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Microbial electrosynthesis of organic chemicals from CO_2 by *Clostridium scatologenes* ATCC 25775^T

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Abstract

Background: The conversion of CO_2 into high value-added products has a very important environmental and economic significance. Microbial electrosynthesis (MES) is a promising technology, which adopts a bioelectrochemical system to transform CO_2 into organic chemicals.

Results: In this study, *Clostridium scatologenes ATCC* 25775^T, an anaerobic acetogenic bacterium, demonstrated its utility as a biocatalyst in a MES system, for the first time. With the cathodic potential of the MES system decreased from − 0.6 to − 1.2 V (vs. Ag/AgCl), the current density of the MES, and the production of organic chemicals, increased. Combining the genetic analysis and the results of the wet lab experiments, we believe *C. scatologenes* may accept electrons directly from the cathode to reduce CO_2 into organic compounds at a potential of − 0.6 V. The acetic and butyric acid reached a maximum value of 0.03 and 0.01 g/L, respectively, and the maximum value of total coulombic efficiency was about 84%, at the potential of − 0.6 V. With the decrease in cathodic potentials, both direct electron transfer and exogenous electron shuttle, H_2 might be adopted for the *C. scatologenes* MES system. At a potential of − 1.2 V, acetic acid, butyric acid and ethanol were detected in the cathodic chamber, with their maximum values increasing to 0.44, 0.085 and 0.015 g/L, respectively. However, due to the low H_2 utilization rate by the *C. scatologenes* planktonic cell, the total coulombic efficiency of the MES system dropped to 37.8%.

Conclusion: *Clostridium scatologenes* is an acetogenic bacterium which may fix CO_2 through the Wood–Ljungdahl pathway. Under H_2 fermentation, *C. scatologenes* may reduce CO_2 to acetic acid, butyric acid and ethanol. It can also be used as the biocatalyst in MES systems.

Keywords: *Clostridium scatologenes* ATCC 25775^T, Microbial electrosynthesis, CO_2

Background

Rapid global economic growth of human civilization has led to an increase in fuel usage (Huber 2009). As a result, the greenhouse gas emissions, caused by use of conventional fossil fuels, have become a serious issue, in need of solutions (Bajracharya et al. 2017; Liao et al. 2016). On one hand, CO_2 is the major contributor to greenhouse gas, which is responsible for climate change (Liao et al. 2016; O'Neill and Oppenheimer 2002); on the other hand, CO_2 is the most extensive and inexpensive carbon resource in the world. However, due to the extreme stability of CO_2, its reduction cannot occur spontaneously (Pearson et al. 2012). Therefore, the development of novel technologies for the conversion of CO_2 into high value-added products has a very important environmental and economic significance. At present, the major technologies for CO_2 reduction include biotransformation (Nowak and Crane 2002), electrochemical reduction (Olah et al. 2013; Nevin et al. 2010; Zhang et al. 2013; Lovley and Nevin 2013; Lovley 2011a), photocatalytic reduction (Chu et al. 2008), catalytic hydrogenation (Karelovic et al. 2012) and so on.

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Among all those technologies, the MES is considered as a promising technology for production of value-added chemicals and fuels, from CO_2 (Patil et al. 2015). MES is an application of a bioelectrochemical system (Yong et al. 2014; Yang et al. 2017; Si et al. 2015), in which anaerobic microbes can accept electrons, from a cathode, as an energy source and CO_2, as a carbon source, to form high value-added products, including methane (Cheng et al. 2009), hydrogen peroxide (Rozendal et al. 2009), formic acid (Kim et al. 2014), acetic acid (Huang et al. 2014), ethanol (Steinbusch et al. 2010), acetone (Kim and Kim 1988), butanol (Kim and Kim 1988), butyric acid, 2,3-butanedio (Nevin et al. 2010), and so on. Therefore, an appropriate biocatalyst is the key factor affecting the transformation efficiency and the product spectrum in the MES (Bajracharya et al. 2017).

Both pure culture (Nevin et al. 2010; Zhang et al. 2013; Gong et al. 2013; Nie et al. 2013; Giddings et al. 2015; Faraghiparapari and Zengler 2017) or mixed microbial consortia (Gildemyn et al. 2015; Jourdin et al. 2015; Marshall et al. 2013; Jourdin et al. 2014; Batlle-Vilanova et al. 2016) can be used as biocatalysts in the MES system. The mixed community-driven MES illustrated a higher acetate production rate than did the pure culture. However, due to the limited knowledge as to the roles and functions of individual species, within the mixed community, optimization of the production rate, through regulating the community mixture, is still a black-box operation (Song et al. 2017). Compared to mixed communities, the production rates of pure cultures, in the MES, is relatively low. However, since there is only one strain as the biocatalyst of the MES, the MES system is relatively simple, which is helpful for us in further understanding the mechanism and regulation of the biocatalyst, within the MES system.

Since Nevin et al. first demonstrated that *Sporomusa ovata* can take electrical current, as an energy source, for the reduction of CO_2 into multi-carbon organic compounds, much effort has been directed toward the mechanisms and regulation of acetogens, inside a MES system (Nevin et al. 2010). *Sporomusa silvacetic* and, *Sporomusa sphaeroides* were both reported to be capable of activity, as biocatalysts, in MES, although their electrical conversion efficiency is 20–30 times lower than that of *Sporomusa ovate* (Lovley and Nevin 2013). Later, other acetogens, *Clostridium ljungdahlii*, *Clostridium aceticum*, and *Moorella thermoacetica*, were reported to adopt electrons, from a cathode, using a C-1 block, such as CO_2 or CO, as the carbon source, to synthesize acetyl-CoA via the *Wood–Ljungdahl* pathway. This acetyl-CoA may be further converted to a wide variety of fuels and chemicals, such as formic acid, acetic acid and 2-oxobutyrate (Nevin et al. 2011). However, not all acetogens are able to obtain electrons directly from the surface of the cathode, for MES. *Acetobacterium woodii* has been shown to only use hydrogen, as an electron mediator, to reduce CO_2 (Lovley and Nevin 2013; Lovley 2011b; Schuchmann and Muller 2014).

Clostridium scatologenes ATCC 25775^T is an anaerobic, Gram-positive, spore-forming bacterium (Holdeman 1977). Its genome contains a complete set of genes related to the Wood–Ljungdahl pathway, in addition to a series of metabolic pathways for acetic acid, butyric acid, and ethanol synthesis (Zhu et al. 2015). *C. scatologenes* is a unique model organism, known to be capable of producing 3-methylindole and 4-methylphenol, in addition to its volatile fatty acid production (Smith 1975; Kusel et al. 2000). Therefore, research, on CO_2 reduction by MES, using *C. scatologenes* as a biocatalyst, may enrich the selection of biocatalysts for MES systems, thereby further improving our understanding of these MES systems.

Methods

Bacteria and growth medium

Clostridium scatologenes ATCC 25775^T was purchased from the American Model Culture Collection (ATCC, Manassas, VA, USA). The strain was activated anaerobically in ATCC 2107 medium. The ATCC 2107 medium comprised the following (per liter of deionized water): 10 g peptone, 10 g beef extract, 3 g yeast extract, 5 g glucose, 5 g NaCl, 3 g sodium-acetate, 1 mL azure (0.025%), 0.5 g Cys-HCl, pH 6.8. Once the strain had been activated, the hydrogen autotrophic fermentation was carried out in growth medium. The growth medium (Song et al. 2017) comprised the following (per liter of deionized water): 50 mL PETC salt solution, 10 mL trace element solution, 10 mL Wolfe's vitamin solution, 1.0 g yeast extract, 0.5 g Cys-HCl and 0.5 mL azure (0.1%), pH 6.8. The PETC salt solution comprised the following (per liter of deionized water): 20 g NH_4Cl, 2 g KCl, 4 g $MgSO_4 \cdot 7H_2O$, 16 g NaCl, 2 g KH_2PO_4, 0.4 g $CaCl_2$. The trace elements solution was comprised in the following manner (per liter of deionized water): 2 g Nitrilotriacetic acid, 1.3 g $MnCl_2 \cdot 4H_2O$, 0.4 g $FeSO_4 \cdot 7H_2O$, 0.2 g $CoCl_2 \cdot 6H_2O$, 0.2 g $ZnSO_4 \cdot 7H_2O$, 0.02 g $CuCl_2 \cdot 2H_2O$, 0.02 g $NiCl_2 \cdot 6H_2O$, 0.02 g $Na_2MoO_4 \cdot 2H_2O$, 0.02 g Na_2SeO_3, 0.025 g $Na_2WO_4 \cdot 2H_2O$. The Wolfe's vitamin solution comprised the following (per liter of deionized water): 2 mg Biotin, 2 mg Folic acid, 10 mg Pyridoxine hydrochloride, 5 mg Thiamine · HCl, 5 mg Riboflavin, 5 mg Nicotinic acid, 5 mg Calcium D-(+)-pantothenate, 0.1 mg vitamin B12, 5 mg *p*-Aminobenzoic acid, 5 mg Thioctic acid. In the MES experiment, the cathode chamber was also filled with growth medium. The medium used for the anode chamber

consisted of 50 ml L PETC salt solution, 6 g L NaCl and 2 g L KCl.

Autotrophic fermentation of *C. scatologenes*

The hydrogen autotrophic fermentation experiment was carried out in a 1 L serum bottle containing 400 ml of growth medium. The inoculums concentration of hydrogen fermentation experiments was 10% (V/V). Both, a high hydrogen-containing gas mixture (H_2/CO_2 (80/20, v/v)) and a low hydrogen-containing gas mixture ($H_2/CO_2/N_2$, (10/10/80, v/v/v)), were adopted to feed the *C. scatologenes,* for autotrophic fermentation, respectively. The gas fermentation experiments were carried out at 30 ± 2 °C. The growth curve and the major metabolites, in the fermenters, were measured every 12–24 h.

Microbial electrosynthesis of *C. scatologenes* at different potentials

The MES apparatus was set up as previous reports (Song et al. 2017). The MES reactor, with an internal volume of 280 mL in both anode and cathode chambers, was made of glass. The anode and cathode chambers were separated by a proton exchange membrane (Nafion 117, Dupont Co., USA). Carbon felt (CF, 50 mm × 50 mm × 5 mm, length × width × thickness) was used as an anode in all the reactors. The carbon dioxide was fed through the pipeline into the cathode chamber of MES by opening the cylinder valve. The cathode was poised with a potentiostat setting of different potentials. The inoculums concentration of the MES experiment was 10% (V/V). The MES biocathode was constructed via two rounds of autotrophic growth of *C. scatologenes*, along with hydrogen-containing gas mixtures. In the first round, the high hydrogen-containing gas mixture was continuously fed into the cathode, of the reactor, as the electron donor, promoting the growth of a biofilm, on the cathode surface, over a span of 5 days. On the 6th day, the culture was bubbled with the low hydrogen-containing gas mixture for 10 days before switching to 100% CO_2 microbial electrosynthesis.

During the MES stage, the cathode was poised with potentiostat settings of − 0.6, − 0.8, − 1.05 and − 1.2 V (versus Ag/AgCl), respectively. The microbial electrosynthesis experiment lasted 28 days and the experimental period, at each potential, was 7 days. A multi-channel potentiostat (CHI1000C, Shanghai Chenhua Instrument Co. Ltd, China) was used for all experiments. All the MES reactors were maintained at room temperature (25 ± 2 °C). Coulombic efficiencies (*CE*) were calculated as $CE = CP/CT \times 100\%$, where *C*T is the total coulombs consumed, calculated by integrating the area under the current-versus-time curve (*i*–*t* curve). *C*P is the coulombs found in the product, calculated as $CP = b \times n \times F$, where *b* is the number of electrons in the product (8/eqmol), *n* is the number of moles of product, and *F* is Faraday's constant (96,485 C/mol).

Analytical methods

The products of MES were analyzed using gas chromatography (GC-2010 Plus,SHIMADZU). The surface morphologies, of the cathode surfaces, were studied by a scanning electron microscope (SEM; JSM-5900, Japan). The biofilms, on the cathodes, were stained with the LIVE/DEAD BacLight Viability Kit and imaged with a Confocal Scanning Laser Microscope (CSLM, Zeiss LSM710). The current was continuously monitored using a precision multimeter and a data acquisition system (Keithley Instruments 2700, USA). Gas samples were collected, using a gas-tight plastic syringe, and analyzed by gas chromatography (GC-2010 Plus,SHIMADZU) equipped with a thermal conductivity detector (TCD).

Results

Autotrophic fermentation of *C. scatologenes*

The fermentation results, for *C. scatologenes* with high hydrogen-containing gas mixture, are shown in Fig. 1a. The OD_{600} of bacteria increased, from the initial 0.05–0.12, within 48 h of inoculation, and then the OD_{600} began to decline sharply, finally becoming stable. The concentration of acetic acid and butyric acid increased with the growth of *C. scatologenes*, The highest concentration of acetic acid reached 1.25 g/L, on the 6th day, and the highest concentration of butyric acid reached 0.320 g/L, on the 7th day, respectively. The concentration of ethanol was stable in the fermentation growth, reached a maximum value of 0.192g/L on the 7th day.

The results of fermentation, by *C. scatologenes,* with the low hydrogen-containing gas mixture, are shown in Fig. 1b. The OD_{600} increased from the initial 0.05–0.11, then began to decrease and was finally stabilized at 0.08. On day 6, the highest concentration, of acetic acid, was reached, at 0.362 g/L, while the highest concentration, of butyric acid and ethanol, was reached, at 0.145 g/L, on the 4th day, and at 0.083 g/L, on the second day, respectively. No butanol was detected. It can be seen that with the decrease of hydrogen, the yield of metabolites decreased obviously, with acetic acid, butyric acid and ethanol decreasing 70, 55 and 57%, respectively. During the 7 days of gas fermentation, we detected trace amounts of formic acid and no 2-oxobutyrate. The highest concentration of formic acid was 0.023 g/L with high hydrogen-containing gas mixture, while that was 0.008 g/L with low hydrogen-containing gas mixture. We detected trace amounts of 2-oxobutyrate at day 12 when we extended the fermentation time. In the 15 days of hydrogen fermentation, the highest concentrations of 2-oxobutanoic

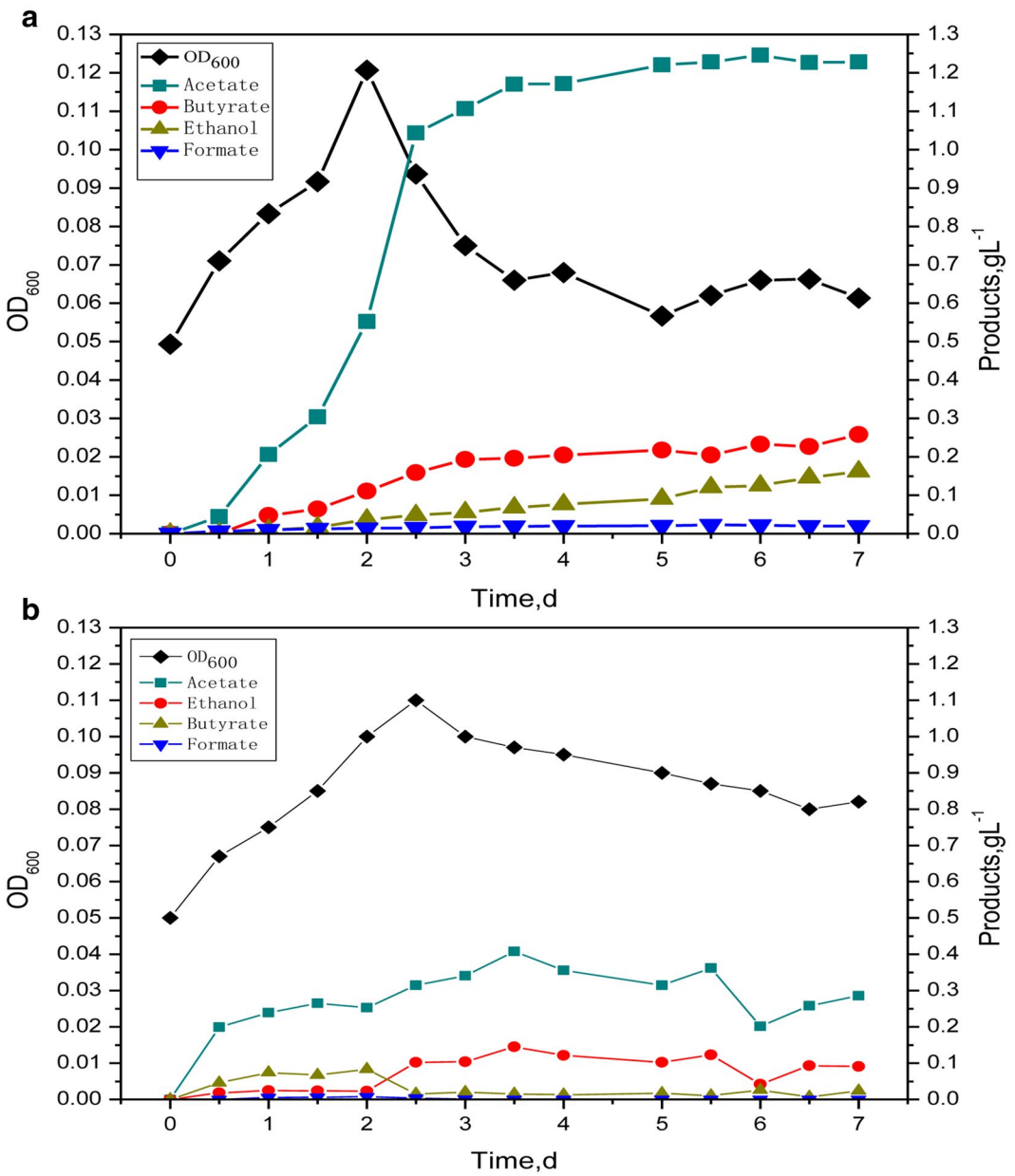

Fig. 1 OD_{600} and products formation from hydrogen autotrophic fermentation using *C. scatologenes* ATCC 25775: (**a**) the autotrophic fermentation of *C. scatologenes* ATCC 25775 by mixture gas (H_2/CO_2,(80/20, v/v)) (**b**) the autotrophic fermentation of *C. scatologenes* ATCC 25775 by mixture gas ($H_2/CO_2/N_2$,(10/10/80, v/v/v))

acid in the high and low hydrogen autotrophic fermentation were 0.007 and 0.004 g/L, respectively.

Microbial electrosynthesis by *C. scatologenes* at − 0.6, − 0.8, − 1.05, − 1.2 V

Acetic acid and a small amount of butyric acid were observed in the cathodic solution, while no ethanol was detected and no H_2 is produced, when the cathodic potential was set up at − 0.6 V, in the MES system. As shown in Fig. 2, acetic acid concentration showed a constant rate of increase, during the first 3 days, to a maximum value of 0.03 g/L, then decreased a little bit, eventually stabilizing, over the 7 day reaction period. Different from the pattern of acetic acid, the concentration of butyric acid continued to grow to a maximum value of 0.01 g/L, throughout the entire reaction period. The OD_{600} reached a maximum value, of 0.052, on the 6th day and subsequently remained stable. The current density

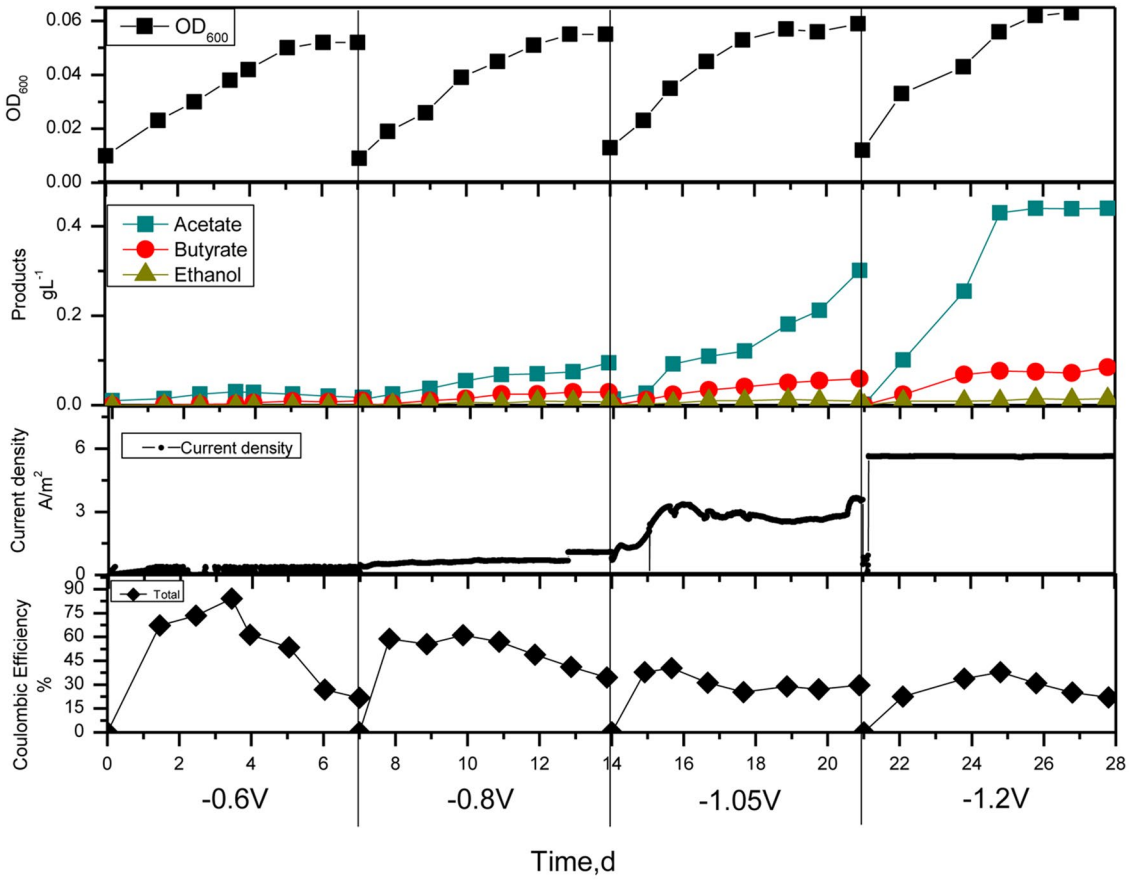

Fig. 2 Microbial electrosynthesis experiments of *C. scatologenes* ATCC 25775 at different potentials: (**a**) OD_{600} of *C. scatologenes* ATCC 25775 at four different potentials (**b**) product formation by *C. scatologenes* ATCC 25775 at four different potentials (**c**) current density of *C. scatologenes* ATCC 25775 at four different potentials (**d**) coulombic efficiency of *C. scatologenes* ATCC 25775 at four different potentials

of this MES system was relatively low, at about 0.2 A/m^2, while the maximum value of total coulombic efficiency was achieved at day 3, at about 84%.

The potential was increased to − 0.8 V, from day 8 through day 14. During this period, 4.7% H_2 was produced, acetic acid and small amounts of butyric acid and ethanol were observed in the cathodic chamber (Fig. 2). The concentration of acetic acid and butyric acid increased continuously over the whole reaction stage. On the 14th day, the concentration of acetic acid reached a maximum value of 0.095 g/L, while that of the butyric acid reached a maximum value of 0.029 g/L. Differing from the − 0.6 V stage, ethanol was detectable, at − 0.8 V, reaching a maximum value of 0.009 g/L, on the 12th day of the reaction (Table 1). At − 0.8 V, the OD_{600} of *C. scatologenes* continuously grew, reaching 0.055 on the 14th day. The current density of the MES system, at − 0.8 V, was relative stable. The current density was maintained at about 0.76 A/m^2, through the entire − 0.8 V stage. Compared to that, at − 0.6 V, the maximum total coulombic efficiency of the MES system deceased 61% at − 0.8 V.

When the potential of the MES system was increased to − 1.05 V, 9.7% H_2 was produced, the production of acetic acid, butyric acid and ethanol, in the cathodic chamber, increased as well. The concentration of acetic acid, butyric acid and ethanol increased continuously, over the whole reaction stage, reaching maximum values of 0.301, 0.059 and 0.013 g/L, respectively, on day 21 (Fig. 2). Due to this increase, of the potential, the current density of the MES system increased to 2.4 A/m^2. However, the total coulombic efficiency of the MES system dropped dramatically. The maximum value, of the total coulombic efficiency, was about 40.5%.

When the potential of the MES system was increased to − 1.2 V, 13.1% H_2 was produced, the production of acetic acid, butyric acid and ethanol also continuously increased. The concentrations of acetic acid, butyric acid and ethanol reached their maximum values on day 28, of the entire reaction period, which were 0.44, 0.085

Table 1 Results of hydrogen autotrophic fermentation and microbial electrosynthesis experiments

	$OD_{600,max}$	Formate (g/L, max)	Acetate (g/L, max)	Butyrate (g/L, max)	Ethanol (g/L, max)	Rate of acetate (g/L/d)	H_2 (%)
80%H_2–10%CO_2	0.120	0.023	1.250	0.320	0.192	0.170	80
10%H_2–10%CO_2–80%N_2	0.110	0.008	0.362	0.145	0.083	0.050	10
− 0.6 V	0.052	–	0.030	0.010	0	0.001	0
− 0.8 V	0.055	–	0.095	0.051	0.010	0.012	4.7972
− 1.05 V	0.059	–	0.301	0.059	0.013	0.041	9.7412
− 1.2 V	0.063	–	0.440	0.085	0.015	0.060	13.1777

and 0.015 g/L, respectively (Fig. 2). Not surprisingly, the current density of the system increased as well. During this stage, the current density was quite stable and maintained a value of about 5.6 A/m^2. The total coulombic efficiency of the MES system continuously dropped, reaching 37.8% at − 1.2 V.

Analysis of biofilm

At the end of the experiment, the morphology of the electrode surfaces was analyzed, by SEM, at different scales. As shown in Fig. 3, *C. scatologenes* was fully adhered to the carbon fiber, forming a biofilm. CSLM measurements (Fig. 4) were also performed, to further understand the composition of the *C. scatologenes* biofilm. CSLM demonstrated that a mixture of live and dead *C. scatologenes* (yellow) were dominant on the electrode, followed by live (green) and dead (red) cells. The average thickness of the *C. scatologenes* biofilm was about 20 μm, which was thinner than the mixed consortia biofilm, on the cathode, which was around 35 μm (Song et al. 2017). This might be due to differences in the strain component and in the cathodic potential settings adopted in the MES experiments.

Discussion

Clostridium scatologenes is a strictly anaerobic, Gram-positive, spore-forming bacterium (Holdeman 1977). The whole genome sequencing of *C. scatologenes* ATCC 25775 was carried out by our group (Zhu et al. 2015). The complete genome sequence of *C. scatologenes* ATCC 25775 was deposited in the GenBank with accession number CP009933. *C. scatologenes* was shown to utilize H_2/CO_2 or CO (Kusel et al. 2000). Its genome sequencing confirmed the presence of all the enzymes and proteins, referred to in the Wood–Ljungdahl pathway, in its chromosome (Table 2, Fig. 5). Apart from formate dehydrogenase and carbon-monoxide dehydrogenase complex, the remaining genes were organized in a cluster, whose gene

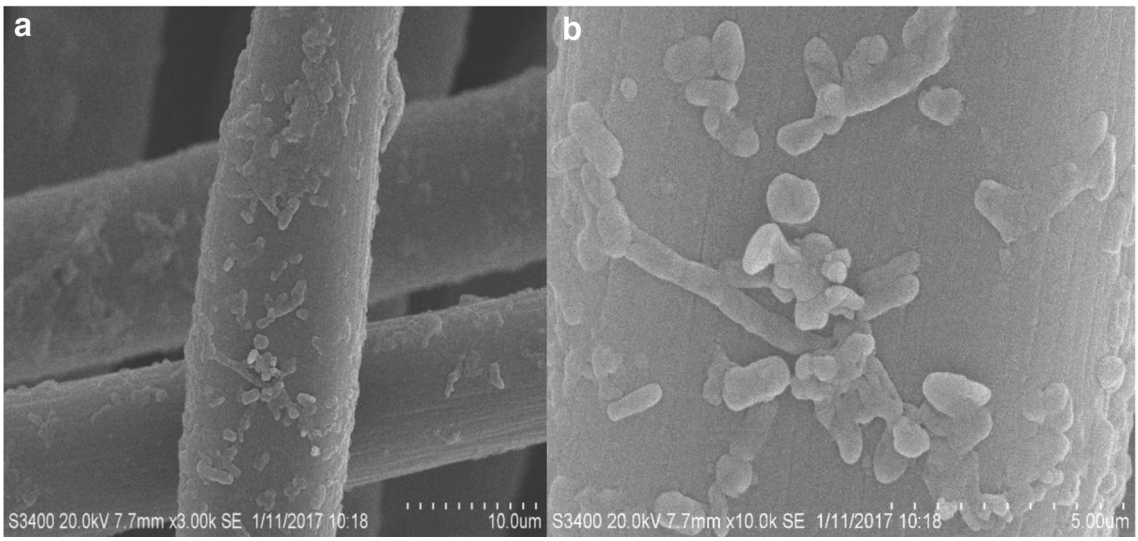

Fig. 3 SEM (**a**, **b**) of *C. scatologenes* ATCC 25775 after microbial electrosynthesis experiments ended in low and high scale, respectively

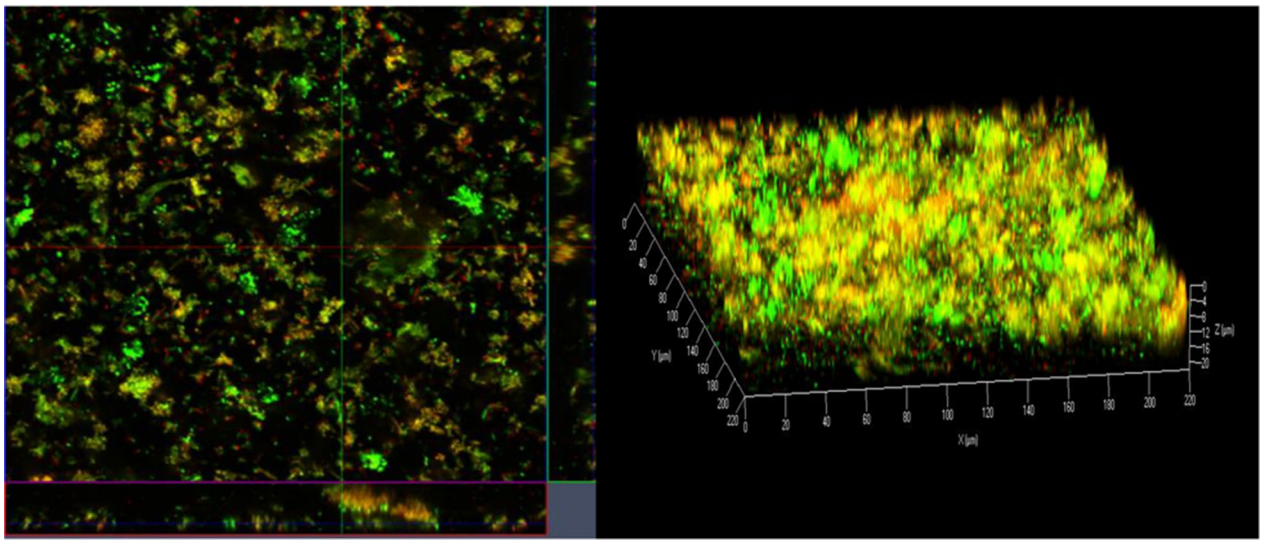

Fig. 4 Microscopy of *C. scatologenes* ATCC 25775 after microbial electrosynthesis experiments ended: top-down and side-view confocal laser scanning micrograph of LIVE/DEAD BacLight viability-stained biofilm

Table 2 Genes predicted to be involved in Wood–Ljungdahl pathway of *C. scatologenes* ATCC 25775

Locus tag	Predicted function
CSCA_2819	Acetate kinase
CSCA_2820	Phosphate acetyltransferase
CSCA_3687	Electron transfer flavoprotein alpha-subunit
CSCA_3688	Electron transfer flavoprotein beta subunit
CSCA_3689	Butyryl-CoA dehydrogenase
CSCA_3690	Acetyl-CoA acetyltransferase
CSCA_3691	3-hydroxybutyryl-CoA dehydrogenase
CSCA_3692	3-hydroxybutyryl-CoA dehydratase
CSCA_4347	Phosphate butyryl transferase
CSCA_4348	Butyrate kinase
CSCA_1267	Acetaldehyde dehydrogenase
CSCA_0076	Bifunctional acetaldehyde-CoA/alcohol dehydrogenase
CSCA_2937	NADH-dependent butanol dehydrogenase A
CSCA_2951	NADH-dependent butanol dehydrogenase
CSCA_5280	CoA-acylating aldehyde dehydrogenase

content and organization were identical to that found in *C. ljungdahlii* (Si et al. 2015) and *C. difficile* (Yang et al. 2017). In the Wood–Ljungdahl pathway, hydrogen provides the electrons and protons required for the synthesis of the products. Therefore, the adequacy of the hydrogen supply should positively correlate to the production efficiency of the acetate, during the CO_2 fixation reaction, by *C. scatologenes*. Our autotrophic fermentation experiments, with different hydrogen contents, confirmed this hypothesis. Under the high hydrogen-containing gas mixture, the maximum production of acetic acid was 1.250 g/L, after 7 days, with a production rate of 0.170 g/L/d, while under the low hydrogen-containing gas mixture, the maximum production of acetic acid was 0.362 g/L, after 7 days, with a production rate of 0.050 g/L. However, no significant difference in the OD_{600} was observed from either high or low hydrogen-containing autotrophic fermentations. The acetogens, such as *Sporomusa* sp., *Clostridium ljungdahlii*, *Clostridium aceticum*, *Moorella thermoacetica* and *Acetobacterium woodii* were reported to be used as biocatalyst in the MES system (Nevin et al. 2010, 2011). They reduced C-1 blocks, such as CO_2 or CO, to acetyl-CoA, through the Wood–Ljungdahl pathway, and acetyl-CoA could be further converted to a wide variety of fuels and chemicals. *C. scatologenes* is also an acetogenic bacterium that can fix CO_2, through the Wood–Ljungdahl pathway, and produce a variety of chemicals. However, its ability for CO_2 fixation and the production of volatile acids, through MES, was unknown. Therefore, the use of *C. scatologenes* as a cathodic biocatalyst, in the MES system, at different potentials, and its electron transfer mechanism, were systematically studied in this research.

H^+-dependent Rnf complex, ATPase and hydrogenase were proposed to play a major role in direct electron transfer (DET) in MES system (Shin et al. 2017) According to the genetic approach, H^+-dependent Rnf-complex (CSCA_2967-2972), H^+-ATPase (CSCA_3911-3918) and hydABCDEF (CSCA_0918-0921,CSCA_1667,CSCA_1668) were found in *C. scatologenes*, which are homologous to those in *C.*

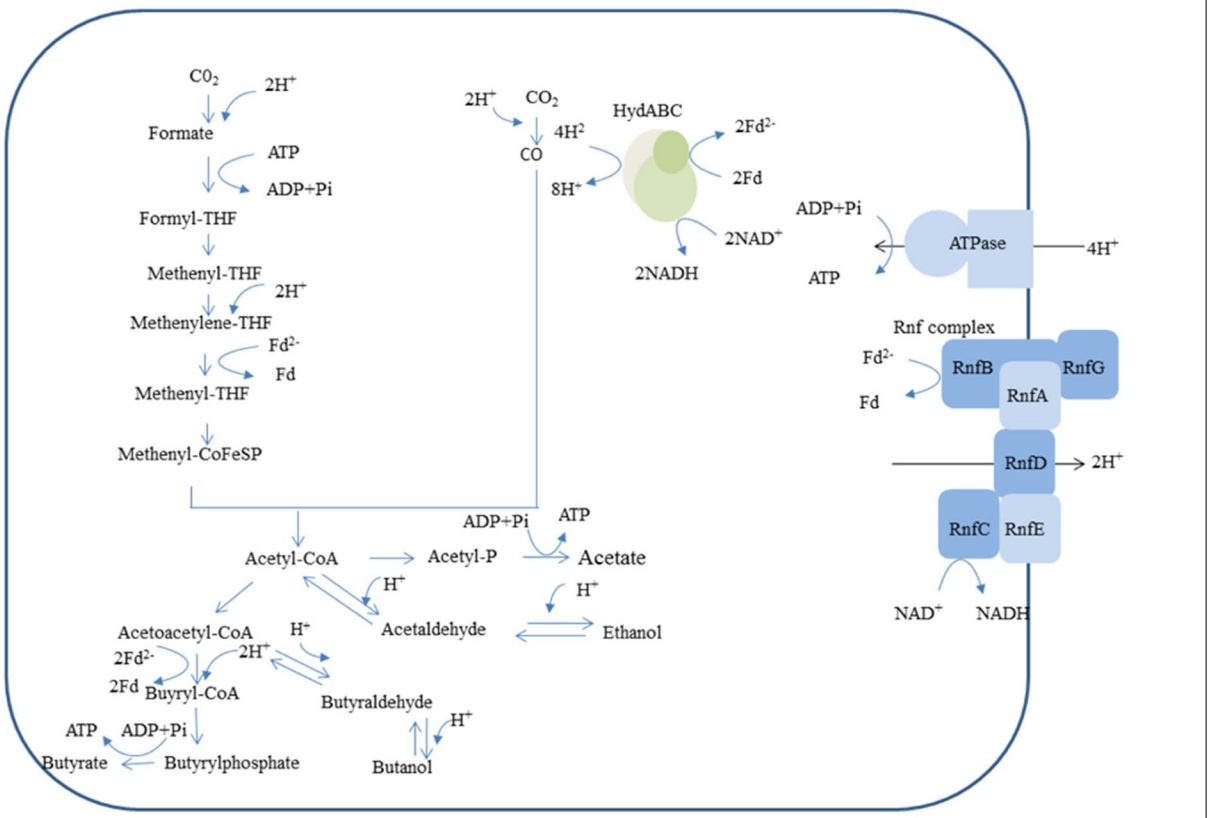

Fig. 5 The Wood–Ljungdahl pathway of *C. scatologenes* ATCC 25775 based on genome sequence

ljungdahlii and *C. aceticum* (Fig. 5). Therefore, theoretically, *C. scatologenes* should be capable of receiving electrons directly from an electrode, just as *C. ljungdahlii* and *C. aceticum* do. To confirm our hypothesis, we carried out our MES at potential of − 0.6 V. A potential of − 0.6 V is sufficiently low for microbial electrosynthesis to occur, without the significant production of hydrogen (Nevin et al. 2010). Thus, the successful production of volatile acids, at this potential, might indicate the direct electron acceptance by the biocatalyst, in the MES system.

At potential of − 0.6 V, with 100% CO_2, the autotrophic growth and production of acetic acid and butyric acid, by MES with biocatalyst *C. scatologenes*, was observed, which indicated that *C. scatologenes* may obtain electrons directly from the cathode surface, in the MES system, thereby reducing CO_2 into organic acid. Although the maximum production of acetic acid was achieved in the third day, and then started to decrease, the production of butyric acid and the OD_{600} of *C. scatologenes* were continuously increasing. This phenomenon proves that *C. scatologenes* may successfully utilize electrons from the cathode to reduce CO_2 into multi-bound chemicals. The decrease in production of the acetic acid might be attributed to the utilization of this acetic acid as the substrate in this process (Yin et al. 2017).

As the cathodic potential, of an MES system, decreases, the hydrogen evolution in the cathodic chamber of the MES rises. At potentials of − 0.8, − 1.05 and − 1.2 V, the hydrogen concentration, in the cathode chamber, may accumulate to 4.7, 9.7 and 13.1%, respectively. In these cases, both DET and indirect electron transfer through exogenous electron shuttle, H_2 may be performed in the MES system(Tremblay and Zhang 2015). Combining the effects of DET and H_2, the maximum acetate production reached 0.095, 0.301 and 0.440 g/L, at potentials of − 0.8, − 1.05, and − 1.2 V, respectively. However, we noticed that the total coulombic efficiency, of the MES system, decreased with the decrease in cathodic potential. At a potential of − 0.6 V, the total coulomb efficiency was 84%, while at a potential of − 1.2 V, the total coulombic efficiency dropped to only 37.8%. This drop might be due to the relative low CO_2 transformation efficiency of the *C. scatologenes* planktonic cell. As the cathodic potential decreases, the H_2 evolution rate increases. The H_2 utilization rate of the *C. scatologenes* planktonic cell was

lower than the H_2 evolution rate in the cathodic chamber, thereby leading to the accumulation of H_2 and the decrease in coulombic efficiency of the MES system. CO_2 fixation is an important function of *C. scatologenes* biotransformation, however, H_2 is not sufficient in natural environment. Here, we successfully demonstrated that by adopting MES system, CO_2 can be reduced by electrons, directly or through H_2, and accomplish with the storage of electrical energy into chemical bond.

Due to the differences in cathodic potentials, number of operating days, and cathode material, it is difficult to compare our maximum acetate concentration with those in most of the other literature. However, we made a rough comparison of the acetate production rate of *C. scatologenes* with that of *S. ovata* (Nevin et al. 2010), *C. ljungdahlii*, *C. aceticum,* and *M. thermoacetica* (Nevin et al. 2011), at a potential of − 0.6 V. The acetate production rate of *C. scatologenes* (0.001 g/L/D) is lower than that of *S. ovate* (0.008 g/L/D) (Nevin et al. 2010), but more or less in the same order of magnitude with *C. ljungdahlii* (0.0009 g/L/D), *C. aceticum* (0.0007 g/L/D) and *M. thermoacetica* (0.0008 g/L/D) (Nevin et al. 2011). MES has been limited to the production of short-chain carboxylates (mainly acetic acid) which, though marketable, has a very low commercial value and limited application. The products of MES by *C. scatologenes* mainly are acetic acid, ethanol and butyric acid. Although the yield of butyric acid is not high, the production of C-4 compounds from CO_2 as sole carbon source, expanding significantly the potential for MES implementation. Due to the expansion of the product varieties in MES system, the separation technologies of products mix, for example, membrane extraction (Batlle-Vilanova et al. 2017) might be focused on in our future study.

Conclusion

C. scatologenes is an acetogenic bacterium which may fix CO_2 through the Wood–Ljungdahl pathway. Under H_2 fermentation, *C. scatologenes* may reduce CO_2 to acetic acid, butyric acid and ethanol. It can also be used as the biocatalyst in MES systems. At a potential of − 0.6 V, the maximum production of acetic acid and butyric acid achieved, in the cathodic chamber, was 0.03 and 0.01 g/L, respectively. The maximum value of total coulombic efficiency was about 84%. With the decrease in cathodic potentials, via both DET and exogenous electron shuttle, H_2 was adopted by the *C. scatologenes* MES system. Due to the increase in redox potential, ethanol was also detected in the product spectrum. However, because the H_2 utilization rate, by the *C. scatologenes* planktonic cell, was lower than the H_2 evolution rate, in the MES system, dropping the total coulomb efficiency to 37.8% at a potential of − 1.2 V. Overall, the production of butyric acid, a C-4 compound, from CO_2 by *C. scatologenes*, significantly expands the potential for MES implementation.

Authors' contributions

HL conducted the experiments. HL, TS and JX wrote the manuscript. TS, KF, HW, and JX provided advices on the experimental design and data analysis. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets supporting the conclusions of this article are included in the main manuscript. The authors promise to provide any missing data on request.

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