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Acetate-to-bioproducts by chain elongation microbiome catalysis under applied voltage regulation

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ABSTRACT

Acetate is the main component in the anaerobic digestion broth of organic waste, which is difficult to be extracted and further recovered because of its completely miscibility. A novel technology convert acetate to bioproducts (like ethanol, butyrate, and caproate) in electro-fermentation system by chain elongation microbiome has recently attracted attention. This work focused on the fate of acetate as substrate and sole electron acceptor under different applied voltage regulation. Products spectrum analysis showed that acetate was effectively converted and accumulated to a maximum concentration of 4.26 g L^{-1} ethanol firstly at 0.6 V, then butyrate (2.40 g L^{-1}) and caproate (0.12 g L^{-1}) were synthesized respectively. With the increase in voltage intensity, the bioconversion performance of acetate gradually deteriorated. The monitoring results of cathode chamber indicated an appropriate applied voltage creating a more favorable conversion environment for chain elongation microbiome, and a biocathode with better redox capability could act as an effective catalyst, contributing to the hydrogen-mediated electron transfer for chain elongation process. The correlation analysis between cathode multi-parameters and bioproduct performance at 0.6 V showed that the chain elongation process may be more dependent on the fermentation environment than the reduction of acetate to ethanol, especially for pH and redox potential. This study provides a sustainable and energy-saving platform for producing bioproducts from readily available acetate under the regulation of applied voltage.

1. Introduction

Given the increasing demand for energy and sustainable development, the production of bioproducts is an imperative [1]. In recent years, volatile fatty acids (VFAs) production from organic waste by anaerobic digestion has been considered as an effective biorefinery technology [2–4]. Acetate, as the main component of the VFAs in fermentation broth, has an abundant source of generation [2]. However, the complete miscibility of acetate makes the subsequent separation process energetically costly, thus posing an obstacle to its further exploitability [5–7]. Fortunately, electro-fermentation (EF) system using carbon chain elongation (CE) microbiome as biocatalyst offers a reliable alternative to overcome this obstacle [8,9], as this novel technology is achieved by upgrading organic compounds (e.g., acetate) to higher value bioproducts, like medium chain carboxylic acids (MCCAs) [9–11]. MCCAs feature richer energy as well as stronger hydrophobicity than its precursors, which make it economically attractive to be separated from fermentation broth to manufacture products [7]. In addition, ethanol is considered to be an intermediate or by-product of MCCAs production from acetate, which is also regarded as an additive to fossil fuels [7]. Van et al. [10] firstly reported that ethanol (27 mg L^{-1}), butyrate (263 mg L^{-1}), caproate (739 mg L^{-1}), and caprylate (36 mg L^{-1}) were synthesized by CE microbiome from acetate (6.0 g L^{-1}) at a fixed cathode potential of –0.9 V vs standard hydrogen potential (SHE). Therefore, the highly efficient conversion of acetate to ethanol and MCCAs is one of the environment-friendly, energy-saving, and economy-sustainable platforms for the production of bioproducts [12].

Furthermore, EF has been widely praised for its special purpose of converting electrical energy into chemicals [8,9,13]. The remarkable advantage of this system ensures that microorganisms can catalyze

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acetate conversion by direct or indirect electrons transfer with the electrode without exogenous chemical electrode donors (EDs) [14–16]. A few studies have evaluated in detail the effects of EF on the mixed culture CE process [9,10]. For example, Jiang et al. [9] suggested that microorganism interacted electrochemically with the electrode for CE, and the synergistic effect was largely limited by various parameters, like substrate concentration and biofilm maturity. In addition, Jiang et al. [9,11] strengthened the chain elongation performance of acetate by exogenously supplementing ethanol as an electron donor to unite the electrodes into a binary electron donor design. Although ethanol can also be generated from waste biomass, it has been shown cumulative toxicity to microorganisms, so it is essential to maintain appropriate ethanol concentration and to ensure that the fermentation environment is favor for CE reactions [17,18]. Therefore, more efficient and optimized treatments need to be developed before a viable technology for EF-coupled CE becomes possible.

Up to date, the cathode potential was generally controlled at the same level by a multichannel potentiometer in the previous studies, which undoubtedly increased the cost of operation and limited the selectivity of products spectrum [9–11]. Schievano et al. [14] proposed an imposed electrical field influences the fermentation environment and microbial metabolism in either a reductive or oxidative manner, and the most outstanding benefit of regulating applied voltage intensity is to produce target bioproducts. Similarly, the accumulation of acetate or methane by different applied voltages regulation has been widely reported in the microbial electrosynthesis system with carbon dioxide as the sole substrate [15,19–21]. However, the effects of voltage on the acetate conversion by CE microbiome in EF system is still not clear. Van et al. [10] have indicated that the electron flows for the conversion of acetate to ethanol and MCCAs consists mainly of hydrogen mediated electron transfer (like electrochemical and biological hydrogen evolution) and direct electron transfer. Thermodynamic calculation shows that the bio-hydrogen evolution potential (H^+/H_2, $2e^-$) is around at −0.41 V vs SHE under standard condition [22], which makes the cathode potential generally controlled at −0.8 to −0.9 V vs SHE in previous studies [9–11], a more negative level to overcome the overpotential for hydrogen production. Yet, some studies have indicated excessive voltages could inhibit the activity of cathode microorganisms and the formation of biofilm by damaging cell membranes [22–24]. Therefore, these studies indicate that an optimal applied voltage is required to regulate bioproducts production from acetate by CE microbial catalysis in the EF system.

In this study, an EF system inoculated with CE microbiome was constructed and fed with acetate as the sole carbon source, and then focused on the fate of acetate under the regulation of different applied voltage. Meanwhile, the effects of cathode multi-parameters (like pH, hydrogen partial pressure, and cathode potential) on the CE process were reported. In addition, the cathode biofilm formation and redox capability after different applied voltage stimulation were evaluated by cathode characterizations. This study could be valuable for promoting and expanding the application of the bioelectrochemical systems in bioproducts production, development, and sustainability fields.

2. Materials and methods

2.1. Seed sludge and inoculum acclimation

Samples of seed sludge in this study were obtained from a citric acid production company in Wuxi, China. Some physical–chemical characteristics of collected sludge were measured according to standard manners, and the data were as follows: pH ~7.64 ± 0.05, total solid content ~7.87 ± 0.2%, dissolved chemical oxygen demand ~13150 ± 150 mg L^{-1}, and polysaccharide concentration ~190 ± 20 mg L^{-1}. After the seed sludge was collected, it was stored at 4 °C for 2 days, then was taken (1 L) to water bath heating for 3 h. The acclimated inoculum was obtained by digesting the pretreated sludge with dosing 6.0 g L^{-1} anhydrous ethanol, 2.78 g L^{-1} sodium acetate (acetate substitute), and some nutrients [25,26], after purging with N_2 prior for 20 min to achieve an anaerobic condition. Ultimately, the mixture continuously and stably operated four batches in the shaker (150 rpm, 36 °C), which could produce MCCAs by CE using ethanol and sodium acetate as substrates [26], then the acclimated enrichment was stably cultivated all the time and used to as the inoculation source of the following batch experiments. In addition, the activities of *Methanogen* were inhibited by preheating of seed sludge and by dosing 2.0 g L^{-1} sodium 2-bromoethanesulfonate as a methanogenic inhibitor [9], and no methane was detected in all operations.

2.2. Construction of the EF-CE scheme

As shown in Fig. 1, each reactor has two chambers which were separated by proton exchange membrane (PEM, 3 cm × 3 cm, Nafion-117, DoPont, USA), and total volume of each chamber was 0.2 L. Carbon felts (2 mm thick, projection area of 15 cm^2, TOKAI CARBON CO., LTD, Japan) were used as anode and cathode working electrodes and pretreated in 1 M NaOH for 12 h and 1 M HCl for 12 h, and then washed with deionized water to a neutral state before being used [9]. Working electrodes were connected by a resister (10 Ω, Svenska Tanso, Swiss) and fixed on the cover of the rubber reactor by titanium wire (9 mm in diameter, Alfa aesar, China), to formed an electrical pathway. The reference electrode (3 M KCl, Ag vs AgCl, INESA Scientific Instrument CO., LTD, China) was closed to the cathode working electrode in each reactor [10]. There was a sampling port of the cathode chamber connected with a tee valve (BaiHe Medical CO., LTD, China), and a rubber gas port was used to taking gas and testing pressure (BaiHe Medical CO., LTD, China). The external DC power sources (3645A, Array Electronic, China) were equipped to supply constant voltage for cells.

2.3. Batch experiments

The processes and parameters of batch experiments were shown in Table 1. Two experimental systems were designed and performed as “Stage I: initiating phase” and “Stage II: operating phase”. The Stage I contained four batches, and each batch ran five reactors (R1, R2, R3, R4, and R5) in the open circuit mode, ethanol (6.00 g L^{-1}) and sodium acetate (2.78 g L^{-1}) were used as substrates in each batch of five reactors for preliminary biofilm formation, microbial activation, and operation stabilization. At Stage II, the cathode fermentation broth from all reactors of the fourth batch was collected respectively, centrifuged, and concentrated as the inoculum for the corresponding reactors of the fifth batch. In order to avoid the residual organic components in the fermentation broth, all batches of inoculum need to washed with ultrapure water for three times [9]. It is well known that the theoretical cell voltage of water electrolysis is 1.23 V (O^{2-}/O_2, 1.23 V VS SHE; H^+/H_2, 0 V VS SHE) under standard condition [21,27], and Rabaey et al. [22] calculated that the hydrogen evolution potential of bioelectrochemical system was about – 0.41 V vs SHE under standard condition. In addition, it has been proved that the hydrogen was an important electron mediator for acetate conversion [10]. Therefore, after the operation of Stage I, the above five reactors cells were applied with different voltages accordingly to continue the operation at Stage II: R1 (open circuit), R2 (0.6 V), R3 (1.2 V), R4 (1.8 V), and R5 (2.5 V), the details of voltage application mode and operation details are shown in Fig. 1 and Table 1. Within this voltage ranges, the acetate conversions were compared under mild or stressed hydrogen evolution conditions. Moreover, referring to the previous study [10], the dosage of sodium acetate per reactor was increased from 2.78 g L^{-1} to 8.34 g L^{-1} as the substrate in Stage II, and other nutrients were consistent with Stage I except for no ethanol addition.

Except for the differences of substrates and applied voltage intensities in two stages, the other parameters were consistent in five batch experiments. As shown as Table 1, the working volume of both

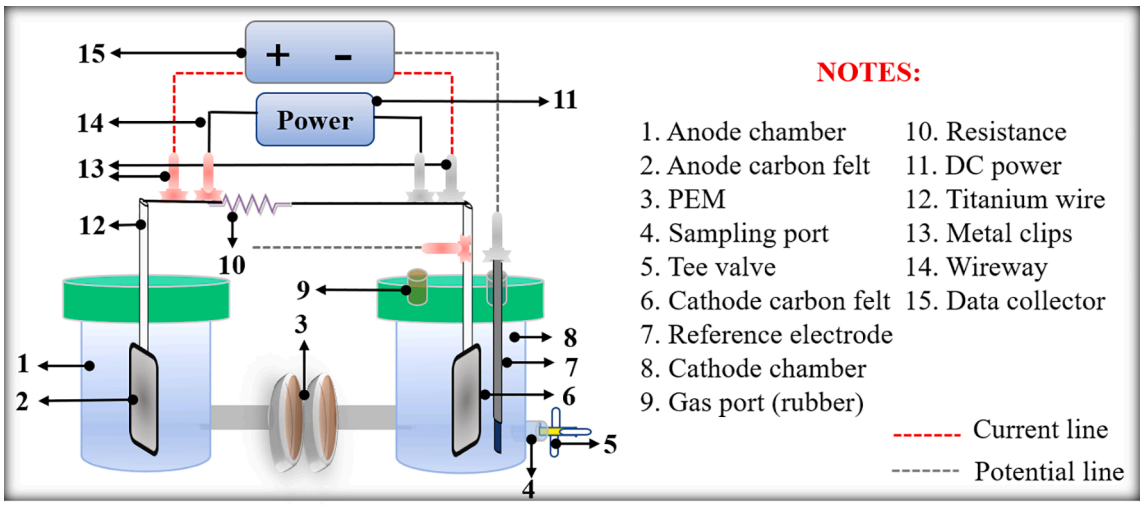

Fig. 1. The diagram of electro-fermentation-chain elongation reactor in this study.

Table 1
Overview of the five batches divided into two stages in this study.

Batch experiments	Stage I: Initiating phase				Stage II: Operating phase
	batch 1	batch 2	batch 3	batch 4	batch 5
Manipulation	Five reactors were performed in each batch for a total of 20 days, and the fermentation broth was centrifuged and concentrated to use as inoculum for each batch.				Five reactors were operated for 12 days per a cycle, and the inoculum obtained from fourth batch, and operating 3 cycles.
Substrates	6.00 g/L ethanol, 2.78 g/L sodium acetate were added in each batch of five reactors.				8.34 g/L sodium acetate was respectively added in this batch of five reactors.
Applied voltage	Open circuit mode				Five reactors cells were respectively operated with open circuit, 0.6 V, 1.2 V, 1.8 V, and 2.5 V applied voltage.
Inoculum	high concentration of bacteria, OD_{600} ~0.20 ± 0.05 after inoculating				
Anolyte	50 mM PBS				
Catholyte pH	Initial pH was 7.0, and pH was not controlled during the operations.				
Working volume	0.17 L				
Electrodes	Carbon felt (3 cm × 5 cm × 0.2 cm)				
Temperature	35–37 °C				
Cathode nutrients	0.31 g/L K_2HPO_4, 0.23 g/L KH_2PO_4, 0.25 g/L NH_4Cl, 0.20 g/L $MgSO_4 \bullet 7\ H_2O$, 1 mL/L Trace metal solution, 1 mL/L Vitamin solution, 1 g/L yeast extract, 0.25 g/L L-cysteine hydrochloride·H_2O, 2.0 g/L sodium 2-bromoethanesulfonate.				

chambers was 170 mL. The anolyte of all reactors was 50 mM phosphate buffer solution (PBS), and the fed medium of catholyte consisted of inorganic salts, growth factors, and yeast extract [9–11]. The trace metal and vitamin solution were referenced DSMZ-52 [10]. The catholyte initial pH was adjusted to 7.0 with 4 M HCl or NaOH solution, and pH was not controlled during the operations. All reactors were placed in a constant temperature room (35–37 °C) [11]. Moreover, it was noted that the catholyte OD_{600} after inoculation with concentrated bacteria fluids was 0.20 ± 0.05 of each batch. The variations of parameters and material concentrations (including substrates, intermediates, and products) in the cathode chamber were investigated by the timing sampling method.

2.4. Process parameter detection

The pH of samples was measured by a pH meter (INESA Scientific Instrument Co., LTD, China). The redox potential (ORP) was detected by Pt-Au composite electrodes (INESA Scientific Instrument Co., LTD, China). TOC and TIC were measured periodically throughout the experiment to analyze the components of catholyte (ASAP 2020, Micromeritics, USA) [28]. The growth of planktonic microorganisms was evaluated by the optical density of the samples using a UV–visible spectrophotometer at 600 nm (UV-1600, MAPADA INSTRUMENTS, China) [9]. As shown in Fig. 1, the cathode potential and current were monitored online by a data collector (Keithley-2700/E, USA) [9–11]. The reactor pressure was detected by inserting a needle barometer (Oxoid, UK) into the rubber gas port at constant temperature room (35–37 °C) [29].

2.5. Analysis and characterization

The concentrations of formic, acetate, propionic, butyrate, caproate, and ethanol were analyzed by gas chromatography (GC-2010, SHIMADZU, Japan) using Rtx-wax polyethylene glycol column with an FID detector, all samples were periodically aspirated from the sampling port with a syringe, and were acidified with 3 mol/L phosphoric acid in equal volume, then filtered through 0.22 μm membrane before testing. The gasification temperature, the column chamber temperature, and the detector temperature were 250 °C, 60 °C and 250 °C, respectively [30]. The gas samples were drawn from the rubber port of each reactor through the syringe, and then the gas compositions were quantified by gas chromatograph (GC-9790 II, FULI INSTRUMENTS, China) equipped with a thermal conductivity detector using nitrogen as the carrier gas [30]. Based on the products concentrations and process parameters data obtained by the above methods, one-way analysis of variance (ANOVA, SPSS, IBM, USA) was used to evaluate the correlation between them, and P-value of significant difference analysis was less than 0.05. After the end of the Stage II operation, a piece of biocathode (1 cm × 1 cm) was cut from the cathode working electrode of each reactors, and then was suspended in PBS (10 mM) of the same volume respectively, and the microorganisms on these tailored biocathodes were stripped by ultrasound method (4 h) [31]. The microorganisms of the catholyte from each reactor were obtained by centrifugation (12000 r/min, 15 min, Thermo Fisher) and concentrated to the same volume with PBS (10 mM) [31]. The protein concentrations of working electrode and catholyte microorganism (above samples) were measured with a bovine serum albumin standard (BCA Protein Assay Kit, BEYOTIME, China) using a microplate reader at 562 nm (Molecular DEVICES, USA) [9].

After the end of the Stage II operation, a three electrodes system consists of counter electrode (platinum plate), reference electrode (saturated calomel electrode), and working electrode (the five biocathodes from five reactors), were constructed for the cyclic voltammetry curve analysis (CHI600E, CHINSTRUMENTS, China) of the remaining biocathodes [24]. The PBS solution (50 mM) was used as the electrolyte [24,32], and the scanning voltage range and scanning rate were determined to be +0.8 V to −1.1 V and 1 mV/s, respectively [9–11]. Meanwhile, the N/P was selected to N [11]. The biofilm on the cathode working electrode was assessed by scanning electron microscopy (SEM, EVO-18, ZEISS, Germany), the another five 1 cm × 1 cm electrode samples were cut from the above remaining cathode before CV analysis and treated with 2.5% glutaraldehyde solution for six hours, then all samples were pipetted with 0.1 mM PBS for three times, and dehydrated in a gradient series of ethanol solutions (20 % for two times, 50% for two times, 75% for one time, 90% for one time, 100% for one time, and soaking for 10 min each time), finally coated with Au before detection [31,33].

2.6. Calculation

The total biomass in five reactors was calculated by Equation S1, including biofilm and planktonic microorganisms [28]. Overall, this is an estimate of the biomass content from the measured protein concentrations [34]. After obtaining the CV curves of different biocathode samples, the capacitances of these samples were calculated based on the area formed by fitting the CV curves (Origin 2019, mathmatics-integrate), the scanning speed and the voltages window (Equation S2) [21]. The carbon and electron conversion rates in R1–R5 reactors were calculated by Equations S3 and S4 respectively after Stage II operation [30,35]. The carbon contents were derived from the substrate and product concentrations measured by the above analysis method, and then electron equivalent of each product was obtained. The total consumptions of electron in five reactors were converted according to the change of acetate concentration and current time curve. The electron conversions were calculated by the ratios of products electron equivalents to electron consumptions.

3. Results and discussion

3.1. Bioproducts production performance in two experimental systems

The concentrations of alcohols and carboxylic acids were measured once a day at Stage I, every two days in Stage II. As shown in Fig. 2a, at Stage I, the majority of ethanol and acetate were consumed by the inoculum for the chain elongation process [7], making a production efficiency of 0.32 g $L^{-1}d^{-1}$ and 0.36 g $L^{-1}d^{-1}$ for butyrate and caproate in five days, respectively. Comparison of the product production performances of the four batches of experiments showed that each reactor was operated stably, indicating that all five reactors were successfully started up, thus the potential parallel differences in reactor configuration and inoculum activity were eliminated for Stage II.

Fig. 2b-f showed the acetate bioconversion performances under conditions of open circuit control and different applied voltage intensities. Firstly, neither the cathode nor inoculum could drive the acetate bioconversion in R1 due to the lack of electron flow from the external circuit and internal chemical EDs. Then, acetate exhibited different conversion performances in the other four reactors, respectively. Under the low voltage intensities of 0.6 V and 1.2 V, acetate was firstly reduced to ethanol, and the maximum concentrations of ethanol respectively reached to 4.26 g L^{-1} and 1.34 g L^{-1} at the 28th day. After 32 days of operation, butyrate was detected in the catholyte of R2 and

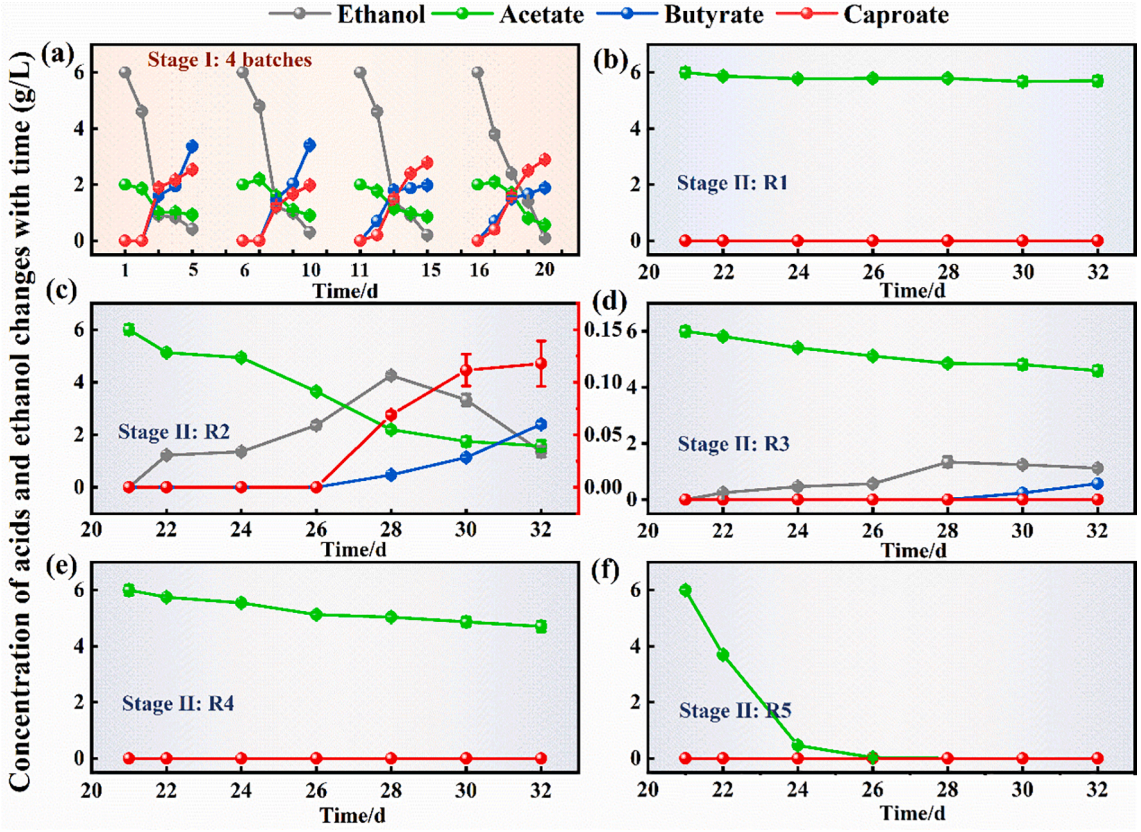

Fig. 2. (a) Concentration of carboxylic acids and ethanol in batches 1–4 at initiating phase (Stage I, 20 days), and the effect of applied voltage on production of carboxylic and ethanol compounds from acetate: (b) R1: open circuit, (c) R2: 0.6 V (the caproate concentration refers to the right red coordinate aixs, g L^{-1}), (d) R3: 1.2 V, (e) R4: 1.8 V, and (f) R5: 2.5 V during 12 days of operating phase (Stage II, ethanol free dosing).

R3, and the maximum concentrations were 2.40 g L^{-1} and 0.57 g L^{-1}, respectively. In addition, caproate was synthesized due to the accumulation of ethanol and butyrate at 0.6 V, although the maximum concentration was 118 mg L^{-1}, the sufficient EDs and EAs ascertained the CE reactions [32,36]. In contrast, there was no caproate was synthesized at 1.2 V. Under the high applied voltage intensities of 1.8 V and 2.5 V, acetate was slowly decreased at 1.8 V, and no other organic components were detected in the catholyte, indicating the ability of electron transfer between microorganisms and solid cathode might be gradually lost. Thereafter, acetate was completely consumed at 2.5 V, and the phenomenon was observed which the catholyte became turbid with the appearance of milky white suspended matter. Similarly, there was no other organic compounds in the catholyte were detected of R5. Therefore, the analysis of the product spectrums of the five EF-CE reactors indicated that the CE performance of acetate was not be strengthened with increasing voltage intensity.

Several studies have shown different mechanisms by which voltage of different intensities affects metabolic activity of microorganisms [21–23]. For example, Lin et al. [21] proved the applied voltage (0.8 V) could boost methane production by enhancing interspecies H_2 transfer in the anaerobic digestion microbial electrolytic system. Chen et al. [23] and Wu et al. [24] suggested that low applied voltage (0.2 V) improved the permeability of cell membrane, and significantly promoted the upregulation of cytochrome protein related to electron transfer, thus ameliorating the redox ability of microbial cells. Hence, the effects of applied voltage on the fate of acetate were supposed to be related to the inoculum activity and cathode reduction power [14,35,37], including the electro-catalytic ability of solid cathode, the bioconversion capacity of biofilm and planktonic microorganisms, and the ability of extracellular electron transfer (EET) [28,31,38]. These mechanisms have been widely reported in bioelectrochemical system that the inoculum activity and electron transfer ability are affected by cathode potential [38]. Accordingly, some conjectures could be put forward in an EF-CE system with applied voltage regulation: 1) acetate conversion was a cooperative process between electrode and inoculum and was regulated by the catalytic capacity of the biocathode [39]; 2) in all reactors, there was no direct bioconversion of acetate to butyrate because ethanol was a necessary intermediate for the CE reactions in this study, which was dissimilar from the reported study in that no ethanol was detected of catholyte in a microbial electrosynthesis system with CO_2 as sole carbon source [33]; and 3) high voltages were likely to cause the deterioration of cathode fermentation environment, resulting in acetate being consumed to provide energy instead of reducing to ethanol [26]. To demonstrate these assumptions about the applied voltage regulation of acetate conversion, multi-parameters variation analysis of cathode chamber, characterization of biofilm formation and redox capability were conducted.

3.2. Interactions of multi-parameters and biocathode reactions

3.2.1. Operational parameters

The pH, OD_{600}, and ORP of catholyte, and the reactor headspace gas components and pressure were analyzed to further clarify the effects of applied voltage on acetate conversion. As shown in Fig. 3a–c, the catholyte pH, OD_{600}, and ORP of all reactors maintained good parallelism at the Stage I: firstly, the catholyte pH rapidly decreased from 7.0 to about 5.5 because of the bio-oxidation of ethanol and bio-synthesis of carboxylic acids [7]. Then, the inoculum grew rapidly making the catholyte OD_{600} increased from 0.20 to 0.40 in 5 days. Finally, the ORP was more negative due to the increase of reducing carboxylic acids [30].

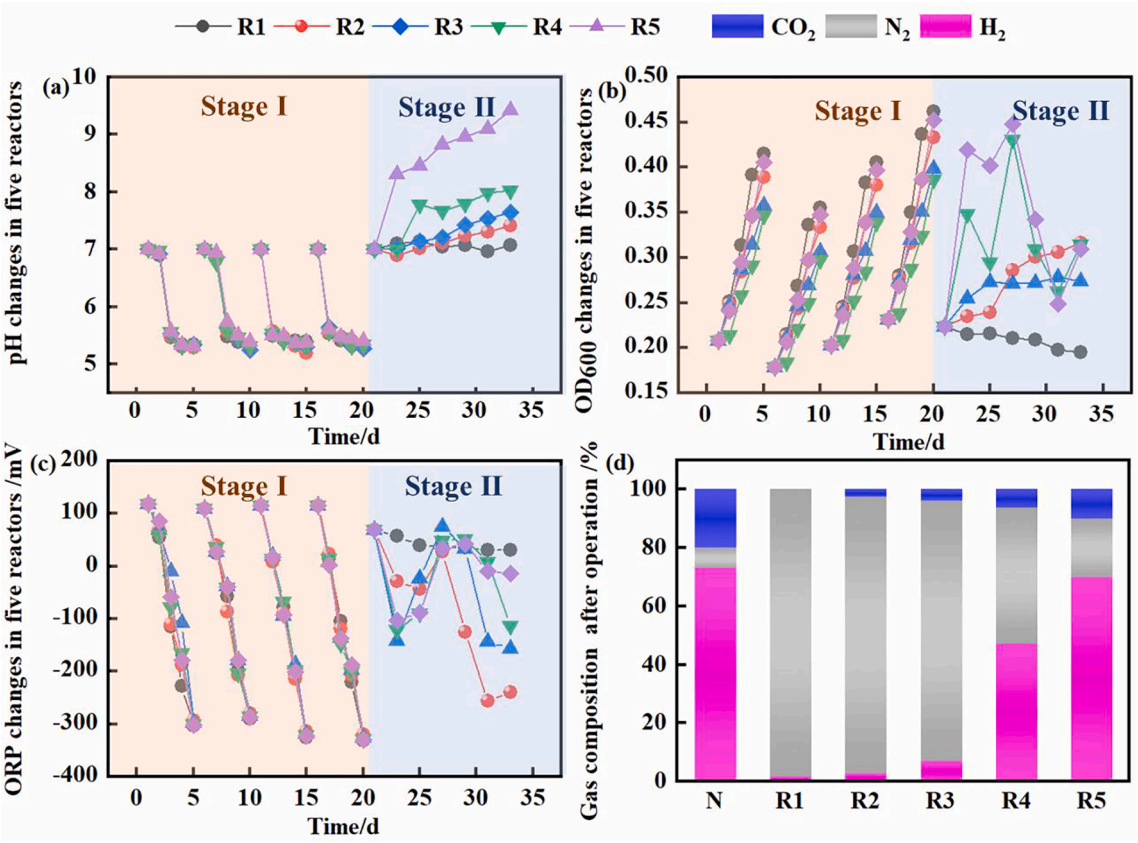

Fig. 3. (a) The pH, (b) OD_{600}, and (c) ORP changes of the five reactors in the Stage I (Initiating phase: acetate and ethanol were used as co-substrates and kept open circuit mode) and Stage II (Operating phase: different voltages were applied to reactors with acetate as sole substrate) are respectively shown in the yellow and blue filling sections; and (d) the headspace gas compositions of N (Stage I) and five EF-CE reactors under different applied voltages (R1: open circuit, R2: 0.6 V, R3: 1.2 V, R4: 1.8 V, and R5: 2.5 V.) at the end of Stage II operation.

As shown in Fig. 3a–c, at Stage II, there was no drastic parameters alteration in R1 merely the OD_{600} slightly decreased due to cell inactivation and decomposition. Compared with R1, the increasing trend of pH in other closed-circuit mode reactors was positively correlated with voltage intensity, but OD_{600} and ORP emerged with different variations. On the one hand, at 0.6 V and 1.2 V, the increase of pH was relatively slow as acetate was partially reduced to ethanol and biosynthesized to carboxylic acids [33], and OD_{600} of catholyte in R2 and R3 were respectively increased by 0.05 and 0.12 in 12 days. Moreover, ORP showed a downward-upward-downward trend of R1 and R2 due to multiple reaction process such as slow acetate reduction, rapid ethanol bio-oxidation (day 21 to 28), and slow carboxylic acids biosynthesis (day 26 to 32). On the other hand, at 1.8 V and 2.5 V, the H^+ obtained electron and was further reduced to H_2, but no other acids were produced [22], causing a rapid rise of pH value (Fig. 3a and d). Obviously, the changes of OD_{600} and ORP were not directly related to the CE microbiome activity in the R4 and R5 because of chain elongation pathways were not performed. Therefore, the changes of parameters in R4 and R5 were likely affected by acetate degradation reactions in catholyte [29].

Fig. 3d and Fig. S1 compared the headspace gas components and cathode pressures in batch reactors at two stages, respectively. In this study, it was noted that no methane was detected in headspaces of all reactors. Firstly, at Stage I, hydrogen was the dominant gas in headspace (73.1%) of reactor, followed by carbon dioxide (19.9%), these gases have been shown to be produced by ethanol oxidation and reverse β oxidation processes [7]. Then, there were trace amounts hydrogen in headspace of R2 and R3 at low voltage intensities (0.6 V and 1.2 V), indicating that the inoculum utilized the released hydrogen from ethanol bio-oxidation and carboxylic acids production via interspecies H_2 electron transfer [10,11]. However, the R4 and R5 showed a high headspace properties for the hydrogen component, suggesting that the inactivated inoculum could not recycle the released hydrogen as the electron mediator in time to produce bioproducts by reducing acetate, even at a high hydrogen partial pressure. Finally, as shown in Fig. S1, at Stage I, the headspace pressure in each reactor reached 0.5 bar (35 ℃), contributing to the chain elongation process [21,26,29]. At Stage II, the headspace pressures in five reactors and the applied voltage intensities showed a positive correlation relationship (R1: 0.10 bar, R2: 0.15 bar, R3: 0.18 bar, R4: 0.23 bar, and R5: 0.32 bar, 35 ℃), which further demonstrated that although the evolution of hydrogen could be enhanced electrochemically, whether hydrogen could be recycled as an electron mediator depends on the activity of the inoculum.

Since the broadest products spectrum was exhibited at 0.6 V, the acetate conversion performance of R2 was further evaluated by correlation analysis between parameters and product concentrations in Stage II. The parameters selected include pH, OD_{600}, and ORP because the sampling points for these parameters coincided with the sample concentration test time points. As shown in Fig. S2, there was no significant correlation between the concentration of ethanol and pH ($P > 0.05$). However, the concentration of caproate concentration showed significant correlations with all three parameters ($P < 0.05$), and butyrate concentration also showed correlations with pH ($P < 0.01$) and ORP ($P < 0.05$). Jiang et al. [30] also found that a direct effect of pH and ORP on the distribution of metabolites in mixed culture electro-fermentation. These results suggested that the chain elongation process may be more dependent on the fermentation environment than the reduction of acetate to ethanol, especially more pronounced for the pH and ORP.

3.2.2. *Electrochemical analysis*

In addition, the change of cathode potential and current also be detected for directly reflecting the electron flows in the entire system. As shown in Fig. 4, the biological metabolic pathways or (bio) electrochemical reactions (like acetate reduction to ethanol, ethanol oxidation, chain elongation, and hydrogen evolution) also showed a significant effect on the potential and current of catholyte. Especially, the changes of cathode potential (Fig. 4c) in 24–32 days showed a similar trend as that of ORP in R2 (Fig. 3c), and the current density of R2 (Fig. 4d) increased gradually and reached a maximum value of 0.10 mA/cm^2 at the peak period of bioproducts production (the days 26–30, Fig. 2c). These results confirmed that the fermentation environment and electron transfer ability were significantly affected by the applied voltage.

It is well known that overpotential is a very important problem in EF system, which often requires energy input higher than thermodynamics to realize redox reactions [10,11]. As shown in Fig. 4, the maximum cathode potentials were −0.3 V vs SHE and −0.5 V vs SHE (converted from vs Ag/AgCl) at applied voltages of 0.6 V and 1.2 V, respectively, and the mixed inoculum might reduce the thermodynamic condition so that the hydrogen mediated electron flow was involved in the reduction of acetate to ethanol [10]. By contrary, there were better thermodynamic conditions (higher hydrogen partial pressure) at the more negative reduction potentials (−0.6 V to −0.9 V vs SHE, converted from vs Ag/AgCl) induced by the higher applied voltages of R4 and R5 [29], but no bioproducts were produced. The accumulation of hydrogen was also confirmed by the rapid increase of the current density of R5 (from −2.1 to −9.2 mA/cm^2) in the initial phase of Stage II (Fig. 4b). Hu et al. [40] showed that hydrogen production was three times higher at 2.0 V than at 0.6 V, but the activity of electroactive biofilm was limited due to the destruction of cell membrane. These results indicated that although high hydrogen partial pressures have been reported to favor acetate

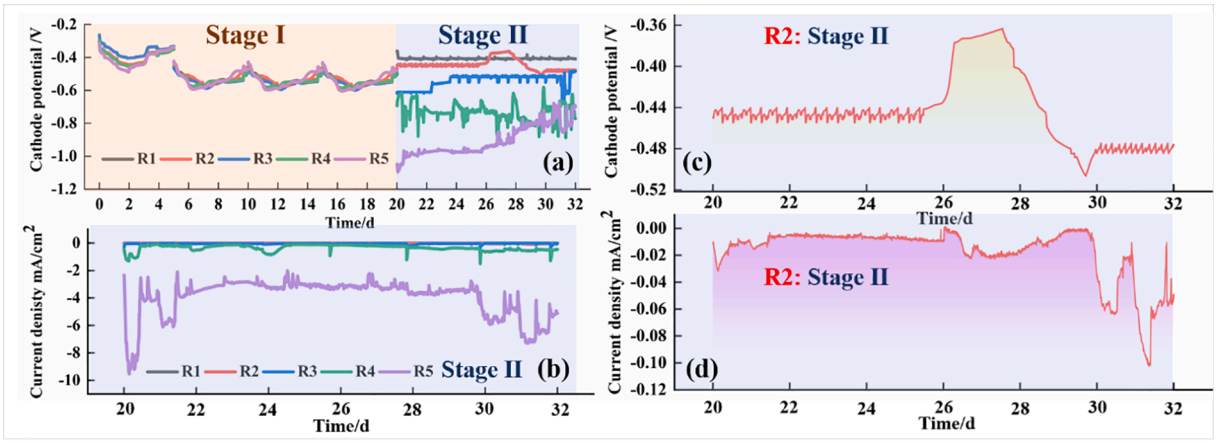

Fig. 4. (a) Comparison of cathode potential (V vs Ag/AgCl) of all reactors in two stages (yellow and blue filling sections were represented respectively the Stage I and Stage II), (b) current density in R1-R5 at Stage II, (c) the cathode potential (V vs Ag/AgCl), and (d) current density changes in R2 during 12 days of the experiment at Stage II.

reduction [10], active and functional microbial catalysis were required in the EF system.

Hence, on the one hand, the low voltage conditions (0.6 V and 1.2 V) favored the stabilizations of multi-parameters and the fermentation environments of cathode, which ensured the acetate reduction to generate ethanol firstly, and then driven the ethanol bio-oxidation and carboxylic acids biosynthesis through interspecies hydrogen transfer. On the other hand, although high voltage conditions (1.8 V and 2.5 V) promoted hydrogen generation, the majority of hydrogen escaped into the reactor headspace and was difficult to use as an electron mediator for bioproducts production, while the rapid rise of catholyte pH value may also inhibited the CE microbiome activity. Meanwhile, these results suggest that pH and ORP of the catholyte continue to be adjusted at optimal voltage to regulate the CE process, and that this pH or ORP based control may present a new approach to regulate mixed culture fermentation and improve the tunability of the products in the future [30,41].

3.3. Characterization of biocathodes

Since the catalytic performances of the five reactors varied, the redox capacity and biofilm distribution of the biocathodes were analyzed by CV and SEM, and the protein contents of the planktonic microorganisms and biofilms were furtherly measured after 32 days operation. As shown in Fig. 5, no redox peaks were evident for the fresh carbon felt and the R1 biocathode, which proved that neither the electrolyte (50 mM PBS) nor potential residual substances (e.g., medium components) on the electrode interfered with the characterization results and accuracy [9], and that the inoculum barely adhered to the electrode surface due to the absence of electron flows.

Compared with the open circuit control, the redox peaks were portrayed of each biocathode from other reactors. Several apparent redox peaks were marked in Fig. 5, and the peak positions and the biocathode capacitances were compared in Table 2. Although CV analysis has been widely used to detect the redox performance of complex biocathode, it is difficult to accurately judge the specific reaction of these peaks in mixed system [9]. Therefore, the overall redox performances of these samples were compared in terms of peak shape, peak position and capacitance of CV diagrams. Firstly, the current densities (mA/cm^2) of peak A1 (0.86), peak B (0.80), peak C (0.85) were obviously higher than peak D (0.62), and the position of peak D obviously shifted to the left (−0.53 V), which

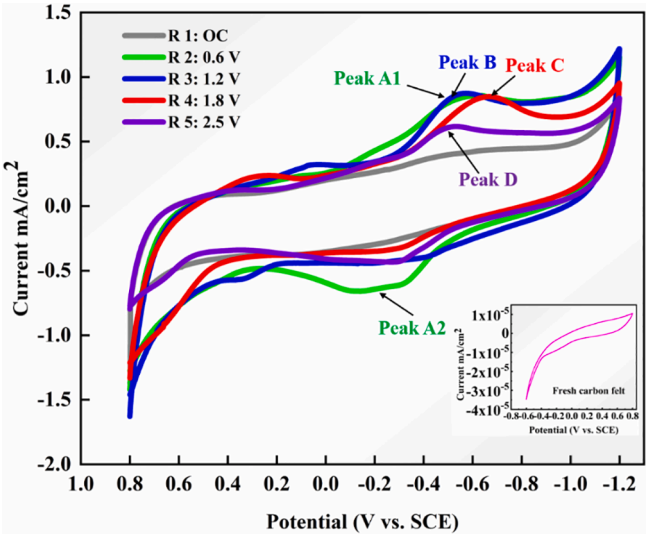

Fig. 5. Cyclic voltammograms are shown for a carbon felt cathodes from R1: open circuit, R2: 0.6 V, R3: 1.2 V, R4: 1.8 V, and R5: 2.5 V after 32 days of operation, and the embedded picture was showed for the fresh carbon felt without inoculum.

Table 2
Comparison of redox peak values and capacitances of cyclic voltammetry curves between EF-CE reactors under different voltages. [α]

EF-CE reactors	R1	R2 Peaks		R3 Peak B	R4 Peak C	R5 Peak D
		A1	A2			
Peaks potential (V)	/	−0.61	−0.27	−0.57	−0.66	−0.53
Peaks current density (mA/cm^2)	/	0.86	0.68	0.80	0.85	0.62
Cp (mA • h • cm^{-2}) [β]	0.97	1.64		1.59	1.29	1.16

Notes: [α] The analysis was detected following 32 days operation, R1: open circuit, R2: 0.6 V, R3: 1.2 V, R4: 1.8 V, and R5: 2.5 V. [β] Capacitance (Cp), was used to represent the electronic quantity per unit area of biocathodes.

indicated the microbial reduction ability of biocathode turned to relatively weak at 2.5 V. Then, a sharp peak A2 (−0.27 V, −0.68 mA/cm^2) was observed on the biocathode from R2 when the curve was scanned from reducing state (−1.1 V) to oxidation (+0.8 V), which indicated the R2 cathode had a better oxidation performance under the stress of higher ethanol concentration [21]. These results also indicated the inoculum responded to the low applies voltage and showed a better CE performance. Finally, compared with the R1, the capacitances of experimental groups increased by 69%, 64%, 24%, and 20%, respectively. More importantly, the biocathode from R2 reached a maximum capacitance of 1.64 mA • h • cm^{-2}, which higher 41% than R5. Thus, the differences of the redox ability performance between five biocathodes, also reflecting the response of inoculum to applied voltage.

Furthermore, the same size cathode felts were tailored at the same position from five reactors for SEM analysis. As shown in Fig. S3a, a thin biofilm was formed on the surface of the R2 carbon felt, which could be clearly observed at the 10 μm scale bar, and some rod-shaped and spherical microbial cells or clusters could be seen in further magnification (1 μm). However, the biocathodes of the other reactors were smoother than the 0.6 V biocathode. In addition, the protein contents of catholyte microorganisms and cathode biofilms were quantitatively determined to accurately evaluated the biocatalytic ability of each biocathode. As shown in Fig. S3b, the protein contents of R2 were the highest whether in catholyte or on the electrode, indicating that the inoculum was adapted to the fermentation environment at 0.6 V. As the voltage intensity was increased to 1.8 V, the protein content of the electrode was even lower than R1, indicating that the microorganisms had been dislodged from the cathode [42]. Moreover, the total biomass of the five reactors was estimated based on the protein contents. As shown in Fig. S4, the total biomass in R2 was 4 times higher than that in the open circuit control, and in the other reactors the total biomass decreased with increasing voltage, which was consistent with above results. Therefore, in terms of the electrochemical performance of biocathode and the growth of microorganisms in five reactors, the applied voltage indeed affected the electrochemical activity and electron transfer ability of inoculum, as reflected in the differences in the bioproducts production from acetate.

3.4. Carbon and electron conversion in EF-CE reactors

Basing on the products spectrum analysis, the carbon and electron balance were conducted after 32 days of operation. As shown in Fig. 6a and Fig. S5a, the carbon recovery rates of R2–R5 were 78.8%, 77.7%, 69.8%, and 76.5%. Actually, the acetate accounted for 74.3% to 96.6% of total carbon consumptions under different applied voltages, indicating that the carbon skeletons of ethanol and carboxylic acids mainly obtained from acetate. Instead, inorganic carbon components like gaseous carbon dioxide occupied a trace amount of carbon recovery at 0.6 V and 1.2 V, but at high voltages, the soluble inorganic carbons produced from acetate were the majority of carbon recovery, which indicated that carbon chain elongation microbiome would not win the

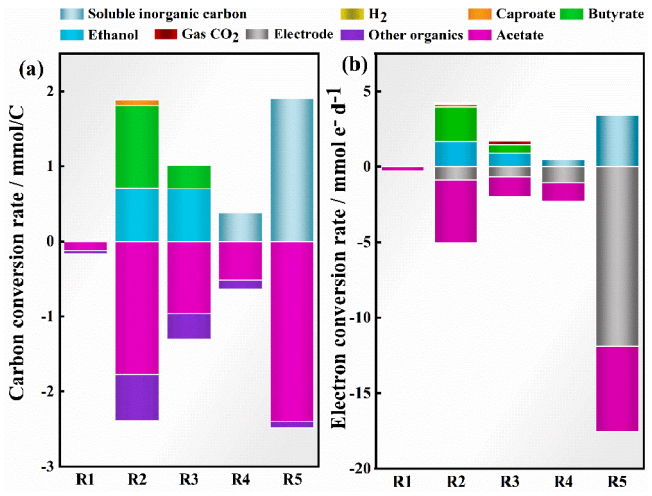

Fig. 6. (a) Carbon and (b) electron conversion rate in five EF-CE reactors: R1: open circuit, R2: 0.6 V, R3: 1.2 V, R4: 1.8 V, and R5: 2.5 V.

heterotrophic electroactive organisms (Fig. 3b showed higher OD_{600} of catholyte at 1.8 V and 2.5 V than lower voltages) in the competition of acetate [11].

In this study, the electron sources directly supplied from the cathode and indirectly provided by generated ethanol from acetate bio-reduction [31]. Fig. 6b and Fig. S5b clearly indicated that the proportion and consumption performances of electrons were provided by both ways. Significantly, in the whole bioconversion process of R2, the majority of consumed electrons originated from the substrate, and about 10% provided by cathode, showing an electron recovery rate of 84%. Instead, the electron recovery rates in R4 and R5 were as low as 22% and 20% respectively. Especially, the cathode provided the majority of electrons (65%) for acetate heterotrophic degradation to produce hydrogen, carbon dioxide, and other carbon components at 2.5 V. Meanwhile, the electrons provide by acetate degradation were likely to be used for heterotrophic growth and thus could not be recovered [10].

3.5. Mechanisms of bioproducts production from acetate regulated with applied voltage

Intuitively, the applied voltage directly caused the multi-parameters changes, making the acetate conversion was regulated by inoculum and electrode; at a deeper level, applied voltage not only affected the cathode redox capacity but also acted on the biofilm formation; ultimately, the bioproducts production performance was diverse in five EF-CE reactors with acetate as substrate under different applied voltage intensities.

The mechanisms of the above results were summarized in Fig. 7, and these mechanisms were clarified from different applied voltage levels in response to the original experimental purposes. It is well known that the acetate reduction and CE reactions require sufficient equivalent, and the cathode (hydrogen mediated or direct electron transfer) has been considered to be an ED for these biological reactions [10]. Therefore, in the open circuit R1 without other chemical ED, acetate could not be driven by the electrode and plankton due to the lack of electron flow. Differently, the entire system was relatively stable at low voltage conditions, and the process parameters were more conducive to the bioproducts production, these advantages benefit from the better fermentation environment and the biofilm formation of the cathode. In addition, both biofilm and plankton were likely to receive electrons from electrodes or hydrogen, ensuring the reduction equivalent for acetate reduction and CE process [10].

By comparison, the system was difficult to be stabilized and controlled under high voltage conditions. Although the stronger electrode reducing power was favorable for hydrogen generation, the CE microbiome activity may be inhibited due to the worse fermentation environment [42]. It was well known the hydrogen was considered to be an electron intermediator for acetate reduction [9–11], but the poor CE microbiome activity could not match the rapid hydrogen synthesis rate under high voltage conditions, making the acetate may be degraded by heterotroph [41]. This imbalance of the multi-reactions triggered the drastic changes of the cathode parameters and even promoted the electrochemical reaction (H^+/H_2) to occupy the dominant position in the entire system rather than biological pathways.

3.6. Prospects

In order to upgrade and transform simple substrate to high valued or sustainable bioproducts in EF with the electrode as the sole ED, Fig. 7 could be used as an operation instruction to supply some references. On the one hand, at low voltage conditions, the mild cathode potential ensured the activity of planktonic microorganisms and the gradual formation of biofilm, nevertheless, the yields of bioproducts require further improving, especially the medium and long chain carboxylic acids, comparing to previous studies with C1/C2 as feedstocks (Table 3)

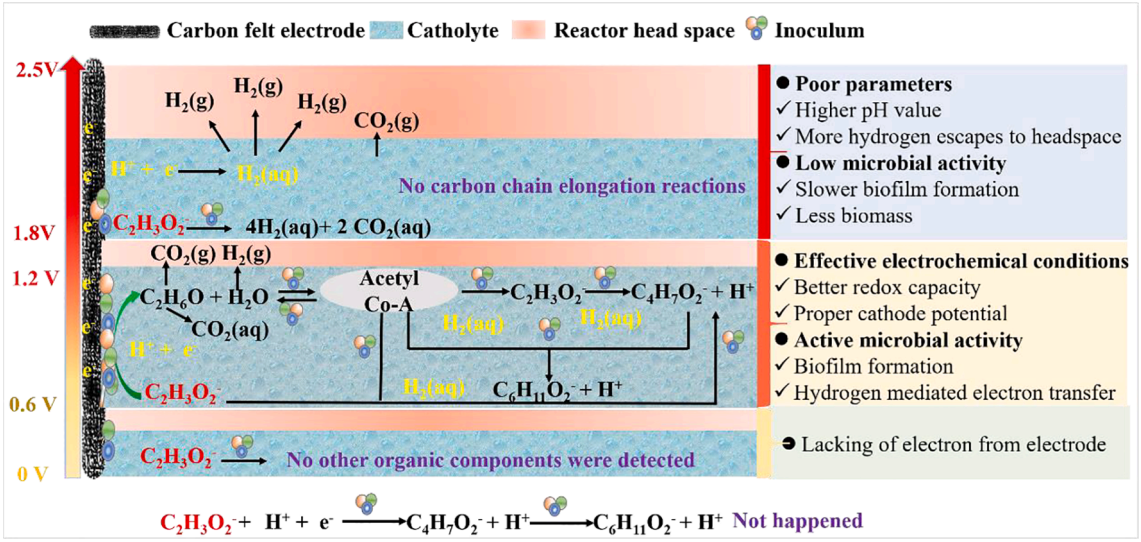

Fig. 7. The mechanism model diagram of carbon chain elongation microbiome assist bioproducts production from acetate with applied voltage regulation.

Table 3
Summary of bioproducts production performance from C1/C2 feedstocks in bioelectrochemical system.

Feedstock	Cathode material	Applied voltage	Electron donor	Maximum production rate (g/L/d), concentration (g/L), or yield	Reference
100 mM ethanol + 50 mM acetate	Carbon felt (3 mm thick, 7 cm^2)	–0.8 V vs SHE constant potential	Electrode + ethanol	Butyrate (2.0 g/L), caproate (2.0 g/L), and other small amounts of odd carbon carboxylic acids.	[7]
100 mM acetate	Graphite felt (250 cm^2)	–0.9 V vs SHE constant potential	Electrode	Ethanol (27 mg/L), butyrate (263 mg/L), caproate (739 mg/L), and caprylate (36 mg/L)	[10]
Regular supplement with pure gas CO_2, sodium bio-carbonate, or CO_2: N_2 (30–70%)	Carbon felt (4 mm thick,198 cm^2)	–0.85 V vs SHE constant potential	Electrode	Acetate (1.83 ± 0.15 g/L/d), n-butyrate (3.2 ± 0.1 g/L/d), and caproate (0.95 ± 0.05 g/L/d)	[11]
Syngas (CO: CO_2 50–50%)	Carbon felt (3 mm thick, 7 cm^2)	10 A/m^2 constant cathode current density	Electrode + CO	C2-C6 alcohols and carboxylic acids all below 0.1 g/L, Acetate (5.47 ± 0.10 g/L)	[35]
100 mM acetate	Carbon felt (2 mm thick, 15 cm^2)	0.6 V	Electrode	Ethanol (4.26 g/L), butyrate (2.40 g/L), and caproate (0.12 g/L)	This study

[7,10,11,35]. In addition, the relationship between voltage intensity and direct EET was still unclear [43,44], and the major work to be done would be on to analyze the microbial community structure and predict the functions of plankton microorganism and electrode biofilm after voltage regulation [43,44]. Reddy et al. [44] concluded that process parameters in bioelectrochemical system significantly affect the microbial composition, particularly under the closed circuit condition, where *Clostridium spp*. might be the dominant acids-producing microorganism and electroactive microorganisms such as *Eubacterium* and *Desulfovibrio* would be higher on biofilms than in planktonic condition. On the other hand, considering that the electrode has a stronger reduction capacity under high voltage conditions, the mass transfer rates among electrons, substrates, and microbial cells will be enhanced when the activity of functional microbiome increases, especially the biofilm thickness and cell density, which may significantly improve the bioproducts production performance of the entire system [45–48].

4. Conclusion

Within this work, we constructed a bioproducts production platform feeding with acetate as the sole electron acceptor and electrode as the sole electron donor, and the effect of applied voltage on acetate conversion was evaluated. The results suggest the cathode fermentation environment is more conducive to the conversion of acetate to bioproducts at 0.6 V. Compared with high voltages conditions, the electrocatalysis plays a dominant role in entire system, makes a dramatic change in cathode multi-parameters, resulting in poor bioproducts production performances at 1.8 V and 2.5 V. The correlation analysis between cathode multi-parameters and bioproduct performance at 0.6 V showed that the chain elongation process may be more dependent on the fermentation environment than the reduction of acetate to ethanol, especially for pH and ORP, and therefore, precise control based on these parameters may propose new approaches to regulate mixed culture fermentation and improve the selectivity of bioproducts in the future. Additionally, from the perspectives of biocathode redox capacity and biofilm formation, the acetate conversion is coordinated by electrode reduction and biocatalysis. This synergistic effect ensures the hydrogen mediated electron transfer builds a bridge between the active biofilm and the electrode at 0.6 V, and also limits the acetate conversion performance due to poor chain elongation microbiome activity at high voltages conditions. Hence, we consider these findings are beneficial to redefine the current situation that bioproducts production by chain elongation pathways from low value chemicals depends on stable cathode potential or current, and we suggest this work is valuable for promoting and expanding the application of the bioelectrochemical systems in bioproducts production, development, and sustainability fields.

CRediT authorship contribution statement

Ping Wu: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **He Liu:** Conceptualization, Project administration, Resources, Supervision, Writing – review & editing. **Jing Li:** Formal analysis. **Peng Ding:** Formal analysis. **Chao Zhang:** Data curation. **Jie Zhang:** Formal analysis. **Qian Jiang:** Writing - review & editing. **Yan Zhang:** Supervision. **Min-hua Cu:** Supervision. **Jia-jie Xu:** Writing – review & editing.

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. Supplementary data

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References

[1] Bhatia SK, Joo HS, Yang YH. Biowaste-to-bioenergy using biological methods–a mini-review. Energy Convers Manage 2018;177:640–60.
[2] Liu HB, Wang L, Zhang XD, Fu B, Liu H, Li YL, et al. A viable approach for commercial VFAs production from sludge: Liquid fermentation in anaerobic dynamic membrane reactor. J Hazard Mater 2019;365:912–20.
[3] Ma HJ, Liu H, Zhang LH, Yang M, Fu B, Liu HB. Novel insight into the relationship between organic substrate composition and volatile fatty acids distribution in acidogenic co-fermentation. Biotechnol Biofuels 2017;10:137.
[4] Bhatia SK, Gurav R, Choi TR, Jung HR, Yang YH, Song HS, et al. Effect of synthetic and food waste-derived volatile fatty acids on lipid accumulation in *Rhodococcus* sp. YHY01 and the properties of produced biodiesel. Energy Convers Manage 2019;192:385–95.
[5] Popiel PO. Designing reactor microbiomes for chemical production from organic waste. Trends Biotechnol 2018;8:747–50.
[6] Spirito CM, Marzilli AM, Angenent LT. Higher substrate ratios of ethanol to acetate steered chain elongation toward n-caprylate in a bioreactor with product extraction. Environ Sci Technol 2018;52:13438–47.
[7] Wu QL, Bao X, Guo WQ, Wang B, Li Y, Luo HC, et al. Medium chain carboxylic acids production from waste biomass: Current advances and perspectives. Biotech Adv 2019;37:599–615.
[8] Chu N, Liang QJ, Jiang Y, Zeng RJX. Microbial electrochemical platform for the production of renewable fuels and chemicals. Biosens Bioelectron 2020;150:111922.
[9] Jiang Y, Chu N, Zhang W, Zhang LX, Zeng RJX. Electro-fermentation regulates mixed culture chain elongation with fresh and acclimated cathode. Energy Convers Manage 2020;204:112285.

[10] Van MCAA, Ter-Heijine A, Grootscholten TIM, Steinbusch KJJ, Sleutels THJA, Hamelers HVM, et al. Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures. ACS Sustain Chem Eng 2013;1:513–8.

[11] Jiang Y, Chu N, Qian DK, Zeng JX. Microbial electrochemical stimulation of caproate production from ethanol and carbon dioxide. Bioresour Techno 2020;295:122266.

[12] Jourdin L, Sousa J, Stralen NV, Strik DPBTB. Techno-economic assessment of microbial electrosynthesis from CO_2 and/or organics: an interdisciplinary roadmap towards future research and application. *Appl.* Energ 2020;279:115775.

[13] Jiang Y, May HD, Lu L, Liang P, Huang X, Ren ZYJ. Carbon dioxide and organic waste valorization by microbial electrosynthesis and electro-fermentation. Water Res 2020;149:42–55.

[14] Schievano A, Sciarria TP, Vanbroekhoven K, Wever HD, Puig S, Andersen SJ, et al. Electro-Fermentation-Merging electrochemistry with fermentation in industrial applications. Trends Biotechnol 2016;34:866–78.

[15] Toledo-Alarcón J, Moscoviz R, Trably E, Bernet N. Glucose electro-fermentation as main driver for efficient H_2-producing bacteria selection in mixed cultures. I Int J Hydrog Energy 2019;44(4):2230–8.

[16] Coma M, Vargas RV, Roume H, Jauregui R, Pieper DH. Product diversity linked to substrate usage in chain elongation by mixed-culture fermentation. Environ Sci Technol 2016;50:6467–76.

[17] Jung S, Shetti NP, Reddy KR, Nadagouda MN, Park YK, Aminabhavi TM, et al. Synthesis of different biofuels from livestock waste materials and their potential as sustainable feedstocks–a review. Energy Convers Manage 2021;236:114038.

[18] Chowdhury R, Ghosh S, Manna D, Das S, Dutta S, Kleinsteuber S, et al. Hybridization of sugar-carboxylate-syngas platforms for the production of bio-alcohols from lignocellulosic biomass (LCB)–a state-of-the-art review and recommendations. Energy Convers Manage 2019;200:112111.

[19] Rojas MDPA, Zaiat M, Gonzalez ER, Wever HD, Pant D. Effect of the electric supply interruption on a microbial electrosynthesis converting inorganic carbon into acetate. Bioresour Techno 2018;266:203–10.

[20] Mohanakrishna G, Vanbroekhven K, Pant D. Imperative role of applied potential and inorganic carbon source on acetate production through microbial electrosynthesis. J CO_2 Util 2016;15:57–64.

[21] Lin CB, Wu P, Liu YD, Wong JWC, Yong XY, Wu XY, et al. Enhanced biogas production and biodegradation of phenanthrene in wastewater sludge treated anaerobic digestion reactors fitted with a bioelectrode system. Chem Eng J 2019;365:1–9.

[22] Rabaey K, Rozendal R. Microbial electrosynthesis revisiting the electrical route for microbial production. Nat Rev Microbiol 2010;8:706–16.

[23] Chen SS, Fang YL, Jing XY, Luo HL, Chen J, Zhou SG. Enhanced electrosynthesis performance of *Moorella thermoautotrophica* by improving cell permeability. Bioelectrochemistry 2018;121:151–9.

[24] Wu P, Zhang LJ, Lin CB, Xie XX, Yong XY, Wu XY, et al. Extracting heavy metals from electroplating sludge by acid and bioelectrical leaching using *Acidithiobacillus ferrooxidans*. Hydrometallurgy 2020;191:105225.

[25] Grootscholten TIM, Steinbusch KJJ, Hamelers HVM, Buisman CJN. Improving medium chain fatty acid productivity using chain elongation by reducing the hydraulic retention time in an upflow anaerobic filter. Bioresour Technol 2013;136:735–8.

[26] Duber A, Jaroszynski L, Zagrodnik R, Chwialkowska J, Juzwa W, Ciesielski S, et al. Exploiting the real wastewater potential for resource recovery n-caproate production from acid whey. Green Chem 2018;20:3790–803.

[27] Lim SS, Fontmorin JM, Izadi P, Daud WRW, Scott K, Yu EH. Impact of applied cell voltage on the performance of a microbial electrolysis cell fully catalysed by microorganisms. Int J Hydrog Energy 2020;45:2557–68.

[28] Izadi P, Fontmorin JM, Godain A, Yu EH, Head IM. Parameters influencing the development of highly conductive and efficient biofilm during microbial electrosynthesis: the importance of applied potential and inorganic carbon source. NPJ Biofilms Microbi 2020;6:40.

[29] Rebaca GC, Juan ML, Jorge R, Robbert K. Linking thermodynamics and kinetics to assess pathway reversibility in anaerobic bioprocesses. Energ Environ Sci 2013;6:3780–9.

[30] Jiang Y, Lu L, Wang H, Shen RX, Ge Z, Hou DX, et al. Electrochemical control of redox potential arrests methanogenesis and regulates products in mixed culture electro-fermentation. ACS Sustain Chem Eng 2018;6:8650–8.

[31] Wu YD, Luo XB, Qin BL, Li FB, Haggblom MM, Liu TX. Enhanced current production by exogenous electron mediators via synergy of promoting biofilm formation and the electron shuttling process. Environ Sci Technol 2020;54:7217–25.

[32] Chu N, Liang QJ, Zhang W, Ge Z, Hao W, Jiang Y, et al. Waste C1 gases as alternatives to pure CO_2 improved the microbial electrosynthesis of C4 and C6 carboxylates. ACS Sustain Chem Eng 2020;8:8773–82.

[33] Jourdin L, Raes SMT, Buisman CJN, Strik DPBTB. Critical biofilm growth throughout unmodified carbon felts allows continuous bioelectrochemical chain elongation from CO_2 up to caproate at high current Density. Front Energy Res 2018;6:7.

[34] Jain K, Parida S, Mangwani N, Dash H, Das S. Isolation and characterization of biofilm-forming bacteria and associated extracellular polymeric substances from oral cavity. Ann Microbiol. 2013, 60, 4553–1562.

[35] Liu C, Luo G, Liu HP, Yang ZY, Angelidaki I, Thong SO, et al. CO as electron donor for efficient medium chain carboxylate production by chain elongation: Microbial and thermodynamic insights. Chem Eng J 2020;390:124577.

[36] Xu JJ, Guzman JJL, Angenent LT. Direct Medium-chain carboxylic acid oil separation from a bioreactor by an electrodialysis/phase separation cell. Environ Sci Technol 2021;55:634–44.

[37] Carvajal-Arroyo JM, Andersen SJ, Ganigué R, Rozendal RA, Angenent LT, Rabaey K. Production and extraction of medium chain carboxylic acids at a semi-pilot scale. Chem Eng J 2021;416:127886.

[38] Hirose A, Kasai T, Aoki M, Umemura T, Watanabe K, Kouzuma A. Electrochemically active bacteria sense electrode potentials for regulating catabolic pathways. Nat Commun 2018;9:1083.

[39] Zhao ZQ, Wang JF, Li Y, Zhu TT, Yu QL, Wang TT, et al. Why do DIETers like drinking: Metagenomic analysis for methane and energy metabolism during anaerobic digestion with ethanol. Water Res 2020;171:115425.

[40] Zeppilli M, Paiano P, Torres C, Pant D. A critical evaluation of the pH split and associated effects in bioelectrochemical processes. Chem Eng J 2021;422:130155.

[41] Hu HQ, Fan YZ, Liu H. Hydrogen production using single-chamber membrane-free microbial electrolysis cells. Water Res 2008;42:4172–8.

[42] Thrash JC, Coates JD. Review: Direct and indirect electrical stimulation of microbial metabolism. Environ Sci Technol 2008;42:3921–31.

[43] Heijine AT, Pereira MA, Pereira J, Sleutels T. Electron storage in electroactive biofilms. Trends Biotechnol 2021;39:34–42.

[44] Reddy MV, Elmekawy A, Pant D. Bioelectrochemical synthesis of caproate through chain elongation as a complementary technology to anaerobic digestion. Biofuels, Bioproducts Biorefining 2018;12(6):966–77.

[45] Tartakovsky B, Mehta P, Santoyo G, Guiot SR. Maximizing hydrogen production in a microbial electrolysis cell by real-time optimization of applied voltage. Int J Hydrog Energy 2011;36(17):10557–64.

[46] Lin R, Cheng J, Murphy JD. Unexpectedly low biohydrogen yields in co-fermentation of acid pretreated cassava residue and swine manure. Energy Convers Manage 2017;151:553–61.

[47] Anzola Rojas MDP, Mateos R, Sotres A, Zaiat M, Gonzalez ER, Escapa A, et al. Microbial electrosynthesis (MES) from C_O2 is resilient to fluctuations in renewable energy supply. Energy Convers Manage 2018;177:272–9.

[48] Zhang K, Abomohra AEF, Xie SX, Yu ZS, Guo Q, Peng L, et al. A sustainable approach for efficient conversion of lignin into biodiesel accompanied by biological pretreatment of corn straw. Energy Convers Manage 2019;199:111928.