Clostridium ljungdahlii* inoculated bioelectrochemical system

Zhen Fang^{a,*}, Qichao Fan^a, Yu Huang^a, Beisong Xu^b, Zhi-Jie Yang^a, Yang Gu^c, Vinod Kumar^d, Yang-Chun Yong^{a,*}

^a Biofuels Institute, School of Emergency Management, School of Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013, China

^b Department of Chemistry and Biochemistry, Ohio State University, Columbus 43210, USA

^c School of Health Science and Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China

^d Faculty of Engineering and Applied Sciences, Cranfield University, Cranfield MK43 0AL, UK

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ABSTRACT

Bioproduction of chemical building block such as propionic acid (propionate) is of great interest in current time as it is a sustainable alternative to petrochemical synthesis. *Clostridium ljungdahlii* as an electroactive homo-acetogen is able to ferment sugars or utilize CO₂ to produce organic acids under bioelectrochemical system (BES). However, few reports evaluated the ability of bioelectrochemically assisted propionate production via *C. ljungdahlii*. In this study, BES equipped with three-dimensional nanostructure electrode and inoculated with *C. ljungdahlii* in cathode chamber was developed to evaluate propionate production. It found that applied negative potential on cathode was necessary to stimulate propionate production from fructose. Bio-electrochemical characterizations of bacteria attached electrode revealed that *C. ljungdahlii* was able to assimilate extracellular electron. Notably, optimization of negative potential on electrode remarkably increased NAD(P)H/NAD(P)⁺ ratio, ATP amounts, and propionate production. Furthermore, BES optimization results showed that propionate achieved 16.29 ± 0.16 mM maximum titer (43 % ratio of total organic acids) and 0.2 g/g mass yield (0.24 mol/mol carbon atom yield) from fructose. A theoretical value (1.6 mmol) of fixed CO₂ was calculated from mass/electron balance analysis. This work not only evaluated the performance of bioelectrochemical technology but also provided a new choice for sustainable and efficient propionate production.

1. Introduction

Propionic acid (propionate), a C3 carboxylic acid and generally regarded as safe (GRAS) chemical, is a promising chemical building block with applications across a wide variety of industries including foods, plastics, and cosmetics. The current propionate production from petrochemical route is non-renewable and unsustainable and generates many environmental pollutants. Impressively, microbial cell factories based on anaerobic fermentation is a green and sustainable process to produce chemicals [19,28,47]. There are many types of microorganism owing propionate-producing pathways, such as *Propionibacterium* using Wood-Werkman cycle and *Clostridium propionicum* using acrylate pathway to convert the pyruvate (glycolytic metabolite) into propionate [38]. About 15 years ago, it has realized ~100 g/L production of propionate via *P. acidipropionici* after more than 2000 h⁺ fermentation [49]. However, over these years, there are no significant improvements (such

as production and productivity) by using traditional propionate-producing microorganisms [2]. It is still urgent to evaluate new strategies or strains for propionate bioproduction.

Bioelectrochemical systems (BES) have received much attentions because of their versatile abilities to electrochemically drive pollution removal, sensor [12,53], and chemical biosynthesis [32,36]. Of particular interest is to develop cathodic applications such as electro-fermentation and microbial electrosynthesis based on the extracellular electron uptake to regulate redox cofactor level of electroactive bacteria as many biochemical reactions are constrained by availability of electrons [10]. Electroactive bacteria are capable of electron exchange with electrode or other bacteria. Those bacteria with substantial transmembrane c-type cytochromes or conductive proteins can capture electrons from electrode and change carbon flux target chemical overproduction [32,42,57]. Electroactive bacteria could be introduced to accomplish CO₂ reduction and synthesize bulk chemicals

* Corresponding authors.

E-mail addresses: zhenfang@ujs.edu.cn (Z. Fang), yeyong@ujs.edu.cn (Y.-C. Yong).

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such as organic acids, biofuels and biopolymers) in BES [15,44]. One interesting report have tried bioelectrochemically assisted propionate production and simultaneous CO₂ recycling with lactate as substrate and microbial community mixture as main producer [24]. Recently, mixed microbial cultures were explored as cathodic inoculum that converted organic substrates (glucose, ethanol, and glycerol) or even CO₂ into short chain organic acids such as lactic acid, butyric acid, isobutyrate using commercial electrodes [17,46,48]. Though mixed-culture system is versatile to generate various organic acids, it requires long time domestication and strict fermentation process. It inspires that developing pure culture in BES is necessary to produce propionate because it owns more feasible and controllable fermentation process.

Interestingly, the two carbon-fixing bacteria of *C. ljungdahlii* and *C. autoethanogenum* have been explored as electroactive bacteria and exhibited the ability of organic acid biosynthesis via BES [27,33,56]. It has reported that *C. ljungdahlii* could directly import extracellular electrons without the assistance of hydrogen and was ideal in transfer of electrons from an electrode to microbes [4,16]. More importantly, *C. ljungdahlii* was easy to be engineered with heterologous pathways for value-added chemicals production [45]. However, electricity-assistant anaerobic fermentation via *C. ljungdahlii* was rarely evaluated for propionate production in cathode. Furthermore, it is still unclear whether applied potential on electrode affects the performance of metabolic pathway.

In this study, 3D nanostructured porous indium tin oxide with inverse opal structure (IO-ITO) electrode was fabricated for favorable attachment or colonization of microorganisms [7]. When IO-ITO electrode was installed into a BES, a fructose-consuming and butyrate-producing *C. ljungdahlii* [22] could easily form electroactive biofilm on electrode to strengthen the close contact for potential electron uptake process. Interestingly, the performance of fructose consumption and propionate production were significantly enhanced in an optimized process. Its electron transfer mechanism and metabolic pathway shift were further proposed.

2. Materials and methods

2.1. Electrode preparation

IO-ITO electrode was prepared by co-assembly method as described in previous literatures [14,20]. The ITO glass electrodes (Hefei Nuozhuo Biological Technology Co., LTD), ITO nanoparticles (Sigma-Aldrich, average size 50 nm), and polystyrene (PS) microspheres (Aladdin, Shanghai, 0.75 mL, 2.5 wt%) were used. The PS microspheres and ITO nanoparticles were ultrasonically mixed in an ice bath for 30 min and then 15 μ L PS-ITO mixture was dropped onto the ITO glass electrode. The PS-ITO mixture on ITO electrode was limited to 0.25 cm² and dried in air for 30 min. The electrode was annealed in air at 500 °C for another 20 min using Muffler furnace at a heating/cooling rate of 1 °C/min to obtain IO-ITO electrodes. Those electrodes were treated with UV sterilization.

2.2. Bacterial culture

C. ljungdahlii suspension was cultured in fresh YTF medium (Table S1) until the optical density (OD₆₀₀) reached 0.8–1.0. The bacterial sludge was then washed three times using PBS medium (Table S2). Finally, the cells were inoculated into a two-compartment reaction device with fructose and NaHCO₃ as the carbon source in PBS medium, and the final density (OD₆₀₀) of *C. ljungdahlii* was ~0.6. All liquid medium were deoxygenated by nitrogen blowing for 30 min before sterilization. Experiments such as the inoculation of *C. ljungdahlii* and the assembly of the reactor were carried out in an anaerobic workstation.

2.3. Electro-fermentation process

Cathodic electro-fermentation (CEF) was conducted under a three-electrode double chamber reactor. IO-ITO was the work electrode while Hg/Hg₂Cl₂ electrode (SCE, saturated KCl) was used as reference electrode and carbon cloth (1 cm × 2 cm) as counter electrode. Two-stage CEF was conducted. The first stage was for biofilm formation by applying 0 V (vs. SCE) potential without fructose addition for 12 h. The second stage was for electro-fermentation by adding fructose and NaHCO₃ as the substrate under a poised potential of −0.7 V (vs. SCE). The bioelectrochemical experiment was completed by electrochemical workstation CHI1000C (Chenhua, Shanghai). The organic acid sample was taken once every 12 h and added with 10 % of 1 M sulfuric acid to inhibit the reaction for testing. The samples were detected by high performance liquid chromatography (HPLC, Shimadzu chromatograph) with HPLC-87H column (BIO-RAD Aminex®, 300 mm × 7.8 mm). Three parallel groups of experiments ensured experimental repeatability.

2.4. Electrochemical characterization

Electrochemical characterization was completed using an electrochemical workstation CHI660E (Chenhua, Shanghai). Cyclic voltammetry (CV) scanning range was −0.8 V to +0.2 V (vs. SCE), and the scanning rate was 1 mV/s from negative to positive potential. Linear scanning voltammetry (LSV) scanning range was −0.6 V to +0.6 V (vs. SCE), scanning rate was 1 mV/s. Differential pulse voltammetry (DPV) scanning range was −0.8 V to +0.2 V (vs. SCE), pulse width 0.05 V per second.

2.5. Characterization of electrode morphology

The morphological changes of PS microspheres and ITO nanoparticles after calcination and IO-ITO after CEF process with *C. ljungdahlii* were observed by scanning electron microscopy (SEM, Quanta FEG 250, FEI Titan Tecna). SEM was set at accelerating voltage 10 kV. The sample was prepared by gradient dehydration of ethanol from low to high concentration according to previous report [7].

2.6. Detection of intracellular redox state

ATP content detection kit was purchased from Sangon (Shanghai). Coenzyme I NAD(H) and coenzyme II NADP(H) content detection kits were purchased from Sangon (Shanghai) by using colorimetric methods. Determination processes were according to kit instructions and repeated three times to make sure the data reliability.

3. Results and discussion

3.1. Effects of electrochemical conditions on organic acid production

In this study, obvious accumulations of acetate and butyrate were observed during traditional anaerobic fermentation condition by *C. ljungdahlii* (Fig. 1a). Though acetate and butyrate needed the same precursor of acetyl-CoA, acetate titer was slightly higher than butyrate (1.65 mM vs 1.24 mM at 36 h) because of the shorter pathway for acetate biosynthesis [9]. Besides, 0.47 ± 0.18 mM lactate was also observed after 36 h of anaerobic fermentation (no applied potential). Impressively, when negative potential (−0.7 V vs. SCE) was applied, it obviously changed accumulation speed and patterns of organic acids (Fig. S1). For example, formate and propionate formed in negligible amounts during traditional anaerobic fermentation showed the highest titers (5.9 mM and 4.73 mM) at 36 h and 60 h, respectively. The slight accumulation (0.75 mM) of succinate was observed. More importantly, electro-fermentation stimulated more than 7-fold increase of lactate and 1.88-fold increase of butyrate compared to anaerobic fermentation without applied potential. *C. ljungdahlii* could use glycolytic pathway to

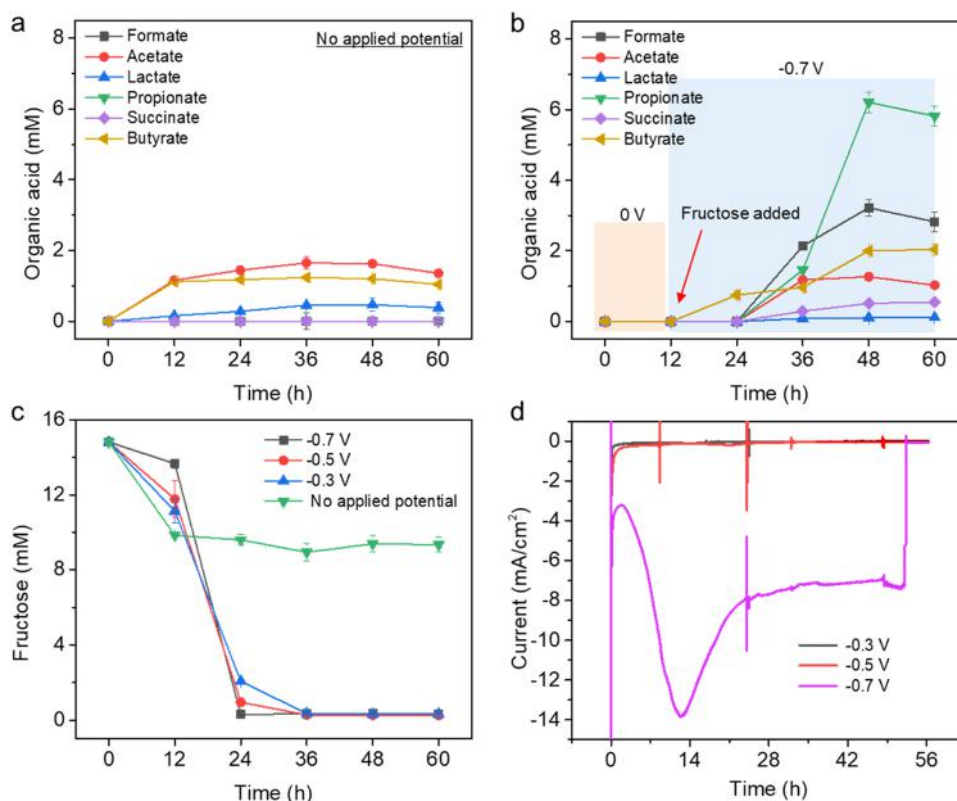


Fig. 1. Organic acid production from fructose via *C. ljungdahliae* with heterogenous butyrate-producing pathway on genome. (a) Anaerobic fermentation (no poised potential), (b) two stages (0 V for 12 h, then -0.7 V was applied and fructose was added) electro-fermentation, (c) fructose consumption under poised potential and no electricity conditions and (d) current time (i-t) curves of cathodic electro-fermentation under various poised potential (-0.7 , -0.5 , -0.3 V vs. SCE). There existed a stage of 0 V for 12 h before monitoring fructose consumption.

metabolize fructose into acetyl-CoA, which is an important precursor to produce butyrate through heterologous butyrate-producing pathway [22]. All the results indicated that organic production by *C. ljungdahliae* was actually benefited from electrochemical conditions.

To investigate whether direct interaction between bacteria and electrode was positive to organic acid production, two-stage cathodic electro-fermentation including potentiostatic pretreatment at 0 V (vs. SCE) without fructose and poised potential at -0.7 V (vs. SCE) with fructose was designed. The first stage was intending to form *C. ljungdahliae* biofilm on electrode and create more efficient electron transfer [7]. Through the above two-stage electro-fermentation (Fig. 1b), propionate quickly achieved the highest titer (6.2 ± 0.3 mM) after 36 h, while formate, acetate and lactate showed insignificant accumulation (especially lactate yielded less than 0.12 mM) compared to the sample of directly poised potential. It deduced that *C. ljungdahliae* biofilm on electrode diverted carbon flux towards propionate by decreasing amount of formate, acetate, and lactate. To prove above hypothesis, increased biofilm on electrode by prolonged potentiostatic time at 0 V (vs. SCE) was conducted (Fig. S2a). Interestingly, remarkable increase of protein concentrations (from 200 to 800 $\mu\text{g}/\text{cm}^2$) on electrode surface and 1 $\mu\text{A}/\text{cm}^2$ current were detected with increasing potentiostatic time and without fructose addition (Fig. S2b). Correspondingly, the group with highest biofilm on electrode achieved the highest concentration of total organic acids (~ 16.5 mM) and ~ 50 % content of propionate after 24 h' electro-fermentation (Fig. S2c). Furthermore, the fructose was completely consumed under electrochemical condition while the open circuit without poised potential only consumed ~ 30 % fructose (Fig. 1c), indicating that direct attachment of *C. ljungdahliae* on electrode was essential to stimulate fructose consumption and organic acid accumulation for cathodic electro-fermentation. Further current monitoring of CEF system showed that -0.7 V (vs.

SCE) poised potential on biocathode achieved quickly increase of reverse current to $-14 \mu\text{A}/\text{cm}^2$ in initial 12 h, which was about two orders of magnitude larger than -0.5 V and -0.3 V groups (Fig. 1d). The obvious reverse current and fructose consumption indicated the possibility of extracellular electron uptake to stimulate organic acid biosynthesis.

3.2. Impact of 3D electrode on extracellular electron uptake

In previous reports, it has been proved that 3D electrode was powerful to augment electroactive biofilm [6,7]. Here, IO-ITO electrode was fabricated by modifying conductive ITO nanoparticles on smooth ITO electrode surface and significantly decreased the internal resistance (Fig. S3). The modified region on ITO electrode showed bright yellow and it changed into yellowish-brown color after 24 h potentiostatic pretreatment (0 V vs. SCE) in *C. ljungdahliae* inoculated cathode (Fig. 2a). Under the SEM observation, electrode surface was covered with 3D porous structure of ITO nanoparticles (Fig. 2b) and the aperture length of honeycomb-like microscale cavities was about 8–10 μm (Fig. 2c). The special three-dimensional structure was powerful to gather more cells on electrode surface, especially those cavities (Fig. 2d), which probably made the cells ($0.5 \times 3 \mu\text{m}$ size) more robust to resist washing off.

To further prove that microbial electron transfer between exoelectrode and electrode surface, bioelectrochemical characterizations of cathode were further investigated (Fig. 3). Under negative hydrogen evolution potential (-0.7 V vs. SCE), the IO-ITO electrode produced negligible hydrogen ($0.17 \mu\text{mol}$ after 36 h) and it seemed that direct electron transfer was necessary to support CEF. To avoid the effect of self-generated mediator (such as riboflavin) on extracellular electron uptake, the IO-ITO and *C. ljungdahliae* biocathodes were washed with PBS buffer after 12–24 h potentiostatic treatment (0 V vs. SCE) and then

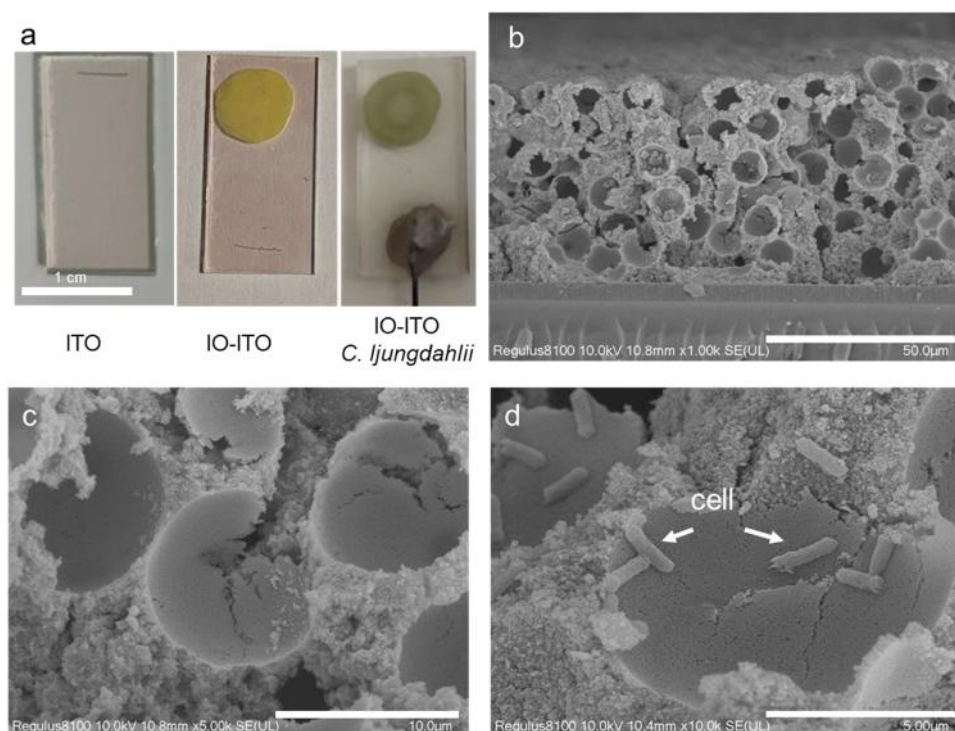


Fig. 2. Morphological features of three-dimensional electrode. (a) Photos of blank ITO, blank IO-ITO, and IO-ITO biocathode (*C. ljungdahliae* bacteria on electrode) electrodes. Scanning electron microscope images of blank IO-ITO electrode (b & c) and IO-ITO biocathode (d). Arrows indicate *C. ljungdahliae* cells attaching in porous structure on IO-ITO electrode.

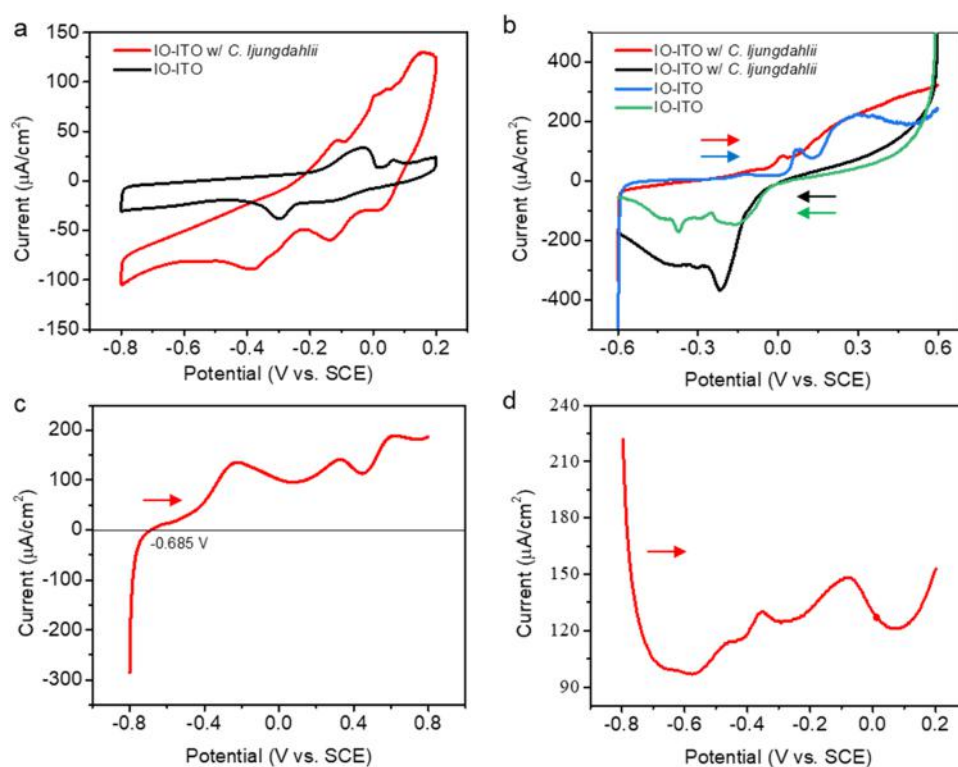


Fig. 3. Bioelectrochemical analysis of biocathode. (a) Cyclic voltammetry analysis and (b) linear sweep voltammetry analysis of blank and *C. ljungdahliae* attached IO-ITO electrodes before electro-fermentation. The control is blank IO-ITO electrode. (c) Linear sweep voltammetry analysis and (d) differential pulse voltammetry of biocathode (*C. ljungdahliae* bacteria on IO-ITO electrode) after electro-fermentation. Arrows indicate the electrochemical scanning direction.

were assayed for bioelectrochemical activities in fructose-free media. Impressively, CV analysis of biocathode showed obvious reverse current (up to $-100 \mu\text{A}/\text{cm}^2$) between -0.8 V and 0 V (vs. SCE) (Fig. 3a). Compared to the blank IO-ITO, there appeared three reductive peaks at -0.8 V , -0.38 V , and -0.12 V , which were probably related to some [4Fe-4S] cluster contained proteins such as membrane-bound hydrogenase, Rnf complex and flavoprotein [1,29,33,52]. On the contrary, planktonic cells, directly inoculated in cathode without biofilm formation by potentiostatic treatment, showed sole redox peak (around -0.12 V) and lower reverse current ($-20 \mu\text{A}/\text{cm}^2$) (Fig. S4). It indicated that direct contact between *C. ljungdahliae* cells and electrode surface was essential to augment extracellular electrons uptake under negative potential.

Next, linear sweep voltammetry analysis was conducted and it was found that *C. ljungdahliae* cells on IO-ITO electrode surface were able to exchange electrons around -0.3 V (vs. SCE) when scanning from negative potential to positive potential (Fig. 3b). Compared to the control group (blank IO-ITO electrode), biocathode exhibited higher

reverse current when potential was shifted from 0 V to -0.6 V (vs. SCE). All the above CV and LSV analysis revealed that IO-ITO and *C. ljungdahliae* biocathode owned significant bioelectrochemical activities to achieve bidirectional electron transfer. More importantly, LSV showed -0.685 V (vs. SCE) was suitable for the extracellular electron uptake of *C. ljungdahliae* in CEF process (Fig. 3c). Cells on electrode exhibited slight changes of reverse electron transfer after CEF application without extra inoculation of *C. ljungdahliae* in cathode. More precise analysis of redox peaks by DPV also revealed that applied potential below -0.685 V could dramatically augment electron exchange (more than $210 \mu\text{A}/\text{cm}^2$) between IO-ITO electrode and *C. ljungdahliae* (Fig. 3d). The redox potential -0.685 V (vs. SCE) was probably related to membrane-bound hydrogenase and formate dehydrogenase complex that could directly utilize electrons for CO_2 -to-formate conversion [1,23]. Other redox peaks (such as -0.45 V , -0.38 V , and -0.1 V vs. SCE) with smaller current density (below $150 \mu\text{A}/\text{cm}^2$) were probably related to membrane-bound proteins such as flavoprotein and NADH dehydrogenase, which were found in another electroactive acetogen of *Moorella thermoacetica* [52].

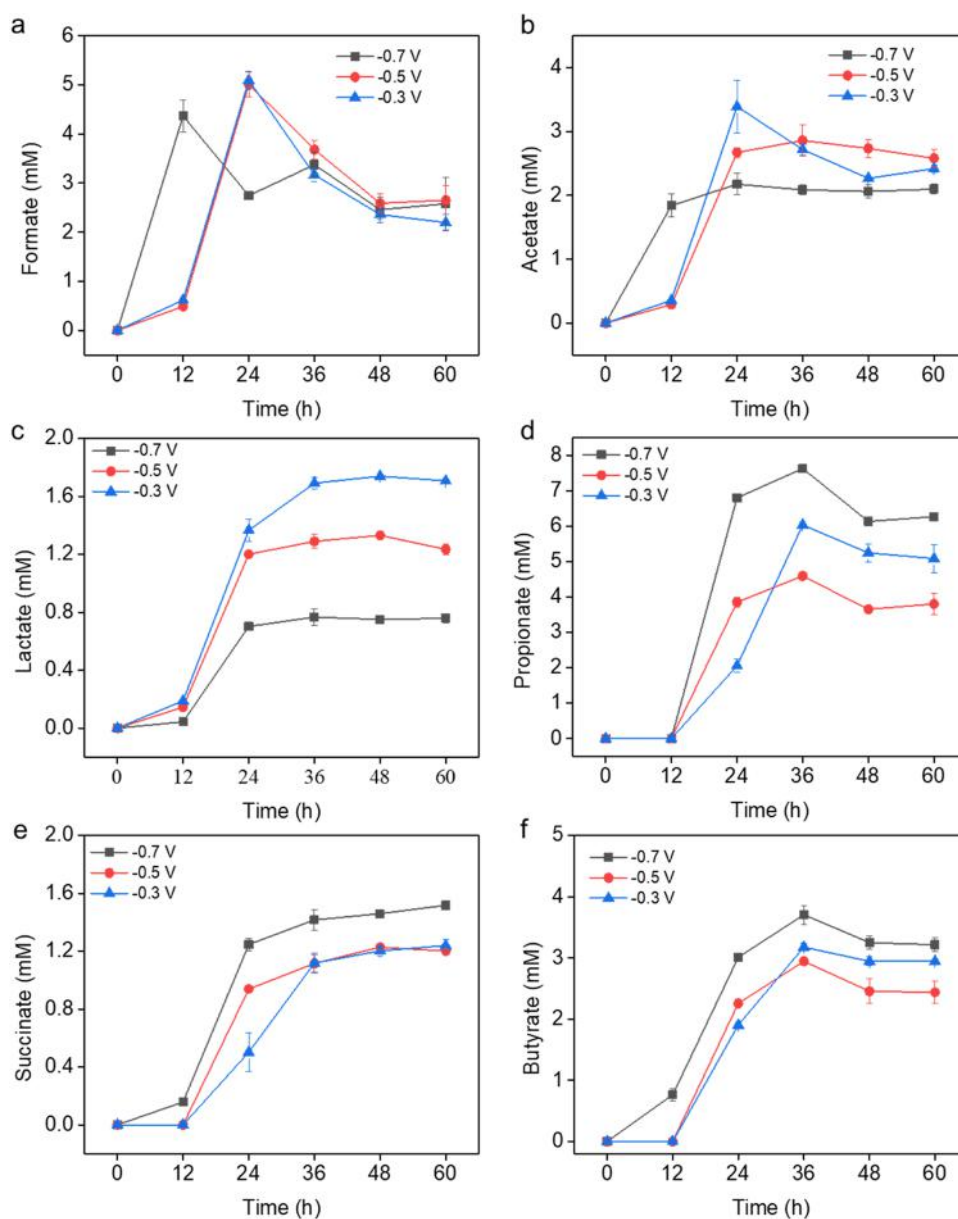


Fig. 4. Organic acids fermentation via *C. ljungdahliae* (with heterogenous butyrate-producing pathway on genome) in cathode chamber under various potential (vs. SCE) on Io-ITO electrode. (a) Formate, (b) acetate, (c) lactate, (d) propionate, (e) succinate, (f) butyrate.

Meanwhile, *C. autoethanogenum*, an acetogen owning 90 % similarity of proteome to *C. ljungdahlii*, have been proved the capability of extracellular electron uptake from electrode in CEF process [27,33]. Though the electron transfer pathway needed more evidences, the above results were enough to explain that potential direct electron transfer could occur in *C. ljungdahlii* but needed more evidences from transcriptome and proteome analysis.

Electrode with the essential role of controlling bacteria's physiological and electrochemical functionalities was possible to adjust microbial electron transfer. Carbon electrodes (such as carbon paper, and carbon cloth) have been frequently applied because of their plug-and-play advantages and electrochemical stability [35]. However, most of carbon electrodes require exogenous mediators (such as methyl viologen, neutral red, and anthraquinone-2,6-disulfonate) to augment extracellular electron uptake [18,21,37,55]. Those exogenous electron mediators are toxic to microorganisms and resulted in complicated product separation or wastewater treatment. Therefore, the biocompatible 3D electrode used in this study could avoid the toxic mediators, improve electron transfer rate, and provide positive effect on adjusting organic acids production.

3.3. Effects of poised potential on metabolites in cathode

Fructose consumption under cathodic fermentation (Fig. 1c) has showed that poised potential could slow down substrate utilization rate in initial 12 h. The increase of reverse current from more negative potential (Fig. 1d) also inferred that more poised potential (−0.7 V vs. SCE) was more suitable for reverse electron transfer (possible for both hydrogen-mediated electron transfer and DET) and probably changed metabolic pathway of *C. ljungdahlii*. Here, effects of various negative potential on organic acids production (including formate, acetate, lactate, propionate, succinate, and butyrate) were investigated along with the CEF process (Fig. 4). The poised potential of −0.7 V (vs. SCE) showed the fast accumulation of formate (4.37 ± 0.33 mM) while −0.5 V and −0.3 V groups only produced ~0.5 mM formate in initial 12 h (Fig. 4a). Similarly, −0.7 V group stimulated the fast accumulation of acetate, about 6 folds of titers compared to −0.5 V and −0.3 V groups in initial 12 h (Fig. 4b). The above data indicated that lower potential probably stimulated the electron-consuming pathway, such as Wood-Ljungdah pathway (an inherent carbon-fixing pathway in *C. ljungdahlii*) and made use of reverse current to support CO₂ reduction into organic acids [11,40,45]. Besides, significant reduction in formate levels with the fermentation time also indicated the possible assimilation of formate into other metabolites via Wood-Ljungdah pathway [15]. Among those organic acids, only lactate production was inhibited by −0.7 V potential and showed about twice less production compared to −0.3 V (Fig. 4c). The decrease of lactate was probably related to the altered metabolic pathway that pyruvate directly diverted into other electron-consuming organic acids, i.e., propionate and butyrate [11].

The propionate is a special organic acid rarely produced in regular anaerobic fermentation by *C. ljungdahlii*. Interestingly, the highest titer (7.63 mM) of propionate was obtained as compared to other organic acids (Fig. 4d). Succinate, another important organic acid from tricarboxylic acid (TCA) cycle, was also detected in cathode solution with the increase of negative potential (Fig. 4e). It has been reported that *C. ljungdahlii* could use succinate as substrate to synthesize propionate [13]. Butyrate was the second largest organic acids produced in CEF and its highest titer was 3.7 ± 0.15 mM at 36 h under −0.7 V, about 640 % improvement compared to the groups without poised potential (Fig. 4f). In total, the poised potential stimulated the production of some organic acids such as propionate and succinate, and it further improved the final titers of butyrate by the *C. ljungdahlii*. The above results indicated that values of negative potential applied on biocathode had significant impact on organic acid production. For example, lower potential at −0.7 V benefitted the reverse electron transfer via membrane-bound hydrogenases and probably facilitated intracellular cofactors regeneration as

well as the propionate/butyrate pathway [8,30].

To prove the positive effects of reverse current on intracellular metabolites, the cofactors including reducing equivalents (NADH/NAD⁺ and NADPH/NADP⁺) and bioavailable energy (ATP) were studied under various poised potential on biocathode (Fig. 5). Compared to the open circuit where no potential was applied on cathode, the NADH/NAD⁺ ratio values at −0.3 V and −0.7 V groups were about 2-fold higher (Fig. 5a). It was also found that NADPH/NADP⁺ ratios of negative poised groups were obviously improved (Fig. 5b), and it probably attributed to the special reverse electron transfer to maintain high level of NADH or NADPH. These reducing equivalents were all necessary to facilitate propionate and butyrate accumulation [22,25]. When using pyruvate as the initial substrate, *C. ljungdahlii* needed two and four NAD(P)H moles to synthesize one mole of propionate and butyrate, respectively. It indicated the possibility that *C. ljungdahlii* harnessed propionate and butyrate biosynthetic pathways to balance intracellular NAD(P)H/NAD(P)⁺ level because excess reducing equivalents were generated at potential of −0.7 V. In this study, propionate achieved the highest production was probably due to the increased ratio of NAD(P)H/NAD(P)⁺ at −0.7 V poised potential. In addition, the ATP, another important intracellular cofactor to determine cell activity, was monitored under CEF process and showed that lower poised potential on

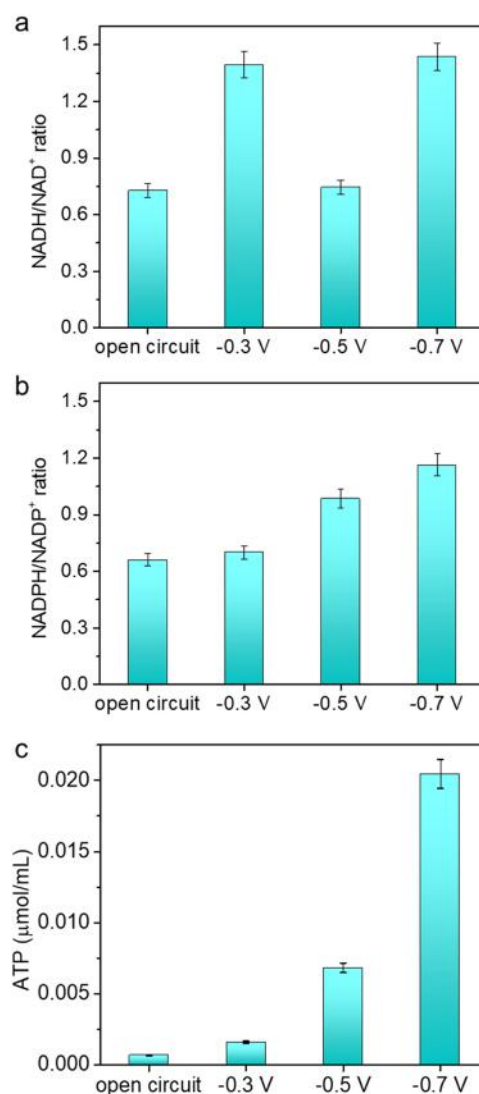


Fig. 5. Effects of open circuit (no electricity supply) and cathodic poised potential on intracellular cofactor levels of *C. ljungdahlii* under fructose-free bioelectrochemical conditions. (a) NADH/NAD⁺, (b) NADPH/NADP⁺, and (c) ATP.

electrode increased ATP levels (Fig. 5c). Notably, the negative potential of -0.7 V showed the highest ATP content, approaching 29 folds of open circuit group. The above results explained that lower potential on cathode was essential to improve intracellular NAD(P)H and ATP regeneration and propionate overproduction.

Therefore, a special metabolic pathway of *C. ljungdahliae* in CEF process could be proposed (Fig. 6). Formate produced in traditional anaerobic fermentation is usually obtained from pyruvate splitting [11]. However, a fast accumulation of formate was observed under lower poised potential. It is inferred that besides fructose, formate can be obtained from other carbon sources. For example, CO_2 -to-formate pathway via lower potential stimulated Hyt-Fdh complex, where formate dehydrogenase can accept electrons from membrane-bound hydrogenase to drive CO_2 reduction [5]. In previous reports, excessive accumulation of succinate was observed by applying lower potential -440 mV (vs. SHE) to *Citrobacter amalonaticus* [3]. Similarly, *C. ljungdahliae* might harness pyruvate carboxylase and consume ATP and CO_2 to produce oxaloacetate [1]. Due to the incomplete TCA cycle in *C. ljungdahliae* [11], oxaloacetate could be firstly changed into aspartate and then into fumarate, which finally reduced into succinate at high NAD(P)H/NAD(P) $^+$ levels (Fig. 6). There were possible three pathways (succinate pathway, acrylate pathway, and propanediol pathway) for propionate biosynthesis [39]. Acrylate pathway probably was main pathway for propionate formation, in where lactate (consuming one NADH to reduce pyruvate) was used as the substrate to form lactoyl-CoA, acryloyl-CoA, and propionyl-CoA (Fig. 6). Besides, NADH was necessary to reduce acryloyl-CoA into propionyl-CoA that finally contributed propionate accumulation [26]. Therefore, NAD(P)H regeneration played key role for shifting of carbon metabolism towards propionate biosynthesis.

Besides, it deduced that hydrogenases and Rnf complex, a special membrane-bound respiratory enzyme that catalyzes the oxidation of reduced ferredoxin for the reduction of NAD^+ , could directly contact electrode and facilitate extracellular electron uptake [43,51]. Intriguingly, a cytoplasmic Nfn complex called electron-bifurcating and ferredoxin-dependent transhydrogenase was able to adjust NAD(P)H/NAD(P) $^+$ ratio, which was necessary to control intracellular cofactor balance [31]. However, it was still unclear whether the pools of intracellular cofactors (including NAD(P)H and ATP) were increased. To get more information about the gene expression, it might need a comparative transcriptome analysis in future.

In total, the main reason resulting in metabolic shift of *C. ljungdahliae*

was probably related to extracellular electron uptake. The first evidences of SEM (Fig. 2d) and increased protein concentration (Fig. S2a) on electrode surface showed that *C. ljungdahliae* cells could attach on IO-ITO electrode. Generally, bacteria attaching on electrode surface is the first step of extracellular electron uptake. Besides, cyclic voltammetry analysis (Fig. 3a) was the second evidence to show *C. ljungdahliae* attaching on electrode were able to realize reverse electron transfer. It was corresponding to Fig. 1d that higher reverse current was observed at -0.7 V poised potential. The third evidence was the improved propionate (Figs. 1b and 4d) needed higher reverse current based on -0.7 V potential applied on *C. ljungdahliae* attached electrode. The fourth evidences of metabolic pathway analysis (Fig. 6) and mass/electron balance analysis (Table 1 and S4) all indicated that extracellular electron assimilation was essential to improve propionate production. Finally, extracellular electron uptake augmented propionate accumulation. Nevertheless, key factors optimization was necessary to obtain the target products from CEF fermentation.

3.4. Further improving propionate production via optimization

Though *C. ljungdahliae* could utilize extracellular electrons to regenerate cofactors, the production of propionate was still limited to its optimized fermentation conditions by using fructose as substrate in CEF process (Fig. 1 and Fig. 4). It is inferred that some key factors such as inoculation amount, initial pH, cathodic buffer, and fructose concentration should be optimized in order to achieve high yield of organic acids [33,34,54]. Firstly, various inoculation amounts based on OD_{600} were used to investigate cell density effects on organic acid production (Fig. 7a). It was found that high cell density (initial 1.2 OD_{600}) decreased total titers of organic acids and especially more than 33.3 % decrease in propionate titer was observed. High cell density also improved the speed of formate and acetate production (1.5–7-fold increase of productivity in first 12 h, Fig. S5), which might attribute to the augmented CO_2 reduction via Wood-Ljungdahl pathway. On the contrary, lower cell density (initial 0.6 OD_{600}) resulted in slow increase of organic acids except for propionate. Consequently, CEF process after cell density optimization achieved the highest ratio (about 74 %) of lactate, propionate, succinate, and butyrate. The experiments showed that adding more cells in cathode solution obviously improved the amounts of planktonic *C. ljungdahliae* cells in solutions but not the biofilm on electrode surface (Fig. S5). We deduced that IO-ITO electrode surface was limited for attaching too much cells [7], and that planktonic cells were

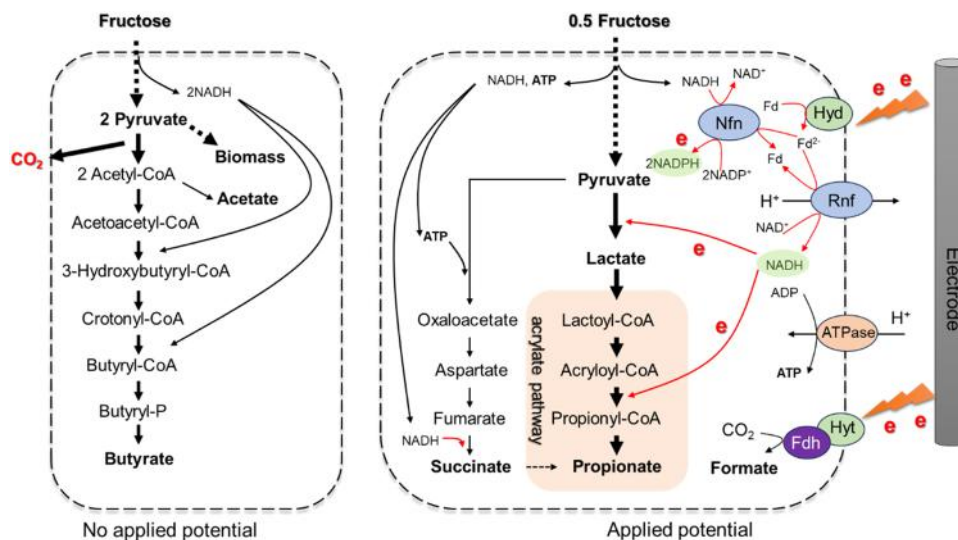


Fig. 6. Proposed metabolic pathway for organic acid production by *C. ljungdahliae*. Nfn, electron-bifurcating and ferredoxin-dependent transhydrogenase; Fd^{2+} , reduced ferredoxin; Fd, oxidized ferredoxin; Rnf, membrane-associated and energy-conserving reduced ferredoxin: NAD^+ oxidoreductase; Hyt and Hyd, hydrogenase; Fdh, formate dehydrogenase. The width of arrows indicates the metabolic flow size.

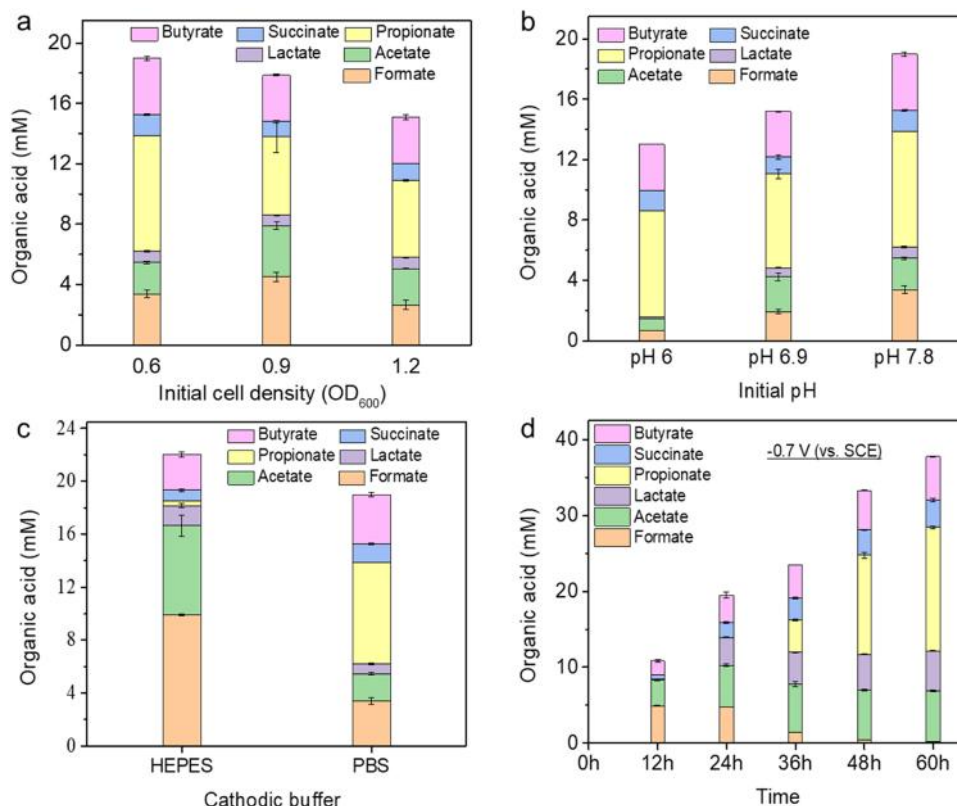


Fig. 7. Effects of various conditions on total organic acid accumulation after 60 h of electro-fermentation (poised potential on cathode is -0.7 V vs. SCE). (a) Initial cell density in cathodic chamber, (b) initial pH of cathodic media, (c) cathodic buffer, and (d) electro-fermentation time with 6 g/L fructose.

failed to efficiently utilize extracellular electron, which probably resulted in pseudo-electro-fermentation and decrease of final organic acid titers. Secondly, effects of initial pH value on CEF were studied (Fig. 7b). It was learnt that weakly alkaline conditions were beneficial for organic acid accumulation and pH 7.8 yielded ~ 19 mM titer of total organic acids, with 46 % improvement as compared to pH 6. More enhancement in the production of propionate and butyrate (approaching 85 % ratio of total organic acids) was noticed with weakly acidic condition (Fig. S6). These results suggested that suitable control of the pH value could dramatically alter metabolic flux of *C. ljungdahliae* in CEF process. Thirdly, the above hypothesis was further proved by comparing organic acid productions with two different pH-stabilizing buffers in cathode (Fig. 7c). HEPES, an excellent pH-stabilizing agent in microbial medium [7], was used to substitute phosphate buffer. The results of HEPES cathodic buffer exhibited higher titers of organic acid accumulation than PBS buffer (Fig. 7c). Nevertheless, large fraction of organic acids (about 77 %) in cathodic solution were formate and acetate, and formate was the major compound (13.59 ± 0.56 mM) after 12 h of electro-fermentation (Fig. S7). It indicated that stable pH buffer in cathode might be suitable for strong organic acids such as formate and acetate [23,33,34]. Therefore, lower cell density (0.6 OD₆₀₀) was used to inoculated *C. ljungdahliae* in weakly alkaline solution (pH 7.8) PBS cathodic media.

Subsequently, substrate concentration optimization was conducted and it was interesting to find that various concentrations of fructose in cathode had different effects on CEF. When low concentration (1.5 g/L, about 15 mM) of fructose was added in cathode, formate and acetate were the main content (around 50 %–65 % ratio of total organic acids) and the highest titer of formate and acetate was 5.74 mM and 3.9 mM, respectively, which was higher in comparison with a fructose concentration of 3 g/L or 6 g/L (Fig. S8 and Fig. 7d). The extra production of formate or acetate could be stem out from CO₂ reduction via Wood-Ljungdah pathway (Fig. 6). Therefore, high concentration of formate

and acetate inhibited the glycolytic pathway and resulted in low regeneration rate of NADH and ATP in *C. ljungdahliae* [11]. Intriguingly, propionate was major product (43 % ratio of total organic acids), achieving the highest value (16.29 ± 0.16 mM), highest productivity (0.27 mM/h), and 0.2 g/g mass yield (0.24 mol/mol carbon atom yield) when supplying 6 g/L fructose into cathodic medium (Fig. 7d and Table 1). The inherent acrylate pathway was probably activated under bioelectrochemical condition and increased propionate production (Fig. 6). Other than acrylate pathway, it was deduced that a few fraction of propionate was synthesized from succinate based on the incomplete TCA cycle in *C. ljungdahliae* [1,11], where oxaloacetate was reduced into succinate and then converted into propionate [39]. Ultimately, 37.5 mM total organic acids were obtained after 60 h electro-fermentation, which exhibited more efficient organic production (0.63 mM/h against 0.04 mM/h of traditional anaerobic fermentation, ~ 16 folds improvement). It was found that high production of organic acids with 6 g/L fructose addition resulted in significant decrease of pH value in cathodic solution (Fig. S9). More importantly, the calculation of mass and electron balances showed carbon atom yield of total organic acids was increased from 0.21 to 0.58 mol/mol by applying -0.7 V (vs. SCE) in cathode (Table S4). When calculating the total carbon atoms of biomass and products together, we found that CO₂ was fixed by with a theoretical value of 1.6 mmol. The carbon fixation ability was attributed to Wood-Ljungdah pathway and 30.27 % contribution of extracellular electricity from electron coefficient calculation (seen in supporting information), which final converted CO₂ into acetyl-CoA, an important precursor for acetate and butyrate biosynthesis. The above results indicated that direct contact between cells and electrode could remarkably improve organic acid accumulation as compared to other diffusing mediators (such as methyl viologen and neutral red) in CEF [18,41,55].

The conventional propionic acid production is mainly dependent on anaerobic fermentation by using glucose and glycerol as carbon source

Table 1

Mass balance of organic acid production from fructose under no electricity and negative potential applied conditions.

Substrate/ Products	No electricity					-0.7 V vs. SCE				
	c (mmol)	Mass yield (g/ g)	c (C atom)	Carbon yield (mol/ mol)	e- (mmol)	c (mmol)	Mass yield (g/ g)	c (C atom)	Carbon yield (mol/ mol)	e- (mmol)
Fructose	12	-	72	-	288	33.33	-	200	-	800
Formate	0	0	0	0	0	0.23	-	0.23	0.001	0.46
Acetate	9.9	0.275	19.8	0.275	79.2	6.64	0.07	13.28	0.07	53.12
Lactate	2.76	0.115	8.28	0.115	33.12	5.28	0.08	15.84	0.08	63.36
Propionate	0	0	0	0	0	16.29	0.2	48.87	0.24	244.35
Succinate	0	0	0	0	0	3.59	0.07	14.36	0.07	50.26
Butyrate	3.5	0.143	14	0.194	70	5.73	0.08	22.92	0.11	114.6

[38,49,50]. *Propionibacterium acidipropionici* is the major industrial strain and can reach 106 g/L production and 0.54–0.71 g/g yield of propionate. However, there are several challenges to apply *Propionibacterium* strains at industrial scale [2]. For examples, the conventional fermentation usually consumes more than 2000 h, and it lacks new strains or strategies for propionate bioproduction. Recently, using CO₂ as one of the renewable substrates is attractive. Though the final titer (1.2 g/L) and mass yield (0.2 g/g) of propionate in this study are far lower than industrial strains, it is still meaningful to evaluate electricity-assisted propionate bioproduction via the carbon-fixing *C. ljungdahliae* since it not only shows new propionate-producing bioprocess but also creates new fermentation strategy to adjust metabolic pathway. We believe this study will new ideas for solving the problems of traditional propionic acid fermentation production industry.

4. Conclusion

This study successfully constructed a 3D nanostructure electrode equipped and exogenous-mediator-free BES. In this BES, *C. ljungdahliae* as the electroactive bacteria used electrons to stimulate propionate overproduction through extracellular electron uptake and intracellular cofactor regeneration. Furthermore, it found that propionate as a rarely accumulated products in conventional anaerobic fermentation was overproduced by *C. ljungdahliae*. We concluded that electro-driven anaerobic fermentation could contribute to sustainable chemical engineering by applying renewable electricity to drive intracellular cofactor regeneration eventually favoring the accumulation of propionate. In future, more studies about transcriptome and metabolome should be conducted to investigate the inherent pathway of propionate production under CEF condition, and it is also important to explain how extracellular electron uptake affects intracellular cofactor balance. In total, this study showed powerful performance of propionate production in BES cathode.

CRedit authorship contribution statement

Zhen Fang: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Qichao Fan:** Writing – original draft, Methodology, Data curation. **Zhi-Jie Yang:** Investigation. **Yu Huang:** Project administration, Methodology, Investigation, Data curation. **Beisong Xu:** Resources, Data curation. **Yang-Chun Yong:** Writing – review & editing, Supervision, Funding acquisition. **Yang Gu:** Resources, Methodology. **Vinod Kumar:** Writing – review & editing, Data curation.

Declaration of Competing Interest

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.procbio.2025.10.003.

Data availability

Data will be made available on request.

References

- [1] M. Akujkar, C. Leang, P.M. Shrestha, M. Shrestha, D.R. Lovley, Transcriptomic profiles of *Clostridium ljungdahliae* during lithotrophic growth with syngas or H₂ and CO₂ compared to organotrophic growth with fructose, *Sci. Rep. Uk* 7 (2017) 13135.
- [2] E.M. Ammar, G.P. Philippidis, Fermentative production of propionic acid: prospects and limitations of microorganisms and substrates, *Appl. Microbiol. Biotechnol.* 105 (2021) 6199–6213.
- [3] K. Amulya, S. Venkata Mohan, Augmenting succinic acid production by bioelectrochemical synthesis: influence of applied potential and CO₂ availability, *Chem. Eng. J.* 411 (2021) 128377.
- [4] S.T. Boto, B. Bardl, F. Harnisch, M.A. Rosenbaum, Microbial electrosynthesis with *Clostridium ljungdahliae* benefits from hydrogen electron mediation and permits a greater variety of products, *Green. Chem.* 25 (2023) 4375–4386.
- [5] H. Chen, Y. Huang, C. Sha, J.M. Moradian, Y.C. Yong, Z. Fang, Enzymatic carbon dioxide to formate: mechanisms, challenges and opportunities, *Renew. Sust. Energ. Rev.* 178 (2023) 113271.
- [6] H. Chen, J.W. Li, Q.C. Fan, T. Zheng, Y.F. Zhang, Y.C. Yong, Z. Fang, A feasible strategy for microbial electrocatalytic CO₂ reduction via whole-cell-packed and exogenous-mediator-free rGO/*Shewanella* biohydrogel, *Chem. Eng. J.* 460 (2023) 141863.
- [7] H. Chen, Y.Q. Wei, M.Y. Xu, M.W. Zhu, J.Y. Liu, Y.C. Yong, Z. Fang, Artificial and biosynthetic nanoparticles boost bioelectrochemical reactions via efficient bidirectional electron transfer of *Shewanella loihica*, *Small* 202400962 (2024).
- [8] O. Choi, T. Kim, H.M. Woo, Y. Um, Electricity-driven metabolic shift through direct electron uptake by electroactive heterotroph *Clostridium pasteurianum*, *Sci. Rep. Uk* 4 (2014) 6961.
- [9] O. Choi, Y. Um, B.I. Sang, Butyrate production enhancement by *Clostridium tyrobutyricum* using electron mediators and a cathodic electron donor, *Biotechnol. Bioeng.* 109 (2012) 2494–2502.
- [10] J.M. Clomburg, A.M. Crumbley, R. Gonzalez, Industrial biomanufacturing: the future of chemical production, *Science* 355 (2017) aag0804.
- [11] M.L. Dahle, E.T. Papoutsakis, M.R. Antoniewicz, 13C-metabolic flux analysis of *Clostridium ljungdahliae* illuminates its core metabolism under mixotrophic culture conditions, *Metab. Eng.* 72 (2022) 161–170.
- [12] C. Dai, X. Huang, D. Huang, R. Lv, J. Sun, Z. Zhang, M. Ma, J.H. Aheto, Detection of submerged fermentation of *Tremella aurantialba* using data fusion of electronic nose and tongue, *J. Food Process Eng.* 42 (2019) e13002.
- [13] P. Dürre, Physiology and sporulation in *Clostridium*, *Microbiol. Spectr.* 2 (2014), <https://doi.org/10.1128/microbiolspec.tbs-0010-2012>.
- [14] X. Fang, S. Kalathil, G. Divitini, Q. Wang, E. Reisner, A three-dimensional hybrid electrode with electroactive microbes for efficient electrogenesis and chemical synthesis, *Proc. Natl. Acad. Sci.* 117 (2020) 5074–5080.
- [15] Z. Fang, H. Chen, Y.Q. Wei, Q.C. Fan, M.W. Zhu, Y.F. Zhang, J.Y. Liu, Y.C. Yong, Bioelectricity and CO₂-to-butyrate production using photobioelectrochemical cells with bio-hydrogel, *Bioresour. Technol.* 398 (2024) 130530.

- [16] Z. Fang, Y.J.J. Tang, M.A.G. Koffas, Harnessing electrical-to-biochemical conversion for microbial synthesis, *Curr. Opin. Biotechnol.* 75 (2022) 102687.
- [17] J. Gavilanes, C.N. Reddy, B. Min, Microbial electrosynthesis of bioalcohols through reduction of high concentrations of volatile fatty acids, *Energy Fuels* 33 (2019) 4264–4271.
- [18] A. Gemünde, E. Rossini, O. Lenz, S. Frielingsdorf, D. Holtmann, Chemorganotrophic electrofermentation by *Cupriavidus necator* using redox mediators, *Bioelectrochemistry* 158 (2024) 108694.
- [19] Q. Guo, H. Zayed, H. Zhang, X. Wang, J. Yun, G. Zhang, M. Yang, W. Sun, X. Qi, Optimization of fermentation medium for a newly isolated yeast strain (*Zygosaccharomyces rouxii* JM-C46) and evaluation of factors affecting biosynthesis of D-arabitol, *LWT* 99 (2019) 319–327.
- [20] N. Heidary, N. Kornienko, S. Kalathil, X. Fang, K.H. Ly, H.F. Greer, E. Reisner, Disparity of cytochrome utilization in anodic and cathodic extracellular electron transfer pathways of *Geobacter sulfurreducens* biofilms, *J. Am. Chem. Soc.* 142 (2020) 5194–5203.
- [21] C.C. Huang, Y.R. Chen, S. Cheng, M.X. Li, L.X. Wang, M.J. Cheng, F. Li, Y.X. Cao, H. Song, Enhanced acetate utilization for value-added chemicals production in *Yarrowia lipolytica* by integration of metabolic engineering and microbial electrosynthesis, *Biotechnol. Bioeng.* 120 (2023) 3013–3024.
- [22] H. Huang, C. Chai, S. Yang, W. Jiang, Y. Gu, Phage serine integrase-mediated genome engineering for efficient expression of chemical biosynthetic pathway in gas-fermenting *Clostridium ljungdahlii*, *Metab. Eng.* 52 (2019) 293–302.
- [23] C. Im, K. Valgepea, O. Modin, Y. Nygård, *Clostridium ljungdahlii* as a biocatalyst in microbial electrosynthesis – effect of culture conditions on product formation, *Bioresour. Technol. Rep.* 19 (2022) 101156.
- [24] M. Isipato, P. Dessi, C. Sánchez, S. Mills, U.Z. Ijaz, F. Asunis, D. Spiga, G. De Gioannis, M. Mascia, G. Collins, A. Muntori, P.N.L. Lens, Propionate production by Bioelectrochemically-Assisted lactate fermentation and simultaneous CO₂ recycling, *Front Microbiol.* 11 (2020) 599438.
- [25] M. Isipato, P. Dessi, C. Sánchez, S. Mills, U.Z. Ijaz, F. Asunis, D. Spiga, G. De Gioannis, M. Mascia, G. Collins, A. Muntori, P.N.L. Lens, Propionate production by Bioelectrochemically-Assisted lactate fermentation and simultaneous CO₂ recycling, *Front Microbiol.* 11 (2020).
- [26] V. Kandasamy, H. Vaidyanathan, I. Djurdjevic, E. Jayamani, K.B. Ramachandran, W. Buckel, G. Jayaraman, S. Ramalingam, Engineering *Escherichia coli* with acrylate pathway genes for propionic acid synthesis and its impact on mixed-acid fermentation, *Appl. Microbiol. Biotechnol.* 97 (2013) 1191–1200.
- [27] F. Kracke, B. Virdis, P.V. Bernhardt, K. Rabaey, J.O. Krömer, Redox dependent metabolic shift in *Clostridium autoethanogenum* by extracellular electron supply, *Biotechnol. Biofuels* 9 (2016) 249.
- [28] E. Kwaw, Y. Ma, W. Tchabo, M.T. Apaliya, L. Xiao, X. Li, M. Hu, Effect of fermentation parameters and their optimization on the phytochemical properties of lactic-acid-fermented mulberry juice, *J. Food Meas. Charact.* 11 (2017) 1462–1473.
- [29] W.J. Li, Y.X. Gao, X. Sun, L. Wan, H.S. Ji, H. Luo, Y. Tian, H. Song, G. Wu, L. Y. Zhang, Direct detection of a single [4Fe-4S] cluster in a tungsten-containing enzyme: electrochemical conversion of CO₂ into formate by formate dehydrogenase, *Carbon Energy* 5 (2023) e304.
- [30] C. Liu, X. Yuan, Y. Gu, H. Chen, D. Sun, P. Li, M. Li, Y. Dang, J.A. Smith, D. E. Holmes, Enhancement of bioelectrochemical CO₂ reduction with a carbon brush electrode via direct electron transfer, *ACS Sustain. Chem. Eng.* 8 (2020) 11368–11375.
- [31] Z.-Y. Liu, D.-C. Jia, K.-D. Zhang, H.-F. Zhu, Q. Zhang, W.-H. Jiang, Y. Gu, F.-L. Li, Ethanol metabolism dynamics in *Clostridium ljungdahlii* grown on carbon monoxide, *Appl. Environ. Micro* 86 (2020) e00730-20.
- [32] B.E. Logan, K. Rabaey, Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies, *Science* 337 (2012) 686–690.
- [33] J. Martínez-Ruano, A. Suazo, F. Véliz, F. Otálora, R. Conejeros, E. González, G. Aroca, Electro-fermentation with *Clostridium autoethanogenum*: effect of pH and neutral red addition, *Environ. Technol. Innov.* 31 (2023) 103183.
- [34] J.A. Martínez-Ruano, A. Suazo, F. Véliz, F. Otálora, R. Conejeros, E. González, G. Aroca, Effect of pH on metabolic pathway shift in fermentation and electro-fermentation of xylose by *Clostridium autoethanogenum*, *J. Environ. Manag.* 351 (2024) 119918.
- [35] A.A. Mier, H. Olvera-Vargas, M. Mejía-López, A. Longoria, L. Vereja, P.J. Sebastian, D.M. Arias, A review of recent advances in electrode materials for emerging bioelectrochemical systems: from biofilm-bearing anodes to specialized cathodes, *Chemosphere* 283 (2021) 131138.
- [36] P. Nevin Kelly, L. Woodard Trevor, E. Franks Ashley, M. Summers Zarath, R. Lovley Derek, Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds, *Mbio* 1 (2010), <https://doi.org/10.1128/mbio.00103-10>.
- [37] P. Paiano, M. Menini, M. Zeppilli, M. Majone, M. Villano, Electro-fermentation and redox mediators enhance glucose conversion into butyric acid with mixed microbial cultures, *Bioelectrochemistry* 130 (2019) 107333.
- [38] V. Ranaei, Z. Pilevar, A.M. Khaneghah, H. Hosseini, Propionic acid: method of production, current state and perspectives, *Food Technol. Biotech.* 58 (2020) 115–127.
- [39] N. Reichardt, S.H. Duncan, P. Young, A. Belenguer, C. McWilliam Leitch, K.P. Scott, H.J. Flint, P. Louis, Phylogenetic distribution of three pathways for propionate production within the human gut microbiota, *ISME J.* 8 (2014) 1323–1335.
- [40] M. Roy, R. Yadav, P. Chiranjeevi, S.A. Patil, Direct utilization of industrial carbon dioxide with low impurities for acetate production via microbial electrosynthesis, *Bioresour. Technol.* 320 (2021) 124289.
- [41] M.J. Salar-García, V.M. Ortiz-Martínez, S. Sánchez-Segado, R.V. Sánchez, A. S. López, L.J.L. Blanco, C. Godínez-Seoane, Sustainable production of biofuels and biochemicals via Electro-Fermentation technology, *Molecules* 29 (2024) 834.
- [42] U. Schröder, F. Harnisch, L.T. Angenent, Microbial electrochemistry and technology: terminology and classification, *Energy Environ. Sci.* 8 (2015) 513–519.
- [43] P.-L. Tremblay, T. Zhang, A. Dar Shabir, C. Leang, R. Lovley Derek, The *rnf* complex of *Clostridium ljungdahlii* is a Proton-Translocating Ferredoxin:NAD⁺ oxidoreductase essential for autotrophic growth, *Mbio* 4 (2012), <https://doi.org/10.1128/mbio.00406-12>.
- [44] W. Tu, J. Xu, I.P. Thompson, W.E. Huang, Engineering artificial photosynthesis based on rhodospin for CO₂ fixation, *Nat. Commun.* 14 (2023) 8012.
- [45] T. Ueki, K.P. Nevin, T.L. Woodard, D.R. Lovley, Converting carbon dioxide to butyrate with an engineered strain of *Clostridium ljungdahlii*, *Mbio* 5 (2014), <https://doi.org/10.1128/mbio.01636-14>.
- [46] M. Villano, P. Paiano, E. Palma, A. Miccheli, M. Majone, Electrochemically driven fermentation of organic substrates with undefined mixed microbial cultures, *ChemSusChem* 10 (2017) 3091–3097.
- [47] P. Wu, Y. Guo, M.K. Golly, H. Ma, R. He, S. Luo, C. Zhang, L. Zhang, J. Zhu, Feasibility study on direct fermentation of soybean meal by *Bacillus stearothermophilus* under non-sterile conditions, *J. Sci. Food Agric.* 99 (2019) 3291–3298.
- [48] G. Xue, S. Lai, X. Li, W. Zhang, J. You, H. Chen, Y. Qian, P. Gao, Z. Liu, Y. Liu, Efficient bioconversion of organic wastes to high optical activity of L-lactic acid stimulated by cathode in mixed microbial consortium, *Water Res* 131 (2018) 1–10.
- [49] A. Zhang, S.-T. Yang, Engineering *Propionibacterium acidipropionici* for enhanced propionic acid tolerance and fermentation, *Biotechnol. Bioeng.* 104 (2009) 766–773.
- [50] A. Zhang, S.-T. Yang, Propionic acid production from glycerol by metabolically engineered propionibacterium acidipropionici, *Process Biochem.* 44 (2009) 1346–1351.
- [51] L. Zhang, R. Zhao, D. Jia, W. Jiang, Y. Gu, Engineering *Clostridium ljungdahlii* as the gas-fermenting cell factory for the production of biofuels and biochemicals, *Curr. Opin. Chem. Biol.* 59 (2020) 54–61.
- [52] R.T. Zhang, Y. He, J. Yi, L.J. Zhang, C.P. Shen, S.J. Liu, L.F. Liu, B.H. Liu, L. Qiao, Proteomic and metabolic elucidation of Solar-Powered biomanufacturing by Bio-Abiotic hybrid system, *Chem. Us* 6 (2020) 234–249.
- [53] W. Zhang, C. Liu, F. Liu, X. Zou, Y. Xu, X. Xu, A smart-phone-based electrochemical platform with programmable solid-state-microwave flow digestion for determination of heavy metals in liquid food, *Food Chem.* 303 (2020) 125378.
- [54] Y. Zhang, J. Li, Y.-c. Yong, Z. Fang, W. Liu, H. Yan, H. Jiang, J. Meng, Efficient butyrate production from rice straw in an optimized cathodic electro-fermentation process, *J. Environ. Manag.* 336 (2023) 117695.
- [55] Y. Zhang, J. Li, Y.-c. Yong, Z. Fang, H. Yan, J. Li, J. Meng, Highly selective butanol production by manipulating electron flow via cathodic electro-fermentation, *Bioresour. Technol.* 374 (2023) 128770.
- [56] X. Zhu, J. Jack, A. Leininger, M. Yang, Y. Bian, J. Lo, W. Xiong, N. Tsesmetzis, Z. J. Ren, Syngas mediated microbial electrosynthesis for CO₂ to acetate conversion using *Clostridium ljungdahlii*, *Resour. Conserv. Recycl.* 184 (2022) 106395.
- [57] L. Zou, Y. Qiao, C.M. Li, Boosting microbial electrocatalytic kinetics for high power density: insights into synthetic biology and advanced nanoscience, *Electrochem. Energy Rev.* 1 (2018) 567–598.



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Evaluating propionate production in *Clostridium ljungdahlii* inoculated bioelectrochemical system

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