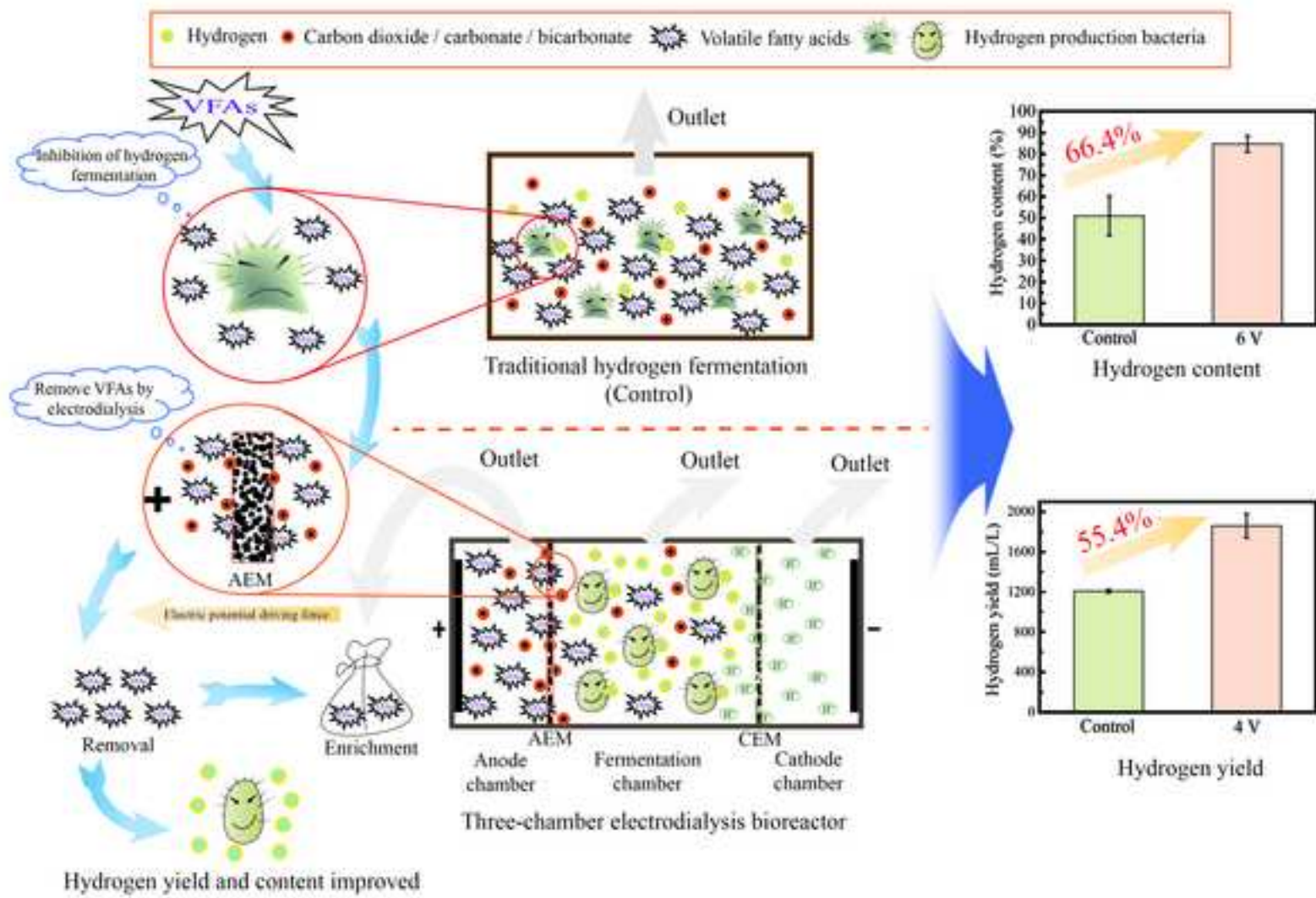


Title	Enhancing fermentative hydrogen production with the removal of volatile fatty acids by electro dialysis
Authors	Wei, Pengfei;Xia, Ao;Liao, Qiang;Sun, Chihe;Huang, Yun;Fu, Qian;Zhu, Xun;Lin, Richen
Publication date	2018-05-08
Original Citation	Wei, P., Xia, A., Liao, Q., Sun, C., Huang, Y., Fu, Q., Zhu, X. and Lin, R. (2018) 'Enhancing fermentative hydrogen production with the removal of volatile fatty acids by electro dialysis', Bioresource Technology, 263, pp. 437-443. doi: 10.1016/j.biortech.2018.05.030
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S0960852418306904 - 10.1016/j.biortech.2018.05.030
Rights	© 2018 Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license. - http://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2025-04-24 10:13:36
Item downloaded from	https://hdl.handle.net/10468/6310



- A three-chamber electro dialysis reactor was used to enhance hydrogen fermentation.
- VFAs and bicarbonate were effectively removed from fermentation chamber.
- Electro dialysis reactor improved specific H₂ yield up to 55.4% at a voltage of 4 V.
- Electro dialysis reactor enhanced H₂ content up to 66.4% with reduced CO₂ content.

1 **Enhancing fermentative hydrogen production with the removal of**
2 **volatile fatty acids by electrodialysis**

3 Pengfei Wei ^{a,b}, Ao Xia ^{a,b,*}, Qiang Liao ^{a,b}, Chihe Sun ^{a,b}, Yun Huang ^{a,b}, Qian Fu ^{a,b},
4 Xun Zhu ^{a,b}, Richen Lin ^c

5 ^a *Key Laboratory of Low-grade Energy Utilization Technologies and Systems,*
6 *Chongqing University, Ministry of Education, Chongqing 400044, China*

7 ^b *Institute of Engineering Thermophysics, Chongqing University, Chongqing 400044,*
8 *China*

9 ^c *MaREI Centre, Environmental Research Institute, University College Cork, Cork,*
10 *Ireland*

11

12

13

14

15

16 *Corresponding author at: Key Laboratory of Low-grade Energy Utilization
17 Technologies and Systems, Chongqing University, Chongqing 400044, China. E-mail
18 address: aoxia@cqu.edu.cn (Ao Xia).

19

1 **Abstract**

2 A three-chamber electro dialysis bioreactor comprising fermentation, cathode and anode
3 chambers was proposed to remove *in situ* volatile fatty acids during hydrogen
4 fermentation. The electro dialysis voltage of 4 V resulted in a volumetric hydrogen
5 productivity of 1878.0 mL/L from the fermentation chamber, which is 55.4% higher
6 than that (1208.5 mL/L) of the control group without voltage applied. Gas production
7 was not observed in the cathode and anode chambers throughout fermentation. By
8 applying different voltages (0-6 V), the hydrogen content accumulated to 54.6%-84.7%,
9 and it exhibited increases of 7.1%-66.4% compared with that of the control. Meanwhile,
10 the maximum concentrations of acetate and butyrate in the fermentation chamber
11 decreased to 10.3 and 13.1 mmol/L at a voltage of 4 V, respectively, which are 68.0%
12 and 62.4% lower than that for the control.

13 **Keywords:** Hydrogen production; Fermentation; Volatile fatty acids (VFAs) removal;
14 Electro dialysis; Bioreactor.

15

1 **1. Introduction**

2 Hydrogen has received increasing attention on account of its clean combustion property
3 and high calorific value by mass (142 MJ/kg) (Noblecourt et al. 2017; Xia et al. 2015).
4 Many conventional methods exist for hydrogen production, such as steam reforming and
5 water electrolysis (Cheng et al. 2012). Nevertheless, such methods may be accompanied
6 by some disadvantages of high temperature, high pressure and high energy consumption
7 (Holladay et al. 2009). In contrast, hydrogen production through dark fermentation of
8 biomass wastes is advantageous due to the low energy demand (Mamimin et al. 2017).
9 Furthermore, a wide range of organic wastes can be degraded by dark fermentation,
10 contributing to significant environmental benefits (Barca et al. 2016). However, the
11 accumulated volatile fatty acids (VFAs), which are generated as a by-product in
12 fermentation, can inhibit the metabolic activity of hydrogen-producing bacteria (HPB)
13 and reduce hydrogen production (Bundhoo & Mohee 2016; Elbeshbishy et al. 2017).

14 The inhibitory effects of VFAs and some control strategies have been investigated in a
15 number of studies. For example, Zhang et al. studied the inhibitory effects of acetate
16 (0-500 mmol/L) and butyrate (0-250 mmol/L) on dark fermentation using glucose as a
17 substrate and *Clostridium bifermentans* 3AT-ma as the HPB (Zhang et al. 2012). They
18 found that the hydrogen production trended to decrease with increased concentrations of
19 acetate or butyrate. Compared with acetate, butyrate exhibited a more significant
20 inhibition on fermentation. When acetate or butyrate was added to 20 mmol/L, the
21 hydrogen production decreased by 15% and 20%, respectively (Zhang et al. 2012).

1 Zheng and Yu studied the inhibitory effect of butyrate (4.2-25.1 g/L) on hydrogen
2 production during fermentation (Zheng & Yu 2005). They found that the hydrogen
3 production decreased by 81.7% with 25.1 g/L of butyrate compared with that without the
4 addition of butyrate (Zheng & Yu 2005).

5 Tang et al. found that the hydrogen production gradually decreased with increasing
6 acetate concentration. When the acetate concentration increased from 0 to 150 mmol/L,
7 the hydrogen production decreased from 2.2 mol H₂/mol glucose to 0.6 mol H₂/mol
8 glucose (Tang et al. 2012). Wang et al. studied the inhibitory effects of ethanol, acetic
9 acid, propionic acid and butyric acid on fermentative hydrogen production at various
10 VFAs concentrations ranging from 0 to 300 mmol/L. They concluded that the hydrogen
11 production and production rate all trended to decrease with increased VFAs
12 concentrations (Wang et al. 2008).

13 The suitable control of VFAs levels during fermentation can contribute to enhanced
14 hydrogen production. Noblecourt et al. used a submerged membrane anaerobic
15 bioreactor to avoid VFAs accumulation (Noblecourt et al. 2017). The component of
16 VFAs has similar molecular weights as monosaccharides and amino acids, rendering the
17 effective separation of substrates and by-products difficult. As a result, this technology
18 could cause a significant loss of small molecules (such as amino acids and
19 monosaccharides), which are favourable substrates for HPB. There are a few literatures
20 indicate that use of electrodialysis technology can remove VFAs and avoid the loss of
21 small molecules of organic components (Arslan et al. 2017; Jones et al. 2017; Tang et al.
22 2014). Jones et al. employed conventional electrodialysis to remove and recover VFAs

1 from model solutions and fermentation broths, resulting in high VFAs removal
2 efficiencies up to 99% at a voltage of 18 V during 60 min of the removal process (Jones
3 et al. 2015). The hydrogen production increased from 0.24 mol H₂/mol hexose to 0.90
4 mol H₂/mol hexose using conventional electrodialysis (Jones et al. 2017). It should be
5 noted that conventional electrodialysis was used for conducting post-treatment on the
6 fermentation effluent, and the fermentation liquor was subsequently circulated to the
7 fermentation reactor. Such a system includes the fermentation unit and the *ex situ* VFAs
8 removal unit, which cannot directly control the concentration of VFAs in the
9 fermentation reaction zone and may increase the system complexity.

10 However, previous studies were mainly focused on the batch VFAs removal in a
11 separated electrodialysis reactor. Continuous *in situ* VFAs removal during dark
12 fermentation by electrodialysis has yet been reported. In this paper, a novel
13 three-chamber electrodialysis bioreactor with *in situ* electrodialysis was proposed, for
14 the first time, to simultaneously remove VFAs continuously and to control the
15 concentration of VFAs in fermentation reaction zone directly, thereby enhancing
16 hydrogen fermentation. The aims of this study are to:

- 17 ● Assess the VFAs removal characteristics using synthetic fermentation liquor.
- 18 ● Compare the performance of hydrogen fermentation at various voltages.
- 19 ● Analyse the changes in concentrations of VFAs during hydrogen fermentation.

20

1 **2. Materials and methods**

2 **2.1. Bioreactor**

3 A three-chamber electro dialysis bioreactor was constructed using polymethyl
4 methacrylate. The inner length, width and height of the reactor are 12, 4 and 5 cm,
5 respectively. This bioreactor has a total volume of 240 mL. It comprises an anode
6 chamber (inner length, width and height are 3, 4 and 5 cm; 60 mL), a cathode chamber
7 (inner length, width and height are 3, 4 and 5 cm; 60 mL), and a fermentation chamber
8 (inner length, width and height are 6, 4 and 5 cm; 120 mL) separated by an anion
9 exchange membrane (AEM, 20 cm²) and a cation exchange membrane (CEM, 20 cm²).
10 AEM and CEM were purchased from Hangzhou Green Environmental Protection
11 Technology Co. LTD (Hangzhou, China). Graphite electrodes were used as the anode
12 and cathode with a thickness of 2 mm and an area of 20 cm² (Beijing Electric Carbon
13 Plant, Beijing, China). A programmable DC power supply (ARRAY 3646A, Bost
14 Electronic Instrument Co. LTD, Shenzhen, China) was used as an external power supply
15 for the electrodes.

16 **2.2. Inoculum and medium**

17 The mixed HPB was isolated and acclimated from the anaerobic digestion sludge
18 derived from a rural digester treating straw and manure in Chongqing, China. The
19 sludge was heated at 100 °C for 30 min to inactivate methanogens and hydrogen
20 consumers, and subsequently enriched three times (3 d each time) to enrich the

1 spore-forming HPB (Xia et al. 2015). The composition of the acclimation medium was
2 described in a previous study (Cheng et al. 2012).

3 **2.3. Experimental procedures**

4 The three-chamber electro dialysis bioreactor was used to assess the VFAs removal
5 characteristics by using a synthetic VFAs solution with an initial acetic acid
6 concentration of 20 mmol/L or a butyric acid concentration of 20 mmol/L. Eighty
7 millilitres of synthetic VFAs solution was added to the fermentation chamber, and 40 mL
8 of deionized water was added to the anode and cathode chambers to ensure an equal
9 liquid surface level in the fermentation chambers.

10 For hydrogen fermentation, 8 mL of acclimated HPB and 72 mL of deionized water
11 mixed with 0.8 g of glucose were added to the fermentation chamber. For all reactors,
12 glucose was used as the substrate at a concentration of 10 g/L. Forty millilitres of
13 deionized water was added to the anode and cathode chambers, respectively. The initial
14 pH value of the fermentation chamber was adjusted to 6.5 ± 0.1 using 6 mmol/L HCl or
15 NaOH solution.

16 The voltage was set at 0-6 V by a programmable DC power supply in the VFAs
17 removal experiments and hydrogen fermentation. A single chamber bioreactor (without
18 voltage and ion-exchange membrane) was used as the control, which was operated with
19 80 mL of fermentation medium with HPB. A three-chamber electro dialysis bioreactor
20 without substrate addition (no glucose) was used as the hydrogen fermentation blank (as
21 shown in Table 1). All fermentation chambers were purged with nitrogen gas for 5 min

1 to ensure an anaerobic environment. The headspace of the fermentation chamber was 40
2 mL. All bioreactors were kept in a thermostat water bath maintained at 35 °C for 96 h.
3 The gas produced was discharged from the headspace of the fermentation chamber and
4 subsequently collected using a graduated container. The gas and liquid samples were
5 collected at a time interval of 12 h for further analysis, and the pH value of the
6 fermentation solution was adjusted to 6.5 ± 0.1 using 6 mmol/L HCl or NaOH at each
7 time interval.

8 **2.4. Analytical methods**

9 The concentrations of acetic acid, propionic acid, butyric acid and hexanoic acid from
10 the fermentation liquor were quantified by a gas chromatograph (Agilent 7890B, USA)
11 equipped with a flame ionization detector (FID) and a polar capillary column (Agilent
12 DB-FFAP Column, 30 m \times 0.25 mm \times 0.25 μ m). The temperatures of the inlet, oven,
13 and FID were 250, 240, and 300 °C, respectively. N₂ was used as a carrier gas at a
14 column flow rate of 1 mL/min. The volume of the liquid samples injected into GC was
15 set as 1 μ L with split mode (split ratio 50). The pH value of the fermentation liquid
16 sample was adjusted with HCl to 2 (Cheng et al. 2012). The total inorganic carbon
17 (including bicarbonate, carbonate concentration and dissolved carbon dioxide) in the
18 liquor phase was measured by a Multi N/C 3000 analyser (Analytik Jena AG, Jena
19 Germany). The glucose concentration in the fermentation liquor was determined using
20 the 3,5-dinitrosalicylic acid method (Miller 1959).

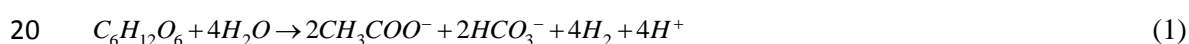
1 The gas composition (H₂ and CO₂) was determined through a gas chromatograph
2 (model Trace 1300; Thermo Scientific) equipped with a micropacked column
3 (ShinCarbon ST Columns, 2 m, OD 1/16", ID 1.0 mm, Mesh 100/120), and N₂ was used
4 as a carrier gas. H₂ and CO₂ concentrations were detected with a thermal conductivity
5 detector (TCD). The temperatures of the inlet, oven, and TCD detector were 120, 110,
6 and 300 °C, respectively. The volume of the gas samples injected into GC was 0.1 mL
7 with split mode (split ratio 29).

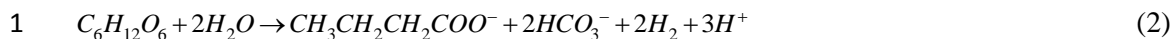
8 The hydrogen production was calculated from the amount and composition of the
9 total volume of hydrogen production in the graduated container at each time interval.
10 Hydrogen content in biogas was expressed as the ratio of the volume of hydrogen to the
11 total volume of hydrogen and carbon dioxide. During fermentation, the gas composition
12 and VFAs were measured every 12 h, and the total inorganic carbon and residual glucose
13 were analysed at the end of the experiment. All of the experimental trials were
14 conducted in triplicate, and the results are expressed as the mean (± standard deviation).

15 **3. Results and discussion**

16 **3.1. Removal characteristics using synthetic fermentation liquor**

17 In dark fermentation, hydrogen gas is usually produced through the acetate and butyrate
18 pathways (as shown in Eqs. (1) and (2)) (Barca et al. 2015; Gupta et al. 2014; Xia et al.
19 2016).





2 Acetate and butyrate have been identified as the major components in fermentation
3 liquor in various dark fermenters. In this study, to assess the VFAs removal
4 characteristics, acetate and butyrate solutions at a typical concentration of 20 mmol/L
5 were used as the synthetic fermentation liquor in the three-chamber electro dialysis
6 bioreactor. Fig. 1a shows the change in acetate concentration during the electro dialysis
7 removal. When the voltage was set as 0 V (without voltage), the concentration of acetate
8 slightly decreased with increasing time. The average removal rate of acetate was 0.09
9 mol/L/h, and the final concentration was 11.0 mmol/L at 96 h, which corresponds to an
10 overall removal efficiency of 44.8%. In this case, the acetate removal was driven mainly
11 by the diffusion force caused by the different acetate concentrations between the AEM.
12 Such a process is slow, and the concentration diffusion force was reduced by decreasing
13 the acetate concentration gradient.

14 When the voltage was set as 2 V, the average removal rate of acetate improved to 0.17
15 mol/L/h. Meanwhile, the final concentration was remarkably reduced to 5.2 mmol/L,
16 corresponding to a removal efficiency of 73.9%. This can be attributed to the enhanced
17 driving force built upon the electric field, in which the process uses an electrical driving
18 force to transfer acetate ions from the fermentation chamber to the anode chamber,
19 thereby improving acetate removal (Mei & Tang 2018; Prochaska et al. 2018).

20 As the voltage further increased to 4 V and 6 V, the final acetate concentration
21 obtained at 2 V (at 96 h) was achieved at approximately 40 h and 20 h, respectively.

1 Meanwhile, the average removal rates of acetate were 0.22 and 0.35 mmol/L/h,
2 respectively, at the voltage of 4 V and 6 V. As a result, the acetate removal efficiency
3 increased to 94.7% and 95.6% at 96 h. This was due to the driving force further
4 enhanced by intensifying the electric field, which significantly improves acetate
5 removal.

6 The accumulation of acetate in the anode chamber is shown in Fig. 1b. The
7 concentration of acetate gradually increased with time in all groups. The acetate
8 concentrations in the anode chamber achieved with voltage application were even higher
9 than those in the fermentation chamber after removal experiments. This can be explained
10 by the fact that the electrodialysis played a dominant role rather than the concentration
11 diffusion. It should be noted that the volume of fermentation chamber was two times
12 larger than that of the anode chamber, leading to a faster concentration change in the
13 anode chamber compared with the fermentation chamber. Nevertheless, the sum amount
14 of acetate in the fermentation and anode chambers was slightly lower than the initial
15 total amount of acetate (20 mmol/L with 80 mL volume = 1.6 mmol). This can be
16 attributed to the partial adsorption of acetate on the AEM.

17 The trend of butyrate removal was similar to that of acetate removal (Fig. 2a). The
18 butyrate concentration in the fermentation chamber also decreased with increased time.
19 As the voltage increased from 0 to 6 V, the average removal rate of butyrate gradually
20 increased and achieved 0.10, 0.15, 0.19, 0.26 mmol/L/h, respectively, and the removal
21 efficiency of butyrate was increased from 47.7% to 94.6%. However, the molecular

1 weight of butyrate is greater than that of acetate, resulting in an increased mass transfer
2 resistance when transferring through the AEM. As a result, the removal rate was lower
3 for butyrate than for acetate. Meanwhile, the butyrate removed from the fermentation
4 chamber was enriched in the anode chamber as shown in Fig. 2b.

5 **3.2. Hydrogen production during fermentation**

6 Dark fermentation in the three-chamber electro dialysis reactor was performed to assess
7 the effects of voltage on hydrogen production performance. When the fermentation was
8 conducted in a single chamber bioreactor without voltage and a membrane (control
9 group), the volumetric hydrogen productivity slowly increased to 61.3 mL/L at the
10 initial fermentation stage (12 h) due to the adaption of HPB (Fig. 3). As the fermentation
11 time increased to 60 h, the volumetric hydrogen productivity rapidly increased to 1116.3
12 mL/L. This suggests a high activity of HPB metabolism and efficient hydrogen
13 production. When the fermentation time further increased to 96 h, the volumetric
14 hydrogen productivity slowly increased to 1208.5 mL/L (corresponding to a specific
15 hydrogen yield of 1.0 mol H₂/mol glucose). The later stage of hydrogen production was
16 less efficient, which may be explained by the depletion of glucose and the inhibitory
17 effect by the accumulated VFAs (Wang et al. 2008; Zhang et al. 2012; Zheng & Yu
18 2005).

19 When the three-chamber reactor was applied without voltage, a slight increase in
20 volumetric hydrogen productivity after 60 h was observed. The accumulation of VFAs
21 in the later stage would be inhibitory for HPB and not advantageous for hydrogen
22 production. This inhibitory effect would be reduced via *in situ* VFAs removal by

1 diffusion across the AEM, thereby improving the volumetric hydrogen productivity. As
2 a result, the final volumetric hydrogen productivity increased from 1208.5 to 1330.4
3 mL/L, and the average hydrogen production rate increased from 12.6 to 13.9 mL/L/h
4 (Fig. 3).

5 When the voltage was set as 2 V, the effect of VFAs removal was enhanced by the
6 electrical driving force. The volumetric hydrogen productivity increased to 1386.4 mL/L,
7 and the hydrogen production rate improved to 14.4 mL/L/h. As the voltage increased to
8 4 V, the electrical driving force was further enhanced and the VFAs removal
9 performance was accordingly improved. The inhibition of VFAs in the late stage of
10 fermentation was dampened, with a significant hydrogen production improvement after
11 36 h. Consequently, the volumetric hydrogen productivity further increased to 1878.0
12 mL/L (corresponding to a specific hydrogen yield of 1.5 mol H₂/mol glucose) with an
13 average hydrogen production rate of 19.6 mL/L/h. As the voltage increased to 6 V,
14 however, the volumetric hydrogen productivity slightly decreased to 1859.3 mL/L with
15 an average hydrogen production rate of 19.4 mL/L/h. This may be attributed to the fact
16 that the ionization of weak acid is enhanced at 6 V, adversely affecting the microbial
17 activity. It could also be explained that the electrical driving force affects the surface
18 charge distribution of the cell and then changes the permeability of the ion to cell.
19 Furthermore, the removal rate of VFAs by electro dialysis was in accordance with the
20 VFAs production rate at 4 V during the fermentation; as a result, the fermentation
21 chamber can be operated stably and efficiently at a low VFAs concentration. The
22 residual glucose concentration in the fermentation effluents for all trials was in the range

1 of 0.22-0.30 g/L, corresponding to 97.0%-97.9% of the glucose utilization efficiency.

2 This suggests that most of the substrate was consumed by HPB during the dark
3 fermentation.

4 It should be noted that no gas production was observed in the anode and cathode
5 chambers during fermentation. Furthermore, a blank group (inoculum without substrate
6 added) was tested, and no gas production was observed in any of the three chambers.
7 These results confirm that the hydrogen production was sourced from the fermentation
8 of glucose rather than from the electrolysis of water.

9 In control group, the hydrogen content was 50.9%. Interestingly, the hydrogen
10 content increased when the voltage was increased from 0 V to 6 V and achieved 54.6%,
11 65.3%, 69.5%, 84.7%, respectively. Apart from hydrogen, carbon dioxide is a major
12 gaseous product that can be easily dissolved in the liquid phase to form bicarbonate and
13 carbonate (Eqs. (3) and (4)). The electrical driving force affects the transportation of
14 bicarbonate and carbonate across the AEM, thereby reducing the bicarbonate and
15 carbonate concentration in the fermentation liquor. This can be confirmed by the results
16 where the total inorganic carbon (including for bicarbonate, carbonate concentration and
17 dissolved carbon dioxide) in the anode chamber were 214.7, 437.5, 796.7, and 1143.4
18 mg/L at a voltage of 0 V, 2 V, 4 V, and 6 V at 96 h, respectively. As a result, carbon
19 dioxide dissolution was promoted and the carbon dioxide content decreased, whereas
20 hydrogen content increased. The increased hydrogen content can reduce the gas storage
21 requirement and lower the gas upgrading cost, which is very beneficial for biohydrogen
22 production at an industrial scale.



3 **3.3. VFAs removal during fermentation**

4 The concentrations of acetate and butyrate in the fermentation chamber are shown in
5 **Figs. 4a and 4b**, respectively. When the fermentation was conducted in the single
6 chamber bioreactor (control group), the acetate and butyrate concentrations rapidly
7 increased to 26.9 and 32.3 mmol/L, respectively, with the increased fermentation time of
8 60 h. This suggests that glucose was quickly metabolized to VFAs during this stage. As
9 the fermentation time further increased to 96 h, the acetate and butyrate gradually
10 increased to 32.2 and 35.0 mmol/L. A number of studies have confirmed that the
11 hydrogen production tends to decrease with increasing concentrations of VFAs (Wang et
12 al. 2008; Zhang et al. 2012; Zheng & Yu 2005). Zhang et al found that with the addition
13 of acetate or butyrate to 20 mmol/L the hydrogen production decreased by more than
14 15% and 20%, respectively (Zhang et al. 2012). Therefore, high levels of VFAs would
15 be inhibitory for hydrogen production. Consequently, a small amount of hydrogen was
16 produced after 60 h (see **Fig. 3**).

17 When the three-chamber electro dialysis reactor was operated without voltage, the
18 concentrations of acetate and butyrate in the fermentation chamber decreased slightly
19 compared with those in the control group (single chamber bioreactor). This can be
20 attributed to the concentration diffusion between the fermentation and anode chambers
21 in which acetate and butyrate can pass through the AEM to the anode chamber. However,

1 the diffusion by the concentration gradient is not efficient; thus, the concentrations of
2 acetate and butyrate in the fermentation remained at high levels, achieving 26.8 and 32.0
3 mmol/L at 96 h.

4 When the voltage was set as 2 V, the concentrations of acetate and butyrate still
5 increased with the fermentation time of 60 h and remained stable with the fermentation
6 time of 96 h. The final concentrations of acetate and butyrate achieved were 18.0 and
7 21.9 mmol/L, which were 32.8% and 31.6% lower compared with the 0 V group,
8 respectively. This implies that the electrical driving force boosted the VFAs removal. As
9 a result, the hydrogen production in the later stage (after 60 h) was significantly
10 improved (Fig. 3).

11 When the voltage was increased to 4 V, the VFAs removal was enhanced. No
12 significant increases in the concentrations of acetate and butyrate were observed during
13 12 h to 60 h. The final concentrations of acetate and butyrate were only 10.3 and 13.1
14 mmol/L, which were 61.6% and 59.1% lower than in the 0 V group, respectively. Wang
15 et al found that with the addition of acetate or butyrate to 10 mmol/L the inhibitory
16 effect on substrate degradation efficiency and hydrogen production decreased just
17 slightly compared with that without the addition of acetate or butyrate (Wang et al.
18 2008). The effective removal of VFAs facilitated hydrogen production at the later
19 fermentation stage. Therefore, the hydrogen production rate during 60-84 h could
20 remain at high level (18.2 mL/L/h) compared with the 22.5 mL/L/h obtained during 0-60
21 h.

1 As the voltage was further increased to 6 V, the concentrations of acetate and butyrate
2 were similar to those obtained at the voltage of 4 V. This suggests that a further increase
3 of voltage could not improve the VFAs removal when the VFAs concentrations were at
4 extremely low levels in the fermentation chamber. The final concentration of acetate and
5 butyrate were decreased slightly to 7.6 and 8.5 mmol/L, respectively.

6 Acetate and butyrate were removed from the fermentation chamber but enriched in
7 the anode chamber. Figs. 4c and 4d show the changes in the concentrations of acetate
8 and butyrate in the anode chamber. When the voltage was set as 0 V, the acetate and
9 butyrate concentrations gradually increased with the fermentation time. The final
10 concentrations of acetate and butyrate achieved were 5.3 and 7.1 mmol/L, respectively.

11 As the voltage was increased from 2 V to 4 V, the VFAs enrichment effect was
12 enhanced, the final acetate concentration increased from 6.8 to 21.4 mmol/L, and the
13 final butyrate concentration increased from 7.9 to 14.9 mmol/L. However, the
14 concentration of acetate in the anode chamber increased slightly in the 6 V group after
15 48 h, and the concentration of acetate at 48 h and 96 h were 17.2 and 17.8 mmol/L,
16 respectively. The concentration of butyrate increased continuously and reached 16.7
17 mmol/L at 96 h. This may be attributed to the shift in metabolism of HPB from the
18 acetate to butyrate pathway, leading to the reduction in acetate production and increase
19 in butyrate production.

20 The final concentration of total VFAs (mainly acetate and butyrate) in the
21 fermentation chamber decreased with increasing voltage (see Fig. 5a). In the control
22 group of the single-chamber reactor, the final concentration of total VFAs was 70.3

1 mmol/L. The experimental results show that the three-chamber electro dialysis bioreactor
2 has obvious effect on control VFAs concentrations (compared with control, total of
3 VFAs decrease by 11.9% to 75.7% when voltage increased from 0 to 6 V). The
4 performance of VFAs removal was greatly improved when a low voltage was applied, as
5 compared with the electro dialysis reactor for post-treatment of fermentation effluent in a
6 recent study (total of VFAs decreased by 26.1% per 24 h at a voltage of 18 V) (Jones et
7 al. 2017). These results indicate that the total VFAs concentrations can be maintained at
8 a desired level by controlling the voltage during the fermentation.

9 **3.4. Utilization of VFAs as substrate**

10 Enhanced hydrogen production can be achieved in the three-chamber electro dialysis
11 bioreactor, in which the accumulated VFAs in the fermentation chamber can be
12 effectively removed. Meanwhile, the VFAs recovered in the anode chamber were
13 considered to be valuable products. As shown in Fig. 5b, the total VFAs concentrations
14 were 13.1, 15.6, 37.5, and 34.5 mmol/L in the anode chamber at voltages of 0 V, 2 V, 4
15 V, and 6 V, respectively. VFAs can be used as an important raw material in various
16 industrial applications (Jones et al. 2017; Motte et al. 2015). For example, VFAs can be
17 used as precursors for biodiesel production. VFAs can also be used as an external carbon
18 source for the biological denitrification of wastewater rich in nitrogen (Motte et al. 2015)
19 and for electricity generation via microbial fuel cells (Pham et al. 2012; Wang et al.
20 2014).

21

1 **4. Conclusion**

2 The three-chamber electrodialysis bioreactor was proposed to effectively remove the
3 VFAs to promote hydrogen fermentation. A volumetric hydrogen productivity of 1878.0
4 mL/L was achieved in the fermentation chamber at a voltage of 4 V, which is 55.4%
5 higher than that (1208.5 mL/L) of the control group. By applying different voltages (0-6
6 V), the hydrogen content accumulated to 54.6%-84.7%, exhibiting increases of
7 7.1%-66.4% compared with the control. Meanwhile, the maximum concentration of
8 acetate and butyrate in the fermentation chamber was maintained at low levels of 10.3
9 and 13.1 mmol/L, respectively, which were 68.0% and 62.4% lower than the control
10 group.

11 **Acknowledgements**

12 This work was supported by the National Science Foundation for Young Scientists of
13 China (No. 51606021), the International Cooperation and Exchange of the National
14 Natural Science Foundation of China (No. 51561145013), the Fundamental Research
15 Funds for the Central Universities (No. 106112015CDJXY140003), and the Venture &
16 Innovation Support Program for Chongqing Overseas Returnees (No. cx2017019). Dr
17 Richen Lin acknowledges the support from the European Union's Horizon 2020
18 research and innovation programme under the Marie Skłodowska-Curie grant agreement
19 No. 797259.

1 **References**

- 2 [1]Arslan, D., Zhang, Y., Steinbusch, K.J.J., Diels, L., Hamelers, H.V.M., Buisman, C.J.N., De Wever, H.
3 2017. In-situ carboxylate recovery and simultaneous pH control with tailor-configured bipolar
4 membrane electrodialysis during continuous mixed culture fermentation. *Sep Purif Technol*, **175**,
5 27-35.
- 6 [2]Barca, C., Ranava, D., Bauzan, M., Ferrasse, J.H., Giudici-Ortoni, M.T., Soric, A. 2016.
7 Fermentative hydrogen production in an up-flow anaerobic biofilm reactor inoculated with a
8 co-culture of *Clostridium acetobutylicum* and *Desulfovibrio vulgaris*. *Bioresour Technol*, **221**,
9 526-533.
- 10 [3]Barca, C., Soric, A., Ranava, D., Giudici-Ortoni, M.T., Ferrasse, J.H. 2015. Anaerobic biofilm
11 reactors for dark fermentative hydrogen production from wastewater: A review. *Bioresour*
12 *Technol*, **185**, 386-98.
- 13 [4]Bundhoo, M.A.Z., Mohee, R. 2016. Inhibition of dark fermentative bio-hydrogen production: A
14 review. *Int. J. Hydrogen Energy*, **41**(16), 6713-6733.
- 15 [5]Cheng, J., Xia, A., Song, W., Su, H., Zhou, J., Cen, K. 2012. Comparison between heterofermentation
16 and autofermentation in hydrogen production from *Arthrospira* (*Spirulina*) *platensis* wet biomass.
17 *Int. J. Hydrogen Energy*, **37**(8), 6536-6544.
- 18 [6]Elbeshbishy, E., Dhar, B.R., Nakhla, G., Lee, H.-S. 2017. A critical review on inhibition of dark
19 biohydrogen fermentation. *Renew Sust Energ Rev*, **79**, 656-668.
- 20 [7]Gupta, M., Velayutham, P., Elbeshbishy, E., Hafez, H., Khafipour, E., Derakhshani, H., El Naggar,
21 M.H., Levin, D.B., Nakhla, G. 2014. Co-fermentation of glucose, starch, and cellulose for
22 mesophilic biohydrogen production. *Int. J. Hydrogen Energy*, **39**(36), 20958-20967.
- 23 [8]Holladay, J.D., Hu, J., King, D.L., Wang, Y. 2009. An overview of hydrogen production technologies.
24 *Catal Today*, **139**(4), 244-260.
- 25 [9]Jones, R.J., Massanet-Nicolau, J., Guwy, A., Premier, G.C., Dinsdale, R.M., Reilly, M. 2015. Removal
26 and recovery of inhibitory volatile fatty acids from mixed acid fermentations by conventional
27 electrodialysis. *Bioresour Technol*, **189**, 279-84.
- 28 [10]Jones, R.J., Massanet-Nicolau, J., Mulder, M.J., Premier, G., Dinsdale, R., Guwy, A. 2017. Increased
29 biohydrogen yields, volatile fatty acid production and substrate utilisation rates via the
30 electrodialysis of a continually fed sucrose fermenter. *Bioresour Technol*, **229**, 46-52.
- 31 [11]Mamimin, C., Prasertsan, P., Kongjan, P., O-Thong, S. 2017. Effects of volatile fatty acids in
32 biohydrogen effluent on biohythane production from palm oil mill effluent under thermophilic
33 condition. *Electron J Biotechnol*, **29**, 78-85.
- 34 [12]Mei, Y., Tang, C.Y. 2018. Recent developments and future perspectives of reverse electrodialysis
35 technology: A review. *Desalination*, **425**, 156-174.
- 36 [13]Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal.*
37 *Chem*, **31** (3), 426-428.
- 38 [14]Motte, J.-C., Sambusiti, C., Dumas, C., Barakat, A. 2015. Combination of dry dark fermentation and
39 mechanical pretreatment for lignocellulosic deconstruction: An innovative strategy for biofuels
40 and volatile fatty acids recovery. *Appl Energ*, **147**, 67-73.

- 1 [15]Noblecourt, A., Christophe, G., Larroche, C., Santa-Catalina, G., Trably, E., Fontanille, P. 2017. High
2 hydrogen production rate in a submerged membrane anaerobic bioreactor. *Int. J. Hydrogen*
3 *Energy*, **42**(39), 24656-24666.
- 4 [16]Pham, T.N., Nam, W.J., Jeon, Y.J., Yoon, H.H. 2012. Volatile fatty acids production from marine
5 macroalgae by anaerobic fermentation. *Bioresour Technol*, **124**, 500-3.
- 6 [17]Prochaska, K., Antczak, J., Regel-Rosocka, M., Szczygielka, M. 2018. Removal of succinic acid from
7 fermentation broth by multistage process (membrane separation and reactive extraction). *Sep*
8 *Purif Technol*, **192**, 360-368.
- 9 [18]Tang, J., Jia, S., Qu, S., Xiao, Y., Yuan, Y., Ren, N.-Q. 2014. An integrated biological hydrogen
10 production process based on ethanol-type fermentation and bipolar membrane electrodialysis. *Int.*
11 *J. Hydrogen Energy*, **39**(25), 13375-13380.
- 12 [19]Tang, J., Yuan, Y., Guo, W.-Q., Ren, N.-Q. 2012. Inhibitory effects of acetate and ethanol on
13 biohydrogen production of *Ethanoligenens harbinese* B49. *Int. J. Hydrogen Energy*, **37**(1),
14 741-747.
- 15 [20]Wang, B., Wan, W., Wang, J. 2008. Inhibitory effect of ethanol, acetic acid, propionic acid and
16 butyric acid on fermentative hydrogen production. *Int. J. Hydrogen Energy*, **33**(23), 7013-7019.
- 17 [21]Wang, K., Yin, J., Shen, D., Li, N. 2014. Anaerobic digestion of food waste for volatile fatty acids
18 (VFAs) production with different types of inoculum: effect of pH. *Bioresour Technol*, **161**,
19 395-401.
- 20 [22]Xia, A., Cheng, J., Ding, L., Lin, R., Song, W., Su, H., Zhou, J., Cen, K. 2015. Substrate consumption
21 and hydrogen production via co-fermentation of monomers derived from carbohydrates and
22 proteins in biomass wastes. *Appl Energ*, **139**, 9-16.
- 23 [23]Xia, A., Cheng, J., Murphy, J.D. 2016. Innovation in biological production and upgrading of methane
24 and hydrogen for use as gaseous transport biofuel. *Biotechnol Adv*, **34**(5), 451-472.
- 25 [24]Zhang, S., Kim, T.-H., Lee, Y., Hwang, S.-J. 2012. Effects of VFAs Concentration on Bio-hydrogen
26 Production with *Clostridium Bifermentans* 3AT-ma. *Energy Procedia*, **14**, 518-523.
- 27 [25]Zheng, X.J., Yu, H.Q. 2005. Inhibitory effects of butyrate on biological hydrogen production with
28 mixed anaerobic cultures. *J Environ Manage*, **74**(1), 65-70.

29

1 **Table caption**

2 Table 1. Experimental design for the VFAs removal and hydrogen production trials.

3

1 **Table 1** Experimental design for the VFAs removal and hydrogen production trials.

	Bioreactor	Voltage	Substrate	Inoculum
VFAs removal experiments	Three-chamber electro dialysis bioreactor	0-6 V	Simulated fermentation broth (acetic or butyric acid)	No
Hydrogen fermentation	Three-chamber electro dialysis bioreactor	0-6 V	Glucose solution	HPB
Hydrogen fermentation blank	Three-chamber electro dialysis bioreactor	0-6 V	No	HPB
Control	Single-chamber	No	Glucose solution	HPB

2 VFAs: volatile fatty acids; HPB: hydrogen-producing bacteria.

1 **Figure captions**

2 **Fig. 1.** Removal experiments of simulated fermentation broth. (a) Acetate concentration in the
3 fermentation chamber; (b) Acetate concentration in the anode chamber.

4 **Fig. 2.** Removal experiments of simulated fermentation broth. (a) Butyrate concentration in the
5 fermentation chamber; (b) Butyrate concentration in the anode chamber.

6 **Fig. 3.** Hydrogen fermentation with electro dialysis.

7 **Fig. 4.** VFAs removal during fermentation. (a) Acetate concentration in the fermentation
8 chamber; (b) Butyrate concentration in the fermentation chamber; (c) Acetate concentration in
9 the anode chamber; (d) Butyrate concentration in the anode chamber.

10 **Fig. 5.** Total VFAs in the fermentation chamber (a); Total VFAs in the anode chamber (b).

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

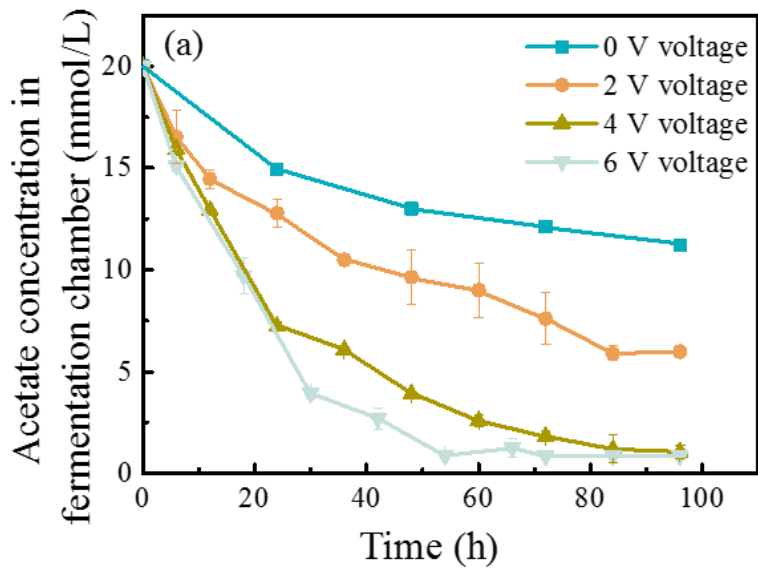
29

30

31

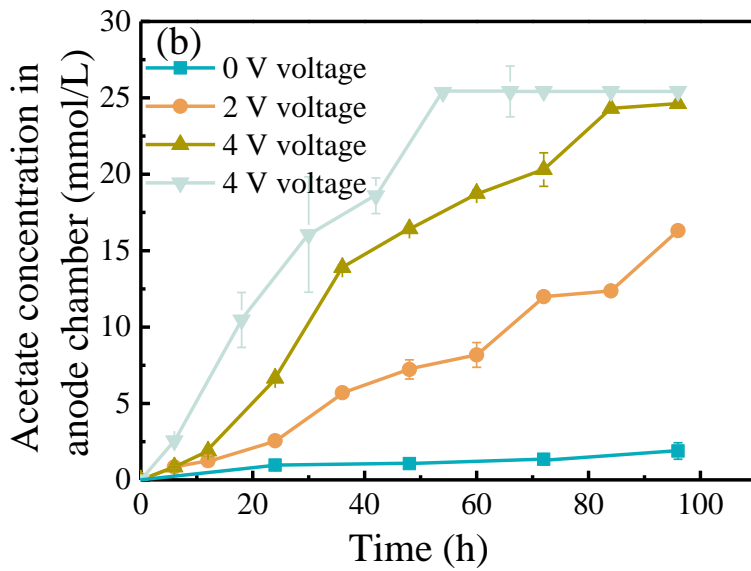
32

1



2

3



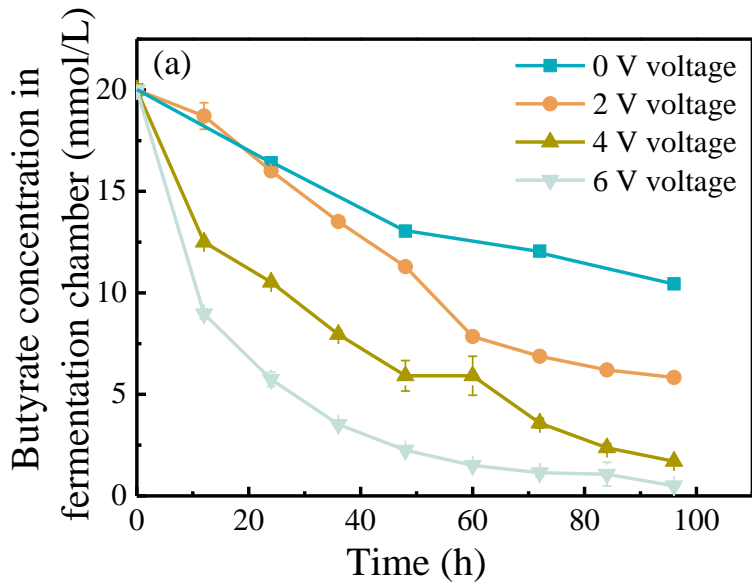
4

5

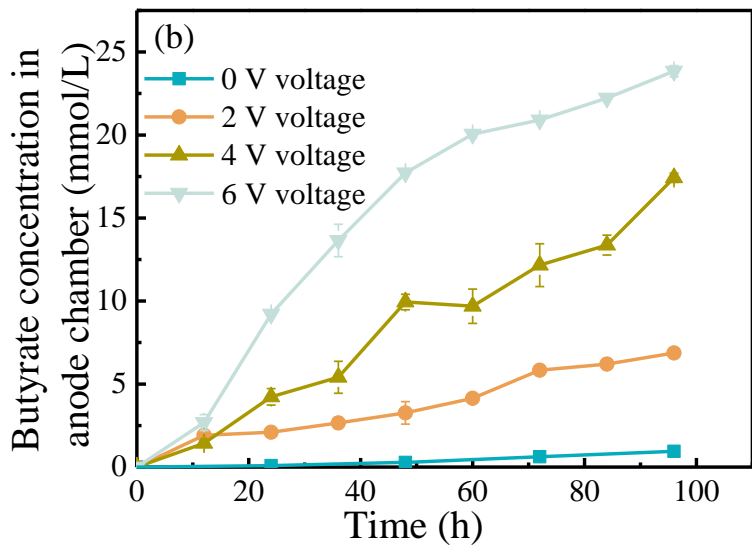
6 **Fig. 1** Removal experiments of simulated fermentation broth. (a) Acetate concentration in the
7 fermentation chamber; (b) Acetate concentration in the anode chamber.

8

1



2

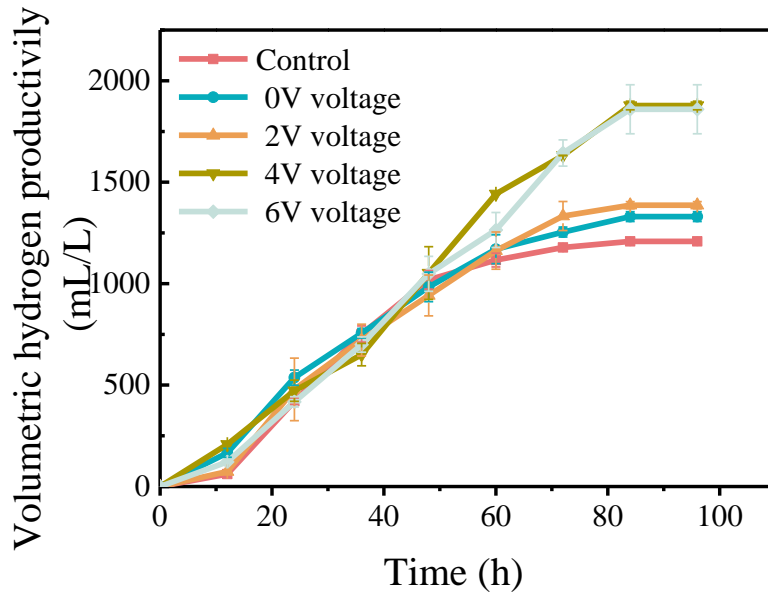


3

4 **Fig. 2** Removal experiments of simulated fermentation broth. (a) Butyrate concentration in the
5 fermentation chamber; (b) Butyrate concentration in the anode chamber.

1

2



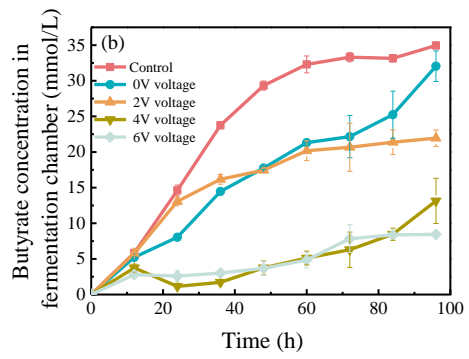
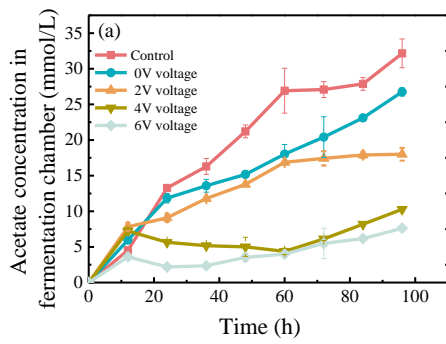
3

4 **Fig. 3** Hydrogen fermentation with electro dialysis.

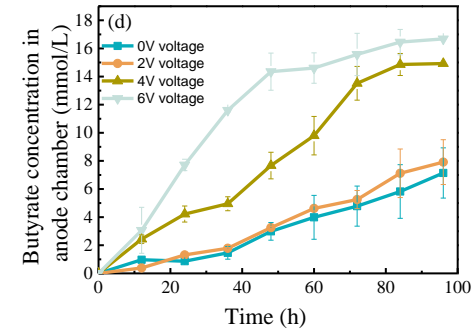
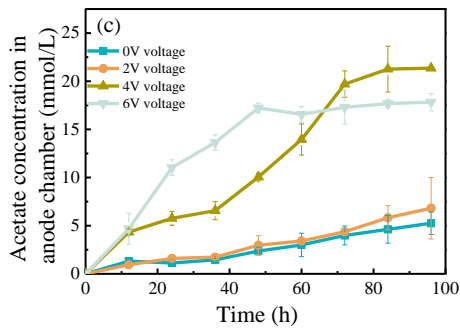
5

6

1



2



3

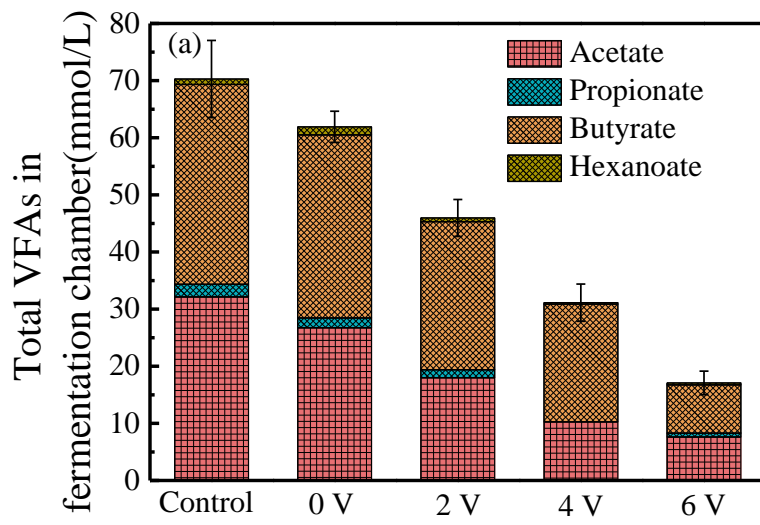
4 **Fig. 4** VFAs removal during fermentation. (a) Acetate concentration in the fermentation
5 chamber; (b) Butyrate concentration in the fermentation chamber; (c) Acetate concentration in
6 the anode chamber; (d) Butyrate concentration in the anode chamber.

7

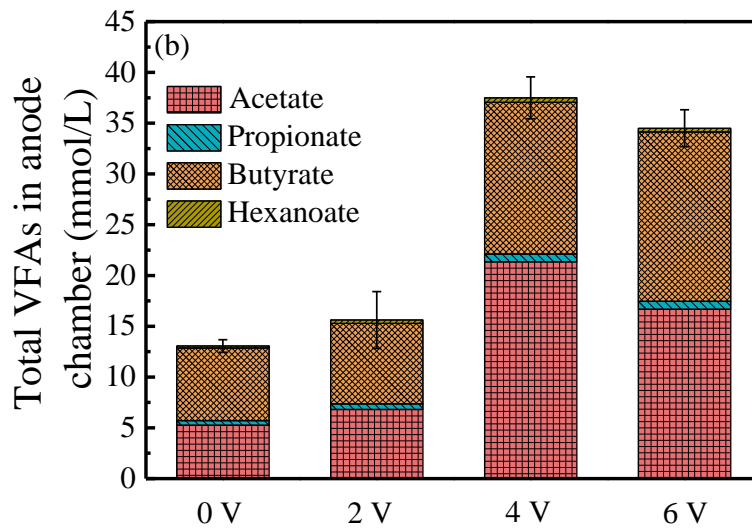
8

9

1



2



3

4

5 **Fig. 5** Total VFAs in the fermentation chamber (a); Total VFAs in the anode chamber (b).

6

7