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# Bioelectrochemical enhancement of anaerobic methanogenesis for high organic load rate wastewater treatment in a up-flow anaerobic sludge blanket (UASB) reactor

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**A coupling process of anaerobic methanogenesis and electromethanogenesis was proposed to treat high organic load rate (OLR) wastewater. During the start-up stage, acetate removal efficiency of the electric-biological reactor (R1) reached the maximization about 19 percentage points higher than that of the control anaerobic reactor without electrodes (R2), and $CH_4$ production rate of R1 also increased about 24.9% at the same time, while additional electric input was 1/1.17 of the extra obtained energy from methane. Coulombic efficiency and current recorded showed that anodic oxidation contributed a dominant part in degrading acetate when the metabolism of methanogens was low during the start-up stage. Along with prolonging operating time, aceticlastic methanogenesis gradually replaced anodic oxidation to become the main pathway of degrading acetate. When the methanogens were inhibited under the acidic conditions, anodic oxidation began to become the main pathway of acetate decomposition again, which ensured the reactor to maintain a stable performance. FISH analysis confirmed that the electric field imposed could enrich the $H_2/H^+$-utilizing methanogens around the cathode to help for reducing the acidity. This study demonstrated that an anaerobic digester with a pair of electrodes inserted to form a coupling system could enhance methanogenesis and reduce adverse impacts.**

Anaerobic methanogenesis is widely used to treat high-concentration organic wastewater with methane as byproduct[1,2]. Methane, the main product of methanogenesis, is produced generally through acetate decomposition by aceticlastic methanogens and $H_2/CO_2$ by hydrogen-utilizing methanogens[1,3]. Since methanogens are susceptible to environment and have a low metabolism rate of degrading organic matters, the acid balance between acidification and methanogenesis is easily broken[4–6]. At high organic load rate (OLR), the accumulation of organic acids may further inhibit the metabolism of methanogens and even lead to a failure of anaerobic methanogenesis.

Bioelectrohydrogenesis using organics (such as acetate) to produce $H_2$ in two-chamber microbial electrolysis cells (MECs) has been widely studied[7–10]. It is a new method to convert cheap or waste carbon sources to recover bioenergy through bioelectrochemical systems[11]. In this system, acetate is firstly oxidized in the anode with producing electrons and protons, and then the electrons are transferred to the cathode through the external circuit. Finally, the electrons combine with protons to form $H_2$[11,12]. Thermodynamically, a potential of at least $E^0 = -410$ mV (normal hydrogen electrode [NHE], pH at 7.0) imposed in the cathode is necessary to produce $H_2$. Considering overpotential and internal resistance, the electrohydrogenesis in MECs usually needs a voltage of 0.5–1.0 V to carry out the overall process[12]. Moreover, the precious metal catalysts such as platinum are necessary for the cathodic reaction[7,9,13]. Recently, methane formation through "electromethanogenesis" was proposed by Cheng et al.[14] who used methanogens as biocathodic catalyst to reduce $CO_2$ into $CH_4$ based on the following reactions:

$$\text{Anode:}\quad \text{acetate}^- + 2H_2O = 2CO_2 + 8H^+ + 8e^- \quad E^0 \approx -0.2\ \text{V}\ (\text{NHE})$$

Cathode: $CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O \quad E^0 \approx -0.44\ V\ (NHE)$

As compared with hydrogen-production MECs, advantages of this electromethanogenesis are obvious. Apart from the free of precious metal catalyst, the potential imposed on electromethanogenesis ($-0.44 - (-0.2) = -0.24$ V) is higher than that in electrohydrogenesis ($-0.410$ V). Namely, electromethanogenesis is easier to happen and more energy-saving than electrohydrogenesis. Electromethanogenesis can be carried out in a single-chamber anaerobic system with no need of an ion exchange membrane[15,16]. Thus inserting electrodes into a UASB can be to construct a methane-production MEC. In this system, organic acids can be decomposed both through aceticlastic methanogenesis and anodic oxidation.

In common MECs, aceticlastic methanogenesis should be avoided because it could decrease the electron production and electron transfer between the two electrodes[17,18]. The Gibbs free energy of acetate-oxidation in the anode is 6 times as high as the Gibbs free energy of aceticlastic methanogenesis[19]. Therefore, aceticlastic methanogenesis is difficult to occur at low concentrations of organics. Thus, methane-production MEC is usually applied for the low-concentration wastewater treatment[15,20]. With the increase of organic concentration, due to the extra electron donor, aceticlastic methanogenesis would gradually increase and even replace anodic oxidation to become the dominant pathway to degrade acetate, which however was unwelcome from the view of MEC because it would decrease anodic Coulombic efficiency[21]. Nonetheless, it might be beneficial to reduce the organics concentration and increase the methane production, which just is the aim of anaerobic wastewater treatment.

In our previous study, for high OLR wastewater treatment, the organics removal efficiency of an anaerobic reactor with a pair of Fe-graphite electrodes inserted increased 19 percentage points and $CH_4$ production rate increased 9 percentage points[22]. The electric field applied in that study was just to enhance the release of $Fe^{2+}$ from $Fe^0$ electrode and further improved the $CH_4$ production[23]. Namely, the enhancement of anaerobic performance was unnecessarily because of the contribution of bioelectrochemical functions. According to the analysis above, it was reasonable to assume that bioelectrochemical system could accelerate the oxidation of organic acids and methane production, especially under acidic accumulation occurring from high organic load or during the start-up stage of the anaerobic digester. Nevertheless, there are few reports focused on using electromethanogenesis associated with aceticlastic methanogenesis for high OLR wastewater[24]. With above consideration, a pair of graphite electrodes was packed into a UASB reactor with the aim to enhance acetate decomposition and $CH_4$ production. We hope to provide a simple and effective method to improve anaerobic treatment of high OLR wastewater using electrochemical technology.

## Results and Discussion

**Comparison of acetate removal and $CH_4$ production during the start-up stage.** In order to assess the effects of electrodes on anaerobic methanogenesis during the start-up stage, the electric-biological reactor (R1) and the control reactors (R2 and R3) were operated continuously for 58 days experiments and the results are showed in Fig. 1. From Fig. 1A, the acetate removal efficiency of R1 increased gradually from 25.2 ± 2.1% to 63.3 ± 2.3%. As compared with R1, the acetate removal efficiency of R2 only increased from 26.1 ± 1.7% to 55.4 ± 3.2% and the acetate removal efficiency of R3 only increased from 24.7 ± 2.2% to 54.2 ± 2.3%. Especially, the acetate removal rate of R1 at day 30 had nearly reached the maximum removal efficiency, about more than 19 percentage points (amount to OLR: 2.4 Kg COD/L·$d^{-1}$) higher than R2 and R3 at the same time. It implied that a faster startup and higher removal efficiency were achieved in R1 with addition of bioelectrochemical system. From Fig. 1B, the $CH_4$ production rate of R1 gradually increased from 31.9 ± 1.2 mL/h to 66.8 ± 2.7 mL/h. Comparatively, the $CH_4$ production rate of R2 increased only from 31.8 ± 1.6 mL/h to 53.5 ± 1.2 mL/h. At the same time, the $CH_4$ production rate of R3 increased only from 30.7 ± 1.9 mL/h to 52.7 ± 2.1 mL/h. Remarkably, during the 58 days experiments in the start-up stage, the average acetate removal efficiency and the average methane production rate of R3 was 39.1% ± 9.7% and 40.2 ± 7.4 mL/h respectively. Comparatively, the average acetate removal efficiency and the average methane production rate of R2 was 40.9 ± 9.9% and 41.3 ± 7.8 mL/h respectively. The statistical analysis of the three reactors is listed in Table S1 and Table S2. These results showed that both acetate removal and methane production in R3 only had less than 5% differences as compared with those in R2, and the correlation coefficient of the two reactors was higher than 0.99 and the P value based on two tailed student t-test (n = 58) was also higher than 0.05. Therefore, it reasonably demonstrated that the electrodes themselves had no significant effects on the performances of the anaerobic system in the acetate removal and methane production, which could be ignored.

The lower $CH_4$ production of R2 was similar to the results of Hao et al.[25,26] who reported that the high initial acetate concentration resulting in the accumulation of organic acids would (>50 mM) inhibited the activity of aceticlastic methanogenesis during the start-up stage. The results indicated that the electrodes might compensate the low rate of methanogenesis during the start-up stage. Remarkably, the only difference between the two reactors (R1 and R2) was the additional electrochemical system. Therefore, it was reasonably assumed that more decomposition of acetate of R1 could be ascribed to the role of anodic oxidation according to the reaction of $CH_3COO^- + 2H_2O = 2CO_2 + 7H^+ + 8e^-$, and the extra $CH_4$ production of R1 could be due to the cathodic reduction based on the reaction of $CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O$.

To further clarify this assumption of the role of additional electrochemical system, anodic Coulombic efficiency and current of R1 had been measured and recorded in Fig. 1C. Theoretically, anodic Coulombic efficiency is a parameter to assess the fraction of electrons available from acetate that ends up as electrical current[21]. Therefore, anodic Coulombic efficiency could be reasonably used to calculate and distinguish the contribution of anodic oxidation and aceticlastic methanogenesis in the acetate removal. From Fig. 1C, the current increased from 0.379 ± 0.012 A to 0.434 ± 0.008 A during the initial 24 days, indicating that both anodic oxidation and cathodic reduction were enhanced which drove the more electron transfer produced from anode to cathode. In this stage, anodic Coulombic efficiency was more than 50% although it appeared a significant decreased trend, implying that anodic oxidation was the main pathway to degrade acetate in the initial start-up stage because aceticlastic methanogenesis was weak. From day 24 to day 58, the change of current was in relatively steady stage, slightly ranging from 0.409 ± 0.012 A to 0.434 ± 0.011A, but anodic Coulombic efficiency still reduced about 13 percentage points (decrease from 45.0% to 32.0%). The results indicated that the percentage of acetate decomposition by anodic oxidation in the total acetate decomposition decreased. In other words, aceticlastic methanogenesis was gradually acclimated to compete with anodic oxidation for acetate decomposition. Considering that the acetate removal efficiency and the $CH_4$ production rate still kept increasing, it suggested that aceticlastic methanogenesis became the main pathway to degrade acetate and produce $CH_4$. At this time, aceticlastic methanogenesis replaced anodic oxidation to obtain more substrates which would decrease anodic oxidation and cathodic methanogenesis. These electrochemical parameters were well in agreement with the performance of the reactor shown in Fig. 1A and Fig. 1B.

During the start-up stage, the average acetate removal efficiency of R1 and R2 was 52.7 ± 11.3% and 41.0 ± 9.9% respectively shown in Table S3 (see Supplementary material). The difference of acetate

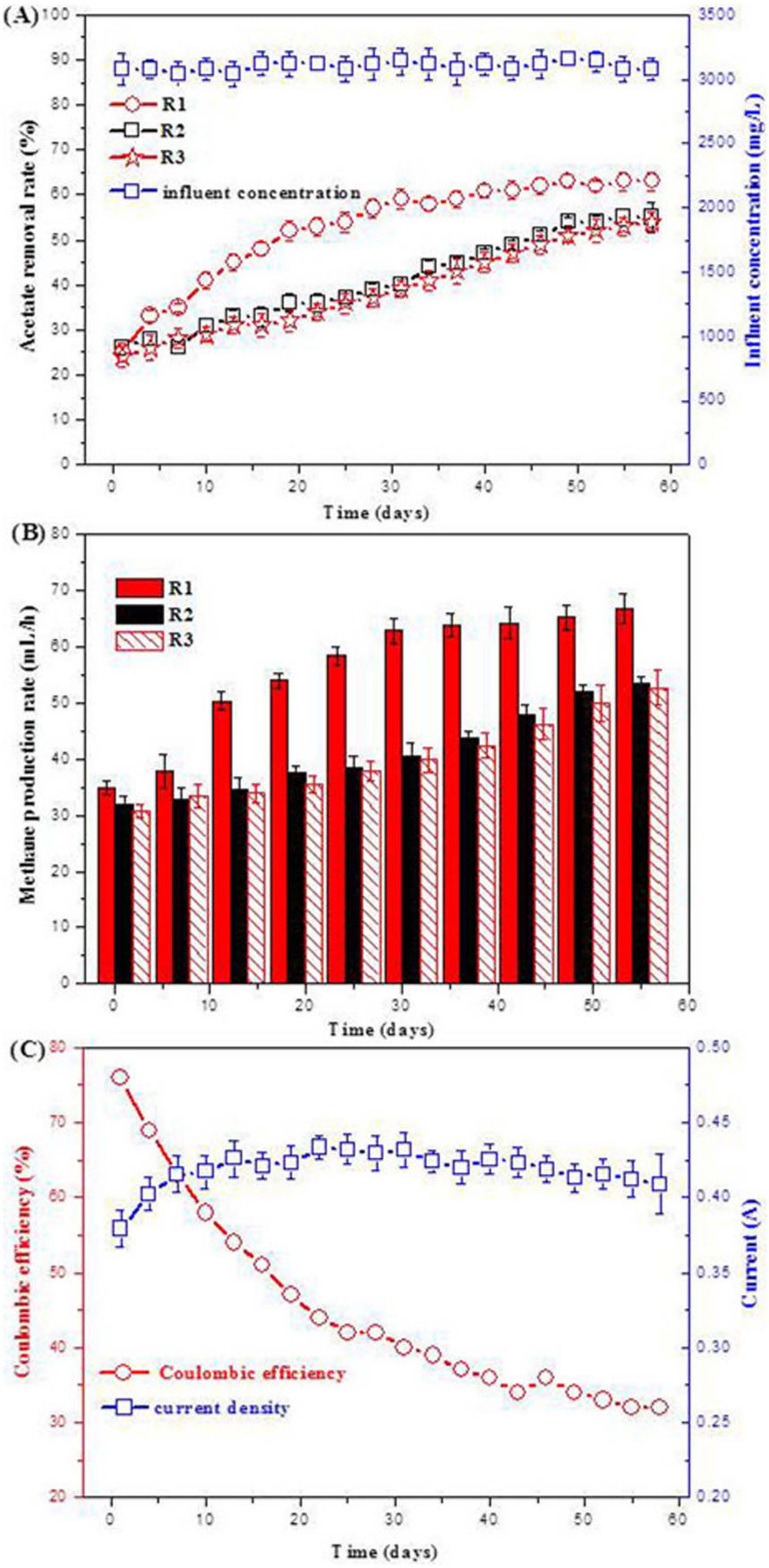


**Figure 1** | Acetate removal efficiency (A) and $CH_4$ production rate (B) of R1, R2 and R3 and change of anodic Coulombic efficiency and current of R1 (C) during the start-up stage. Error bars represent standard deviations of three measurements.

Table 1 | The performance of R1 at different anode potentials

| Anode potential (mV) | Removal rate ± SD[a] (%) | $CH_4$ production rate ± SD[a] (mL/h) | Coulombic efficiency ± SD[a] (%) |
|---|---|---|---|
| −400 | 52.9 ± 2.1 | 55.9 ± 3.3 | 18.6 ± 3.1 |
| −350 | 61.5 ± 1.4 | 63.2 ± 2.8 | 29.1 ± 1.9 |
| −300 | 67.3 ± 2.7 | 66.8 ± 4.4 | 34.0 ± 2.2 |
| −250 | 77.1 ± 3.3 | 77.7 ± 5.4 | 38.1 ± 1.7 |
| P value[b] (−400 mV and −350 mV) | $< 1.64988 \times 10^{-6}$ | | |
| P value[b] (−350 mV and−300 mV) | $< 5.17803 \times 10^{-7}$ | | |
| P value[b] (−300 mV and −250 mV) | $< 5.58934 \times 10^{-6}$ | | |
| P value[b] (−400 mV and −300 mV) | $< 3.0816 \times 10^{-13}$ | | |
| P value[b] (−350 mV and −250 mV) | $< 2.48222 \times 10^{-9}$ | | |
| P value[b] (−400 mV and −250 mV) | $< 4.16116 \times 10^{-17}$ | | |

[a]Data are mean values (n = 7) ± standard deviation (SD).
[b]P value was provided by two tailed student t-test (n = 58).

removal efficiency between R1 and R2 was 11.7%. The average anodic Coulombic efficiency was 45.0 ± 12.9%. The acetate removal efficiency through anodic oxidation of R1 was 23.7% (23.7% = 45.0% × 52.7%). This calculated result was obviously higher than the difference of acetate removal rate between R1 and R2 and demonstrated that more decomposition of acetate of R1 as compared with R2 should be ascribed to the role of anodic oxidation according to the reaction of $CH_3COO^- + 2H_2O = 2CO_2 + 7H^+ + 8e^-$. The more electrons was produced through anodic oxidation, the more methane would be formed according to the reaction of $CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O$. The average acetate removal efficiency of direct methanogenesis (aceticlastic methanogenesis) of R1 was 29.0% ([100% − 45.0%] × 52.7% = 29%). It was assumed that the acetate removal through direct methanogenesis of R1 had a same conversion efficiency of 54.6% with R2 shown in Table S3 (see Supplementary material). The methane production rate through direct methanogenesis of R1 was 30.1 mL/h (30.1 mL/h = 29.0% × 3000 mg/L [influent]/59 × $10^3$ mg/mol × 22.4 × $10^3$ mL/mol/6 h × 54.6%). The average methane production rate of R1 was 59.8 mL/h. Therefore, the methane production rate through cathodic reduction of $CO_2$ into $CH_4$ was 28.7 mL/h. The difference of methane production rate between R1 and R2 was about 11.7 mL/h (11.7 mL/h = 52.7 mL/h − 41.0 mL/h), and this result was obviously lower than that of cathodic reduction of $CO_2$ into $CH_4$. It reasonably implied that the extra $CH_4$ production of R1 should be due to the role of cathodic reduction based on the reaction of $CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O$.

The cathode potential and the potential difference between anode and cathode of R1 were recorded during the start-up stage (shown in Fig. S1). From this figure, the potential difference increased from 0.749 ± 0.002 V to 0.807 ± 0.003 V (vs Ag/AgCl electrode) in the initial 28 days, and then decreased from 0.807 ± 0.003 V to 0.759 ± 0.002 V. The average cathode potential of R1 was −1.081 ± 0.016 V (vs Ag/AgCl electrode) which was significantly lower than the theoretical potential of cathodic reduction of $CO_2$ into $CH_4$ (−0.44 V NHE) and also lower than the needed cathode potential (−0.7 V) for the significant methane production reported by Cheng et al.[14] Especially, during the overall start-up 58 days, the electric energy supply or consumption calculated was 543.2 J/h according to the following formula[37]: $W_E = (IE_{ap} - I^2R)\Delta t$, where $E_{ap}$ is the average potential difference between anode and cathode (0.779 ± 0.023 V) according to Fig. S1, I is the average current (0.418 ± 0.003 A), Δt is per unit time (3600 s) and R is the external resistor (1 Ω). This energy supply was less than the energy harvest from the extra increased $CH_4$ production. The extra increased $CH_4$ production of R1 as compared with that of R2 was averagely 17.5 mL/h. It meant that the extra obtained energy from $CH_4$ was 635.9 J/h (635.9 J/h = $17.5 \times 10^{-3}$ L/h/24.5 L/mol × 890.31 × $10^3$ J/mol), about 1.17 times of the electric energy supply, where 24.5 L/mol was the molar volume of the gas at normal temperatures and pressures and 890.31 × $10^3$ J/mol is the energy content of methane based on the heat of combustion.

**Effects of different anode potentials on the acetate removal and $CH_4$ production of R1.** To further study the effects of different anode potentials on acetate removal and $CH_4$ production in R1, the anode potential was in turn increased from −400 to −350, −300, and −250 mV (vs Ag/AgCl). Table 1 shows the acetate removal efficiency, $CH_4$ production rate and anodic Coulombic efficiency of R1 at different anode potentials and Fig. 2 shows the change of current. With the increase of anode potential from −400 mV to −250 mV, the current increased from 0.142 ± 0.008 A to 0.473 ± 0.013 A, as well as anodic Coulombic efficiency increased from 18.6 ± 3.1% to 38.1 ± 1.7%. The increased anodic Coulombic efficiency meant that the contribution of anodic oxidation to acetate decomposition was raised. This result was consistent that the acetate removal efficiency increased from 52.9 ± 2.1% at −400 mV to 77.1 ± 3.3% at −250 mV (shown in Table 1). Actually, the amount of acetate removal increased from 1629.3 mg/L (1629.3 mg/L = 52.9% × 3080 mg/L [influent]) to 2374.7 mg/L (2374.7 mg/L = 77.1% × 3080 mg/L [influent]). The increased amount of acetate removal was 745.4 mg/L. At the same time, according to the increased anodic Coulombic efficiency shown in Table 1, the increased amount of acetate removal by anodic oxidation was 601.7 mg/L (601.7 mg/L = 38.1% × 2374.7 mg/L − 18.6% × 1629.3 mg/L). It meant that about 81% of increased acetate removal was resulted from the increase of potential from −400 mV to −250 mV. The increase of anode potential might accelerate the electron transport rate, facilitating electrogens to consume more substrates[21]. Therefore, the enhanced acetate decomposition was observed with increase of anodic oxidation.

Theoretically, when more substrates were degraded by electrogens, less substrate was available for aceticlastic methanogens. It would directly reduce the $CH_4$ production from aceticlastic methanogenesis. Reversely, when increasing anode potential from −400 mV to −250 mV, the $CH_4$ production significantly increased from 55.9 ± 3.3 mL/h to 77.7 ± 5.4 mL/h (shown in Table 1). It was reasonably ascribed to the role of cathodic reduction of $CO_2$ into $CH_4$. To further clarify this deduction, it was assumed that the increase of acetate removal by direct methanogenesis was completely converted to methane. Therefore, the increased methane production rate by direct methanogenesis was about 9.1 mL/h (9.1 mL/h = [745.4 mg/L − 601.7 mg/L]/59 × $10^3$ mg/mol × 22.4 L/mol/6 h). Actually, according to the Table 1, with the increase of anode potential from −400 mV to −250 mV, the increased methane rate was 21.8 mL/h (21.8 mL/h = 77.7 mL/h − 55.9 mL/h). Therefore, the contribution of cathodic reduction of $CO_2$ to the increased methane production was higher than 60%. This result implied that the cathodic reduction of $CO_2$ contributed quite a large part of the increased methane production with the increase of anode potential.

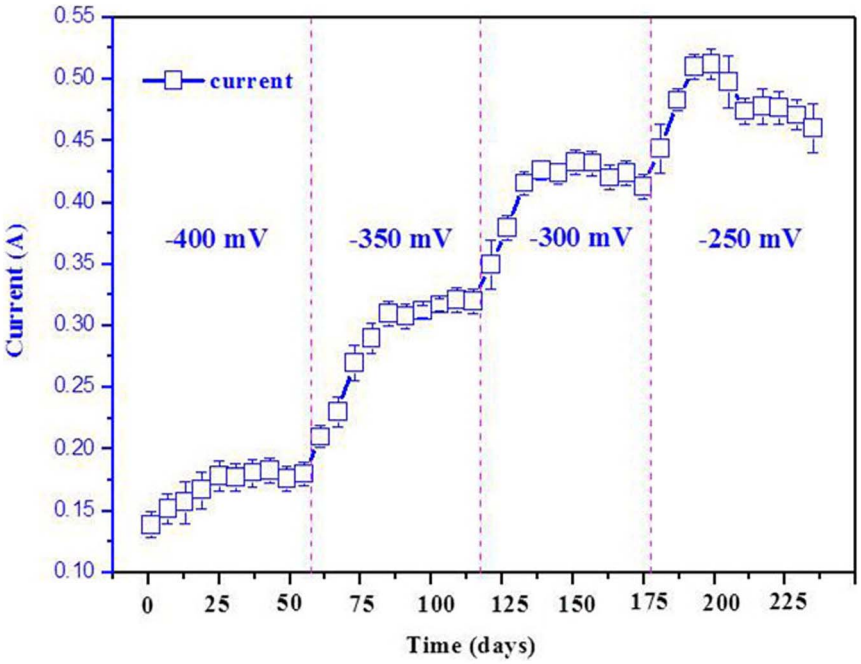


**Figure 2** | **Influences of anode potential on the current in R1.** Error bars represent standard deviations of three measurements.

Together with the above results, this bioelectrochemical enhancement of methanogenesis would be potentially applied to improve the performance of anaerobic digester by gradually increasing anode potential or apply voltage when the treatment efficiency was low.

**Effects of acidic conditions on the performance of R1 and R2.** In order to clarify the contribution of bioelectrochemical system to methanogenesis under the acidity accumulated conditions, the electric-biological reactor (R1) and the control reactor (R2) were operated with the influent pH gradually dropped from 7.0 to 5.0 during the 48 days experiments. Here, the anode potential of R1 was maintained at −250 mV (vs Ag/AgCl).

Methanogens would be inhibited at acidic conditions (pH $<$ 6.2) as reported by Kotsyurbenko et al.[27] who observed that lower pH extended the lag phase for methanogenesis. From Fig. 3A,B, with the influent pH decreased from 7.0 to 5.5, the acetate decomposition and $CH_4$ production of R2 appeared an obvious decreasing trend, at which the acetate removal efficiency dropped from 85.2 ± 2.7% to 34.3 ± 1.5% and $CH_4$ production rate dropped from 92.8 ± 2.6 mL/h to 35.3 ± 3.1 mL/h. Comparatively, R1 was less affected by the acidic pHs. The acetate removal efficiency of R1 was about 9 percentage points (amount to OLR: 1.2 Kg COD/L·$d^{-1}$) higher than that of R2 at influent pH 6.2 and about 20 percentage points (amount to OLR: 2.6 Kg COD/L·$d^{-1}$) higher than that of R2 at influent pH 5.5, while the average $CH_4$ production rate of R1 was about 15 mL/h higher than R2 at influent pH 6.2 and about 25 mL/h higher than R2 at influent pH 5.5. When the influent pH further decreased to 5.0, the acetate removal efficiency of R2 was only 5%–9% and nearly no $CH_4$ produced (shown in Fig. 3A,B), while acetate removal efficiency of R1 was about 30.5 ± 2.1% and $CH_4$ production rate was 14.9 ± 2.8 mL/h. Commonly, in an anaerobic system of feeding with acetate, the acetate decomposition would partially neutralize organic acids. A good performance of $CH_4$ production in R1 was partially due to the more acetate decomposition. This consideration had been further verified by changes of the effluent pH shown in Fig. 3C. With the influent pH decreased from 7.0 to 5.0, the effluent pH of R1 was still maintained at a near-neutral pH (>6.0). Comparatively, the effluent pH of R2 was less than 5.5, causing the activity of methanogens still in a low level.

Fig. 3D shows the change of anodic Coulombic efficiency and current of R1. With the influent pH decreased from 7.0 to 5.0, the average current of R1 decreased from 0.451 ± 0.012 A to 0.302 ± 0.008 A. Compared with the decrease of methane production, acidic pH had less effect on the anodic oxidation. This assumption had been documented in many literatures. Chae et al.[17] obtained an extra 10% hydrogen yield through suppressing methanogens using acidic feeding. Similarly, Liang et al.[28] found that the optimum pH for anodic oxidation in a BES was 4.5. These indicated that anodic oxidation (exoelectrogens) is more accommodative than methanogens in the low pH conditions. With the influent pH decreased from 7.0 to 5.0, the acetate removal efficiency of R1 decreased from 85.6 ± 1.8% to 30.5 ± 2.1%. The amount of acetate removal decreased from 2670.7 mg/L (2670.7 mg/L = 85.6% × 3120 mg/L [influent]) to 976.0 mg/L (976.0 mg/L = 30.5% × 3200 mg/L [influent]). Therefore, the decreased acetate removal of R1 was 1694.7 mg/L. During this time, the anodic Coulombic efficiency increased from 28.1% to 62.3% (shown in Fig. 3D). The decreased acetate removal by anodic oxidation was 142.3 mg/L (142.3 mg/L = 28.1% × 2670.7 mg/L − 62.3% × 976.0 mg/L) accounted for 20.0% (20.0% = 142.3 mg/L/[2670.7 mg/L × 28.1%]) of total acetate removal by anodic oxidation at pH 7.0, while the decreased acetate removal by methanogens was 1552.4 mg/L (1552.4 mg/L = 1694.7 mg/L − 142.3 mg/L) accounted for 80.8% (80.8% = 1552.4 mg/L/{[100.0% − 28.1%] × 2670.7 mg/L}) of total acetate removal by methanogens at pH 7.0. Thus, it was demonstrated that the decrease of acetate removal was caused by both methanogens and anodic oxidation but acidic impacts had a less effect on anodic oxidation than methanogens. The portion of anodic oxidation to acetate decomposition increased and anodic oxidation hereby gradually replaced aceticlastic methanogensis to become the main pathway. This role of anodic oxidation helped the reactor maintain relatively stable performance when aceticlastic methanogenesis got stressed due to the low pHs.

Commonly, the $H^+$ consumption through hydrogenotrophic methanogens played an important role to make the anaerobic reactor adaptable for acidic impact[27,29,30]. Hydrogenotrophic methanogenesis coupling with anodic oxidation might be a major reason for the better performance in R1. One hand, the cathodic hydrogenotrophic methanogens accepted the electron produced from anode

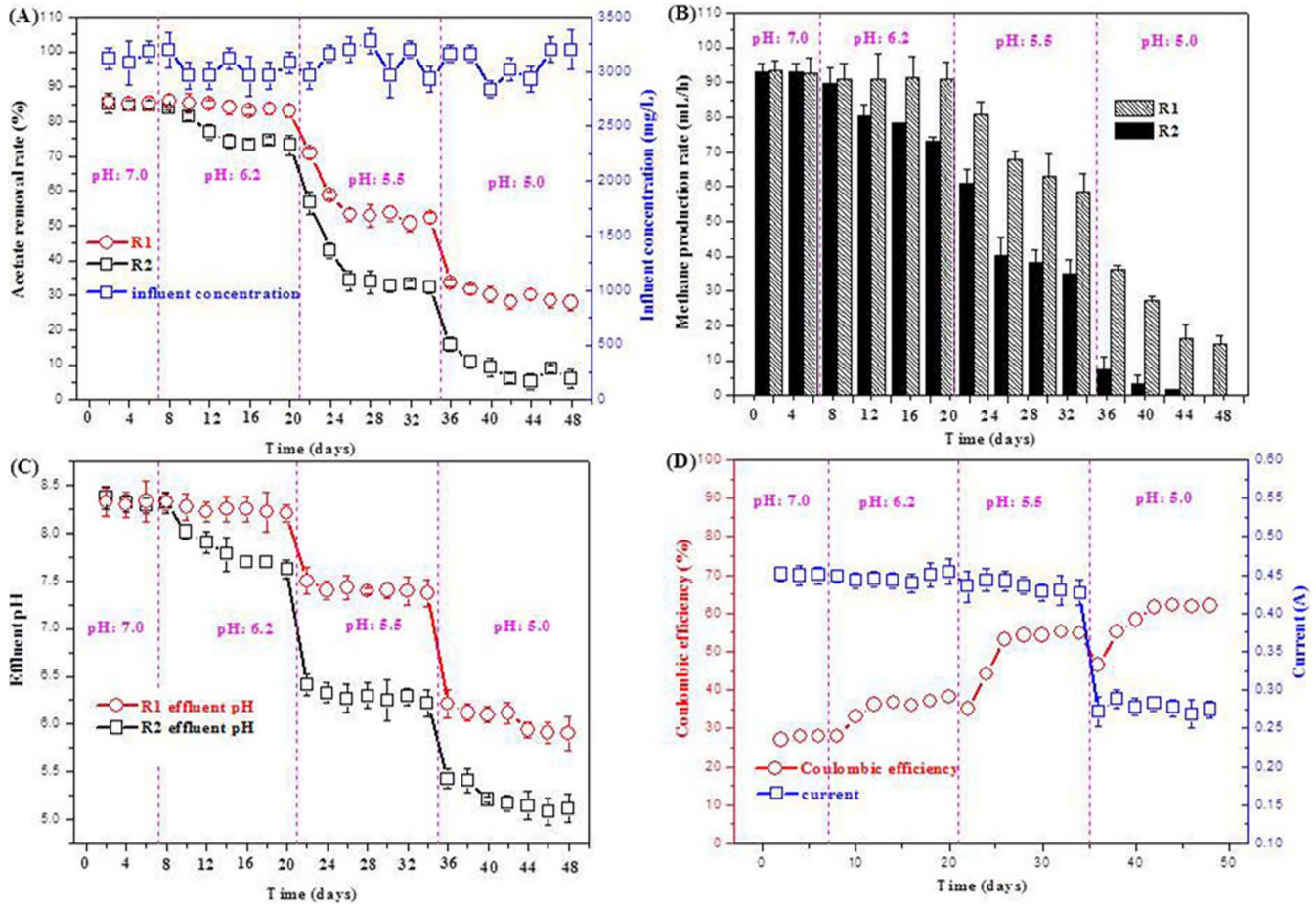


**Figure 3** | Acetate removal efficiency (A), $CH_4$ production rate (B) and effluent pH (C) of R1 and R2 and change of anodic Coulombic efficiency and current of R1 (D) under the acidic conditions (pH: 7.0–5.0). Error bars represent standard deviations of three measurements. (P value [acetate removal] = 0.012957, P value [$CH_4$ production rate] = 0.009679, P value [effluent pH] = 0.009734 based on two tailed student t-test [n = 48]).

to drive acetate oxidation in the anode to happen. In other words, acetate could not be anodically decomposed until the electron produced was accepted by cathodic acceptors such as hydrogentrophic methanogens. On the other hand, anodic oxidation reduced the acidity to gradually create a favorable condition for aceticlastic methanogens. Considering the relationship between the electron and hydrogentrophic methanogens, it was assumed that the electrochemical function was likely to facilitate the cathodic hydrogentrophic methanogens.

To make clear the assumption above, FISH was used to determine the relative abundance of hydrogenotrophic methanogens in the archaea community of R1 and R2 (shown in Fig. 4). From Fig. 4a,b, according to the analysis of using Image-Pro Plus 6.0, the relative abundance of hydrogenotrophic methanogens at the bottom of R1 was 56.25%, about 30 percentage points higher than that of R2 about 26.83%. This finding could explain the difference of methane production between R1 and R2 under the acidic pHs. Cheng et al[14] enriched a high abundance of hydrogen-utilizing methanogens from a mixed culture as the biocathode to produce methane. Villano et al.[31] reported that $H_2$-utilizing methanogens in the cathode were critical for methane production. These indicated that anaerobic methanogenesis coupled with a pair of electrodes could improve the $H_2$-utilizing methanogenesis. To clarify this deduction, the biofilm attached to the cathode of R1 was collected to determine the abundance of hydrogenotrophic methanogens used FISH analysis. From Fig. 4c, according to the analysis using Image-Pro Plus 6.0, the relative abundance of hydrogenotrophic methanogens of biofilm attached to the cathode was 85.01%. It was implied that the dominant methanogenic microbial community was hydrogentrophic methanogens around the cathode and this result was well in agreement with the report by Cheng et al.[14] who found that *Methanobacterium* accounted for 86.7% of the total cells in the cathode. The relative abundance of hydrogenotrophic methanogens around the cathode was obviously higher than that at the bottom of the reactor and also much higher than that in R2. These results demonstrated that the additional electrochemical system could enrich the hydrogen-utilizing methanogens around the cathode to serve as electron acceptors to drive acetate oxidation in the anode and to reduce the acidity. This enhancement of hydrogenotrophic methanogenesis might be an important reason for the stable performance of this coupling system at acidic pHs. Thus, anodic oxidation coupled with hydrogenotrophic methanogenesis created a favorable environment for aceticlastic methanogenesis. This further enhanced aceticlastic methanogenesis to accelerate the acetate decomposition and methane production.

## Methods

**Experimental setup.** The electrochemical experiments were operated in a up-flow anaerobic blanket (UASB) reactor (internal diameter of 70 mm and height of 300 mm) which had a working volume of 1000 mL (hereafter referred to as R1). The graphite-rod anode and cathode (external diameter of 16 mm and height of 180 mm, surface areas $9.05 \times 10^3$ $mm^2$) with a distance of 40 mm were installed into the bottom of the reactor. The anode and cathode potentials were measured using an Ag/AgCl electrode (Yueci, 218, China) also inserted into the reactor as the reference electrode. A potentiostat (Zhenhua, CHI1030C, China) were connected with the electrodes to serve as the electric supply and control the anodic potential.

Two control experiments were operated in this study. One was conducted in a same UASB reactor as R1 but without electrodes (hereafter referred to as R2). The other was

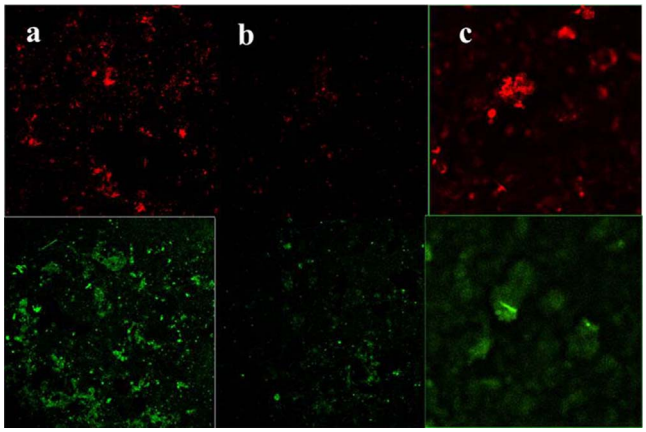


**Figure 4 | FISH images of the sludge samples in different reactors (R1 and R2) at the acid conditions of influent pH below 5.5.** The sludge samples (a, b) was collected from the bottom of the two reactors (R1 and R2). The sludge samples (c) were collected from the surface of the cathode in R1. Respectively, the sludge of R1 and R2 hybridized with specific probes for Archaea and hydrogenotrophic methanogens (ARC915-FITC, green and MB1174-CY3, red).

also conducted in a same UASB reactor as R1 with the same electrodes, but was not connected with the potentiostat (hereafter referred to as R3).

The hydraulic retention time (HRT) of the electric-biological reactor (R1) and the control reactors (R2 and R3) were fixed at 6 h. The influent concentration of the acetate was kept at about 3000 mg/L (namely OLR: 12.8 Kg COD/L·d$^{-1}$). The influent was replaced every three days and stored in a plastic bucket at 4°C before the experiments. Before, this influent fed to the reactors, it was deoxygenated by flushing nitrogen gas for 30 min and then was pumped into the reactors with a peristaltic pump. All the reactors were operated at a room temperature (25 ± 2°C).

**Sludge and wastewater.** Seed sludge was taken from a sedimentation tank in Chunliu municipal sewage plant based on the aerobic activated sludge process in Dalian (China). The ratio of volatile suspended sludge to total suspended sludge (VSS/TSS) was 0.72 with initial TSS of 17100 mg/L. It was cultured in a batch anaerobic reactor which had a working volume of 10 L and fed with glucose and acetate in turn as the substrate (COD: 1000 mg/L), and $NH_4Cl$ and $KH_2PO_4$ as nitrogen and phosphorus sources (at ratio of COD : N : P 200 : 5 : 1), respectively. Then this seed sludge of 500 mL was added to each reactor (R1, R2 and R3). All the reactors were operated in a room temperature (20.0 ± 2.0°C) with a hydrolytic retention time (HRT) of 24 h.

$CH_3COONa$ were used as the substrate in all the experiments of this study, and $NH_4Cl$ and $KH_2PO_4$ were used as nitrogen and phosphorus sources (at ratio of COD : N : P 200 : 5 : 1), respectively. The trace elements were added according to the following composition: 1 mL/L of a trace element solution containing Zn at 0.37 mmol/L, Mn at 2.5 mmol/L, Cu at 0.14 mmol/L, Co at 8.4 mmol/L, Ni at 0.25 mmol/L, $H_3BO_3$ at 0.8 mmol/L and EDTA at 3.4 mmol/L.

**Experimental procedure.** In the start-up stage, anode potential of R1 was maintained at −300 mV (vs Ag/AgCl electrode), which was lasted for 58 days experiments with R2 and R3 simultaneously. Afterwards, anode potential of R1 was controlled in turn at −400, −350, −300 and −250 mV (vs Ag/AgCl electrode) by the potentiostat to investigate the effects of the different anode potentials on the performance of R1. Finally, to study the reactors in response to acidic impacts, the influent pH of the wastewater was adjusted from 7.0 to 5.0 using dilute HCl. In this stage, anode potential of R1 was maintained at −250 mV, which was lasted for 48 days experiments with R2 simultaneously.

**Analysis.** Total suspended solids (TSS) and volatile suspended solids (VSS) used to evaluate the initial seed sludge were measured according to Standard Methods for the Examination of Water and Wastewater. The acetate concentration was calculated by the measured value of chemical oxygen demand (COD) according to Standard Methods for the Examination of Water and Wastewater similarly. The equivalent relationship between COD and acetate are 1.07 g COD/g acetate. Biogas collected from the two reactors was measured with a gas meter (Changchun, LMF-2, China). The contents of the methane were analyzed by a gas chromatograph (Tianmei, GC-7900P/TCD, China) according to the method reported previously[32]. Electrical current data between the electrodes was collected by data acquisition card (Hongge, PCI-821H, China)[33].

Fluorescence in situ hybridization (FISH) was used to determine the abundance of hydrogenotrophic methanogens in the Archea community according to the method described by Wu et al.[34]. Two types of sludge sample in different positions were collected here. One with the same volume (10 mL) was taken from the bottom of the two reactors (R1 and R2) and harvested by centrifugation (110 × 100 g for 15 min at 4°C). The other was collected from the cathode of R1 which were rinsed twice by phosphate-buffered saline (PBS; 0.13 M NaCl and 10 mM $Na_2HPO_4$ at pH 7.2) and harvested by centrifugation (110 × 100 g for 15 min at 4°C). Two genus-specific probes for Archaea[35] (ARC915, GTGCTCCCCCGCCAATTCCT) and *Methanobacteriaceae*[36] (MB1174, TACCGTCGTCCACTCCTTCCTC) were used in this study. All the sludge samples were rinsed thrice by phosphate-buffered saline (PBS; 0.13 M NaCl and 10 mM $Na_2HPO_4$ at pH 7.2), and then fixed with 4% para-formaldehyde for 2 h at 4°C. Hybridizations were performed at 46°C for 1.5 h with buffer (0.9 M NaCl, 20 mM Tris-HCl [pH 7.2], 0.01 sodium dodecyl sulfate and 35% formamide) containing 50 ng probe per microliter and then washed with buffer (15 min at 48°C). The samples were observed under a confocal laser scanning microscope (Leica SP2, Heidelberger, Germany). The FISH images obtained were imported to Image-Pro Plus 6.0 for analysis of the relative abundance of microorganisms.

**Calculation.** Anodic Coulombic efficiency (CE) was used as an indicator to reflect the contribution of acetate-oxidation or aceticlastic methanogenesis to the acetate decomposition. CE was calculated using the following equation (1)[21,37]:

$$CE = \frac{I_{measured}}{nFQ(C_{in} - C_{out})/M} \times 100\% \quad (1)$$

$I_{measured}$ is the measured current (A), n is the amount of the electrons (8 for acetate), F is faradays constant (96485 C/mol), Q is the influent flow rate (here, $4.5 \times 10^{-5}$ L/s), M is the molecular weight of acetate$^-$ ($59 \times 10^3$ mg/mol), and $C_{in}$ and $C_{out}$ are the acetate concentrations in the influent and the effluent respectively (mg/L).


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## Author contributions

Z.Q.Z. and Y.B.Z. conceived and designed the experiments; Z.Q.Z. and Q.L.Y. performed the experiments; Z.Q.Z., Y.B.Z. and X.Q. analyzed data; Z.Q.Z. and Y.B.Z. wrote the manuscript; Y.B.Z. and S.C. contributed reagents and materials, and all authors reviewed the manuscript.

## Additional information

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