Effects of different substrates on microbial electrolysis cell (MEC) anodic membrane: biodiversity and hydrogen production performance

Qiongli Shao, Jianchang Li, Sixia Yang and Helin Sun

ABSTRACT

To investigate the effects of different substrates on the biodiversity and hydrogen production performance of microbial electrolysis cell (MEC) anodic membranes, the vital electroactive microorganisms (e.g. in MEC hydrogen production) were worth identifying. In the present study, single-factor experiments were performed. Sodium acetate, sodium propionate, sodium butyrate, glucose and starch served as different substrates for MEC anodic culture experiments under the same condition. The effects of different substrates on the bioactivity, biomass and hydrogen production performance of MEC anodic films were analyzed. Also, the effects of different microbial communities on hydrogen production were studied using 16S rRNA sequencing. According to the experimental results, all the five substrates here can serve as hydrogen-producing raw materials for MEC. All indicators revealed that sodium acetate, sodium propionate and sodium butyrate are excellent biofilm culture materials. The serious acidification of glucose and starch was identified at the same substrate concentration, and the environment of the culture medium was difficult to control, which affected the growth and metabolism of electroactive microorganisms. In comparison, sodium acetate was the best, achieving a maximum output of 23.4 mA and a maximum hydrogen content of 25.85%. The other four were ranked as sodium butyrate > sodium propionate > glucose > starch. According to the results of high-throughput sequencing, when sodium acetate, sodium propionate, sodium butyrate, glucose and starch served as substrates, the number of operational taxonomic units reached 464, 728, 636, 784, and 1,083, respectively. Furthermore, when MEC was cultured with sodium acetate, sodium propionate and sodium butyrate as substrates, the electroactive microorganism *Desulfuromonas* in the *Proteobacteria* would contribute the most to producing hydrogen. The relative abundance of the five substrates was ranked as sodium acetate > sodium butyrate > sodium propionate > glucose > starch, suggesting that the MEC anodic film cultured with sodium acetate as the substrate exhibited the best hydrogen production performance, and the starch showed the worst. It is noteworthy that *Desulfuromonas* was the most abundant species according to sequencing results. When glucose and starch served as substrates, they exhibited high biodiversity. The anodic membranes cultured with sodium acetate, sodium propionate and sodium butyrate were not as good as those cultured with glucose and starch, yet the electroactive microorganisms were up-regulated.

Key words | biodiversity, hydrogen production, MEC anodic film, substrate

Qiongli Shao
Jianchang Li (corresponding author)
Sixia Yang
Helin Sun
Yunnan Normal University,
Kunming 650500, Yunnan,
China
E-mail: *li.jianchang@aliyun.com*

INTRODUCTION

The rapid economic development in China is facing the dual pressure of energy shortage and environmental pollution. To address such pressure, it is imperative to find a clean and efficient new energy source. Hydrogen energy is a renewable energy source with the characteristics of being non-toxic and non-polluting, and it has high quality combustion performance, a wide range of available forms and high energy. Hydrogen has the highest energy content per unit mass except from nuclear fuel, with a heating value of 143 MJ/kg, which is nearly three times as high as that for gasoline.

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Therefore, hydrogen energy is considered the best energy source of the 21st century. As an efficient, clean and sustainable 'carbon-free' energy source, hydrogen energy has received widespread attention from various countries in the world. Developing a hydrogen economy is a permanent strategic choice for mankind to get rid of dependence on fossil fuels and ensure energy security.

Nowadays, the biggest limitation of hydrogen production efficiency is the thermodynamic problem in the traditional hydrogen production process. The existing methods of hydrogen production primarily include conventional chemical hydrogen production (e.g. hydrocarbon-reforming hydrogen production (Kniep *et al.* 2005), desulfurization hydrogen production (Alptekin *et al.* 2008), ion-reforming hydrogen production (Du *et al.* 2014) and electrolysis of water to make hydrogen), and bio-hydrogen production (e.g. light fermentation hydrogen production (Wen *et al.* 2010), dark fermentation hydrogen production (Yongfeng *et al.* 2009)). Among them, the chemical hydrogen production method (except hydrogen produced by electrolyzed water) has large environmental pollution, low net energy value, and high energy consumption rate. Although the electrolysis-water hydrogen production technology is clean and pollution-free, it requires a higher electrolytic voltage. Tartakovsky *et al.* (2009) showed that the minimum electrolysis voltage required is 1.23 V. The bio-hydrogen production method uses fuel wood, sawdust, wheat straw, rice straw, etc. as raw materials to obtain hydrogen. Although the method uses waste materials, it has the disadvantages of low conversion rate of hydrogen, difficulty in separation of hydrogen, and the like. Therefore, Logan *et al.* (2008) proposed a novel hydrogen production method based on microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for hydrogen production. The MEC is an appropriate modification of the original MFC. At present, there is no definite conclusion on the working principle of MEC. It is generally considered that under anaerobic conditions, an additional external voltage is added to enrich CO_2, protons, and electrons that are accumulated in the MEC anode and electroactive microorganisms, and the electrons pass through the extracellular electrons due to the presence of an applied voltage. The carrier is delivered to the anode and then through an external circuit to the cathode. The protons pass through the proton exchange membrane or directly through the electrolyte to the cathode and are reduced to hydrogen. Compared with other hydrogen production methods, MEC hydrogen production is friendly with the advantages of wide availability of substrate sources, high hydrogen conversion rate, and the combination of electrochemical and microbiological principles. Current researches show that all types of organic wastes (e.g. livestock manure, domestic sewage, activated sludge, and industrial wastewater) can serve as ideal substrate sources for MEC hydrogen production. Ueno *et al.* (1996) used sugar fermentation wastewater to ferment and produce hydrogen in a 5 L anaerobic reactor in continuous operation for 190 days; the hydrogen production rate was 0.76 L H_2/L·d, and hydrogen content in the gas production reached 64%. Yu & Zhu (2002) used a 3.0 L upflow anaerobic reactor for hydrogen production from brewery wastewater. When the pH was 5.5, the hydraulic retention time was 2 h, and the influent chemical oxygen demand was 34 g/L, the hydrogen production rate was 9.33 L/g-VSS.d (VSS: volatile suspended solids).

The electroactive microorganisms dominate the entire microbial electrolysis cell fermentation system. The activity, enrichment degree, and metabolic capacity of electroactive microorganisms will directly affect the hydrogen production capacity of microbial electrolytic cells. However, most current reports are focusing on electroactive microorganisms in MFC. Daniel & Lovley (2003) inoculated *Geobacter sulfurreducens* in the anode compartment of the MFC. According to the experimental results, the *Geobacter sulfurreducens* strain can adsorb the electrode and maintain its activity for a long time, and it can be used during the complete oxidation of organic substrates as well as the quantitative electron transfer to the electrode.

In 2006, Zhang *et al.* (2006) used *Escherichia coli* K12 as functional bacteria and glucose and yeast extract as a carbon source to construct a non-mediator MFC. In 2008, Logan's research group separated and screened two novel strains of electrogenic production, *Ochrobactrum anthropi* YZ-1 (Zuo *et al.* 2008) and *Rhodopseudomonas palustris* DX-3 (Xing *et al.* 2008), respectively, from anodic biofilms of MFC reactors. In terms of the working principle of MEC and MFC, the electroactive microorganisms in both should have certain commonalities, but in fact the community structure among them is different. Currently known electroactive microorganisms such as *Desulfuromonas* (Bond *et al.* 2002), *Desulfovibrio* (Zhao *et al.* 2008), *Geobacter* (Lovley *et al.* 1993), and *Geopsychrobacter* (Holmes *et al.* 2004) belong to *Deltaproteobacteria*; many bacteria of the genus *Geobacter* can produce electricity and are an important class of production. *Geobacter sulfurreducens* (Bond & Lovley 2003), for which genome sequencing has been completed, and *Geobacter metallireducens* (Bond *et al.* 2002) are currently the most well-known electrogenic bacteria. In the operation of MFCs, the Coulombic efficiency can even reach 99%, and electricity production is the main

way to obtain energy (Methé *et al.* 2003). Even so, the causative factor of the different community structure between electroactive microorganisms in MEC and MFC is unknown. To clarify the mechanism underlying this status, the study here is devoted to investigating the biodiversity of a MEC anode membrane. Moreover, a series of in-depth analyses were performed to explore the effect of different substrates on the anodic membrane biodiversity and the effect of different biological communities on the hydrogen production performance, and to select the most suitable raw materials for the growth and metabolism of electroactive microorganisms in MECs.

MATERIALS AND METHODS

Materials

Inoculum

The inoculum was taken from the anaerobic activated sludge after the normal biogas fermentation at the Biomass Energy Research Laboratory of the School of Energy and Environmental Sciences, Yunnan Normal University. The pH was determined as 7.79, total solids was 15.04% and volatile solids was 8.14%.

Substrates

Five substrates were used: sodium acetate, sodium propionate, sodium butyrate, glucose, and starch.

Material ratio

Buffer solution

The buffer solution contained calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) 10 mg/L, magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H2O$) 100 mg/L, potassium chloride (KCl) 2.22 g/L, potassium dihydrogen phosphate (KH_2PO_4) 0.61 g/L, ammonium chloride (NH_4Cl) 0.28 g/L, yeast extract 0.1 g/L, and dipotassium hydrogen phosphate (K_2HPO_4) 0.96 g/L. The solution pH was 7.

Trace element liquid

The composition of trace element liquids is as follows: ferrous chloride ($FeCl_2{\cdot}4H_2O$) 2 g/L, ammonium molybdate ($(NH_4)6Mo_7O_{24}{\cdot}4H_2O$) 0.05 g/L, boric acid ($H_3BO_3$) 0.05 g/L, zinc chloride ($ZnCl_2$) 0.05 g/L, copper chloride ($CuCl_2$) 0.03 g/L, manganese chloride tetrahydrate ($MnCl_2{\cdot}4H2O$) 0.05 g/L, cobalt chloride hexahydrate ($CoCl_2{\cdot}6H_2O$) 0.05 g/L, aluminum chloride ($AlCl_3$) 0.05 g/L, nickel chloride ($NiCl_2$) 0.05 g/L and a multivitamin.

Experimental device

The experimental device is composed of a fermentation unit and a record detection unit, an electrolysis unit and a gas collection unit. As shown in Figure 1, a 500 mL wide-mouth bottle serves as the electrolysis fermentation tank.

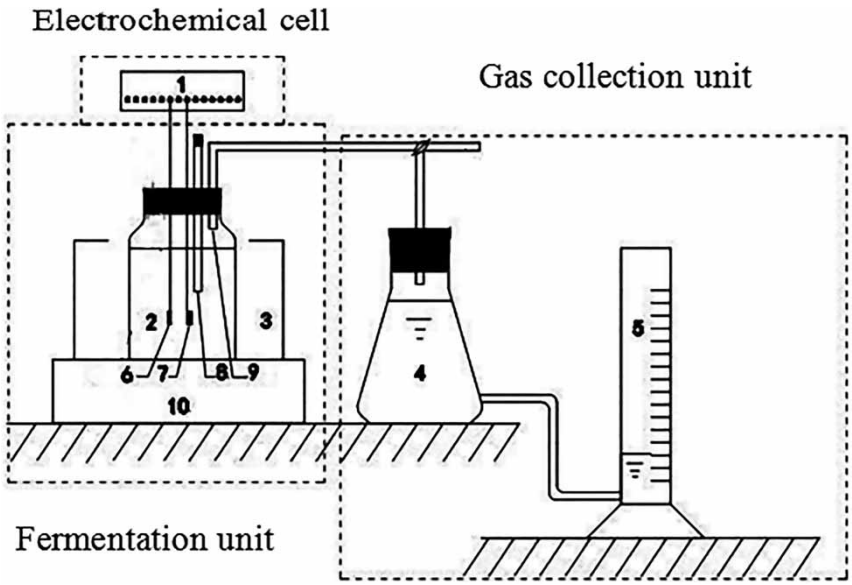

Figure 1 | Single-chamber electrolytically assisted bio-hydrogen production device. 1. Electrolysis power supply and current records; 2. Electrolysis fermentation tank; 3. Constant temperature water tank; 4. Gas collection bottle; 5. Measuring cylinder; 6. Pt electrode; 7. Carbon felt electrode; 8. Sampling tube; 9. Air outlet pipe; 10. Magnetic heating stirrer.

Table 1 | The equipment used in this experiment

Instrument name	Model	Company
Gas chromatograph	GC9790II	Zhejiang Fuli Analytical Instrument Co., Ltd
Electrochemical workstation	CHI600E	Shanghai Chenhua Instrument Co., Ltd
UV spectrophotometer	UV-5100	Shanghai Yuan Analysis Instrument Co., Ltd
Magnetic heating stirrer	780-1	Changzhou Lang Yue Instrument Manufacturing Co., Ltd
Ultrasonic cleaner	DSA	China Fuzhou Desen Precision Industry Co., Ltd
HAOSHI pH/ORP meter	H-1008A	Dongguan House Instrument
DC operating power	TXN-1505A(D)	Shenzhen Zhaoxin Electronic Instrument Equipment Factory
Ultra-thin widescreen paperless recorder	SIN-R7000A	Joint Measuring Instrument Co., Ltd
Autoclave	YXQ-LS-50SII	Shanghai Kexiao Scientific Instrument Co., Ltd
Electric blast drying box	GZX-P146MBE	Shanghai Bo Xun Industrial Co., Ltd Medical Equipment Factory
Beckman high-temperature low-speed centrifuge	BECKMAN GS-15R	Nanjing Beideng Electromechanical Equipment Co., Ltd
pH electrode	H-201	Dongguan House Instrument

The distance is 3 cm between the cathode and anode electrodes. The two electrodes are placed in parallel symmetry, soaked in the electrolyte and connected to the current recorder. The electrolytic power supply is achieved using the wires. Each fermentation cell is outfitted with a reference electrode to measure the electrode potential, and the gas is collected using a drainage method.

Experimental equipment

A list of experimental equipment used is given in Table 1.

Methods

Experimental methods

First, in a fermentation tank of the single-cell electrolysis-assisted fermentation hydrogen production plant, sodium acetate, sodium propionate, sodium butyrate, glucose, starch (6 g), inoculum (120 g), buffer nutrient solution (100 mL) and trace elements (10 mL) were added, respectively. Thus, each total mass of the material in the wide-mouth bottle was 400 g, and then the pH of the fermentation broth in the jar was adjusted to 7. Next, the air in the fermentation bottle was replaced with nitrogen, and the bottle was quickly processed in an anaerobic state. Finally, the MEC was started with an applied voltage of 1.0 V and an ambient temperature of 35 °C. During the stage of hydrogen production, electrode potentials, electrolysis voltages, pH values, currents, electrochemical activity of microorganisms, changes in gas production and analytical gas content, and organic acid content were measured and recorded for each fermentation unit. After the culture was completed, the membranes were submitted for a series of analyses: electron microscopy, membrane biomass, and biodiversity analysis.

Experimental design

A total of five experimental groups were set up in this experiment. All groups were controlled under the same conditions except for substrate. Each experimental group had three parallel groups. Detailed information is listed in Table 2.

Experimental analysis

Electrolytic voltage and current

Electrolytic voltage and current can be read directly on the wide-screen paperless recorder SIN-R7000A. The variations of current were recorded by the ultra-thin widescreen paperless recorder per 4 min, and a value was taken out every 24 h.

pH

The pH was read directly from a pH meter and the wide-screen paperless recorder SIN-R7000A.

Table 2 | Experimental design scheme of microbial membrane culture

	Groups				
	I	II	III	IV	V
Inoculum volume (g)	120	120	120	120	120
Buffer nutrient solution (mL)	100	100	100	100	100
Substrate (6 g)	Sodium acetate	Sodium propionate	Sodium butyrate	Glucose	Starch
Applied voltage (V)	1.0	1.0	1.0	1.0	1.0
Temperature (°C)	35	35	35	35	35
Total mass (g)	400	400	400	400	400

Gas production

The gas was collected using the drainage and gas collection method. The gas production was measured by the daily cylinder reading.

Gas content

The gas content was determined using a GC9790 II gas chromatograph.

Column

The chromatographic conditions for the packed column include: column pressure 1 refers to the sample column pressure, and column pressure 2 the reference column pressure. According to the analysis requirements, the steady flow valve was adjusted, and the appropriate column flow was selected, so that the gas flow of the thermal conductivity detector (TCD) two-way carrier can range from 25 to 40 mL/min. When the chromatograph gas was measured, the injector temperature was 200 °C, the TCD temperature was 200 °C, the column temperature was 110 °C, the total pressure was 300 kPa, the column pressure 1 was 200 kPa, and each experiment took 200 μL of gas sample. The gas detection cycle was 10 min.

During the experiment, the organic acid was determined using the GC9790II gas chromatograph, KB-FFAP (30 m*0.32 mm*0.25 m) capillary column, and hydrogen flame ionization detector.

Cyclic voltammetry assay

Anode biofilms were analyzed by cyclic voltammetry (CV) assay to understand the electrochemical activity of electrogenic microorganisms. The instrument used for the analysis was an electrochemical workstation (CHI660E) produced by Shanghai Chenhua.

Membrane biomass determination

The phospholipid colorimetric method was used to obtain the corresponding number of microorganisms.

Electron microscope scanning and biodiversity analysis

After being cultured, the sample was scanned under a scanning electron microscope. After scanning, the sample was stored in an ultra-low temperature refrigerator at −80 °C, and then submitted to 16S rRNA sequencing and analysis. The sample information and sample relationship of the submitted samples are shown in Table 3.

RESULTS AND DISCUSSION

Effect of different substrates on hydrogen production performance and electrochemical activity of MEC

During the experiment, with the consumption of substrates, the current daily gas production, gas content, organic acid content, and electrochemical activity are gradually changing as shown in Figure 2.

When cultivating MEC anodic membranes, due to the existence of electroactive microorganisms, there is always the generation and change of current at the beginning of the experiment. The change of current is a dynamic process, which is inseparable from the growth activity of microorganisms in the whole system. By monitoring the variations of current during the formation of the MEC anodic film under different substrate conditions, the results show that the ability to produce electricity is sodium acetate > sodium butyrate > sodium propionate > glucose > starch

Table 3 | Sample information table

Sample serial number	Sample name	Bioinformatics analysis name	Biometric analysis name – group name (after training, the environment is divided into one group)	Species source
1	Sodium acetate MEC anodic film	MYSN	MSN	Swine manure inoculum
2	Sodium propionate MEC anodic film	MBSN		Swine manure inoculum
3	Sodium butyrate MEC anodic membrane	MDSN		Swine manure inoculum
4	Glucose MEC anodic membrane	MPTT	MT	Swine manure inoculum
5	Starch MEC anodic film	MDF		Swine manure inoculum

(Figure 2(a)). The maximum current produced by each experimental group is 23.4, 21.1, 19.2, 8.3, and 7.4 mA, respectively.

As for the change in daily gas production, the result in Figure 2(b) shows the microbial growth from another perspective. Also, the results of composition and content of gas (Figure 2(c) and 2(d)) reflect which microorganisms are dominant. At the beginning, the methane content of all experimental groups was gradually increasing, indicating that the methanogenic bacteria in each experimental group grew normally. However, in the experimental process, the methane content of any experimental group was always greater than the hydrogen content, indicating that the methanogens account for certain advantages during the membrane culture stage. Compared with traditional hydrogen production methods, MEC's hydrogen production performance has improved. Using gas chromatography to analyze the content of gas produced every day, the hydrogen content of the gas produced by each experimental group was consistent with the change of current. The highest hydrogen content in the produced gas was: 25.85, 21.41, 20.18, and 9.12% 8.96% for the first, second, third, fourth and fifth experimental group, respectively. Combined with the changes of current, this further shows that the generation of hydrogen has a direct relationship with the generation of current. According to the results, it is noteworthy that the higher the current, the higher the hydrogen content of the gas will be, which verifies the existence of electroactive microorganisms. For the beginning of the experiment, no hydrogen was detected until the current was generated. Hydrogen was detected in the gas, indicating that no electroactive microorganisms were present in the reactors of the experimental groups. However, with the assistance of electrolysis voltage, electroactive microorganisms emerged as the experiment progressed. The dynamic changes of electroactive bacteria and methanogens could also be seen from the changes in hydrogen and methane content.

Here, in the analysis of organic and acid content, the results reflect a gradual decrease over time (Figure 2(e)). The reduction in the organic acid content indicates that the substrate is consumed and the substrate consumption must be accompanied by the growth of the microorganism and the production of the target product, and the electrochemical activity indicates the growth of the microorganism. At a certain stage, if the electrochemical activity is higher, the less target product is generated and vice versa. Regardless of whether it is the growth of microorganisms or the production of target products, the contribution of substrates to the whole reaction system by the formation of electroactivity or hydrogen and electricity cannot be unilaterally assessed, combining the results of hydrogen production and electricity production. The analysis of the results of chemical activity shows that there is no strict linear relationship between the changes of organic acids, hydrogen production, and electrochemical activity, but they are inextricably linked.

The CV curve obtained by scanning the solution of the anodic membrane of the experimentally cultured MEC in the buffer plus substrate was measured by CV as shown in Figure 2(f). The figure shows that the highest oxidation peak appeared in the sodium acetate experimental group, directly reflecting the activity of the electroactive microorganisms, and indicating that sodium acetate is most beneficial to the growth and reproduction of electroactive microorganisms, followed by butyric acid, sodium propionate, glucose, and starch. Together with the results of changes in current and hydrogen content, the effect of sodium salt appears to be always better than that of sugar, since the hydrolysis of sugar raw materials does not promote the growth and reproduction of electroactive microorganisms.

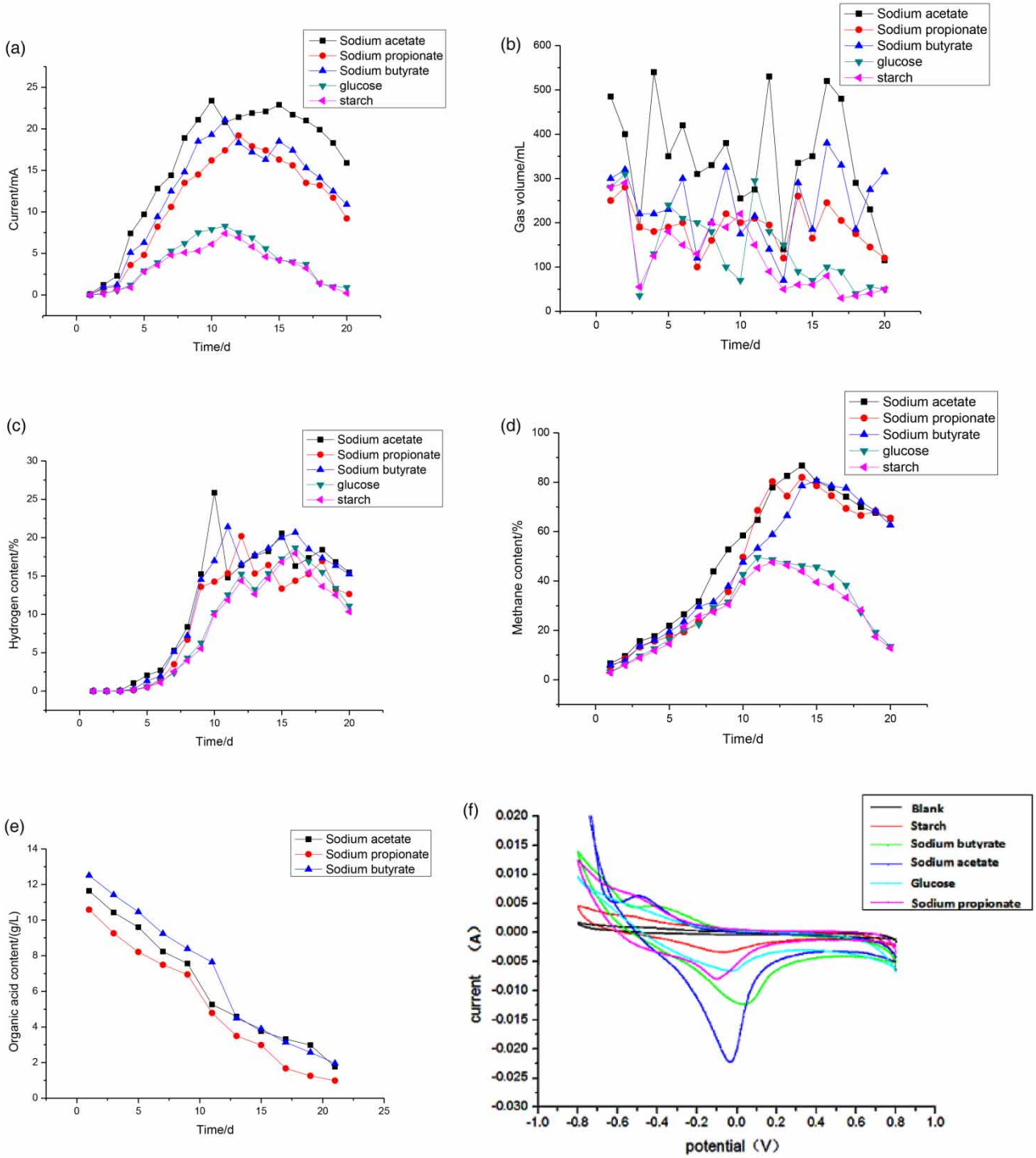

Figure 2 | The curve of each performance index with time: (a) the variation of current; (b) the variation of daily gas production; (c) the variation of hydrogen content; (d) the variation of methane content; (e) the variation of organic acid content; (f) CV curve of anode film.

Determination of anode membrane biomass formation in experimental groups with different substrates

In the study of MFCs, it was confirmed that there was a direct relationship between the number of anodic microorganisms and the generation of currents. At the same time, the most important contribution to voltage was the microbe on the electrodes, and the microorganisms in solution were used to produce batteries. The electrical

Table 4 | Phosphorus content in anodic film of each experimental group

Test group	Sodium acetate	Sodium propionate	Sodium butyrate	Glucose	Starch
Absorbance (A)	0.181	0.073	0.042	0.056	0.040
Phosphorus concentration (mg/L)	5.74	2.01	3.94	1.43	0.88

contribution was small and did not show electrical activity. Also belonging to the bioelectrochemical systems, MEC is developed based on the MFC. In principle, MEC and MFC have the same functional bacteria, so the method of evaluating MFCs could provide an important reference for the development of MECs.

To study the relationship between the membrane biomass and the electrochemical activity on the electrode, the anodic membranes cultured in each experimental group were taken out, and their phosphorus content was measured using the phospholipid method. The graphite anode of each experimental group was removed, and after sample pretreatment, the absorbance after the digestion was measured and is shown in Table 4. The phosphorus content was measured on the surface of the anodic film of each experimental group. The amount of phosphorus content is related to the biomass of the anodic film surface. The higher the phosphorus content, the greater the biomass of the anodic film surface and the greater the abundance of electroactive microorganisms. In combination with the electrochemical oxidation peaks, the peak of oxidation increases with the increase in the phosphorus content, which generally shows a linear relationship. It is verified that the greater the phosphorus content of the anodic membrane of the MEC, the greater the corresponding electrical activity; and the better the MEC anode membrane growth, the better the electrical activity expressed. Measured results of membrane biomass indicated that the biomass of anodic films cultured with sodium acetate as substrate was the largest. Phosphorus content was 5.74 mg/L, 6.5 times that of the starch experimental group.

Characterization of anodic films cultured on different substrates

After a period of cultivation, the electroactive microorganisms will be concentrated on the anodic membrane, and the enrichment of microorganisms can be visually observed by electron microscopy scanning of the membrane. The scanning results are shown in Figure 3.

According to the morphology of the above electron microscopy scans, the anodic membrane cultured with sodium acetate and sodium butyrate as substrates shows the best microbial enrichment, followed by sodium propionate,

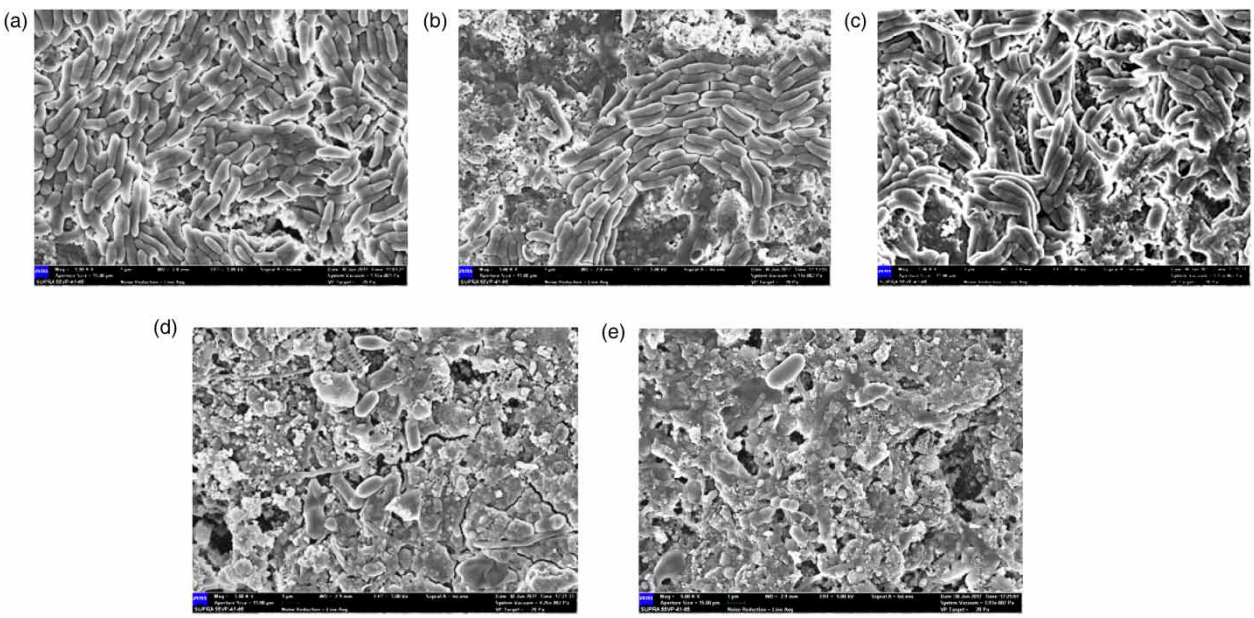

Figure 3 | Anodic membrane characterization of each substrate culture: (a) morphology of microbial membrane in sodium acetate culture; (b) microbial membrane morphology in sodium propionate culture; (c) microbial membrane morphology in sodium butyrate culture; (d) microbial membrane morphology in glucose culture; (e) microbial membrane morphology in starch culture.

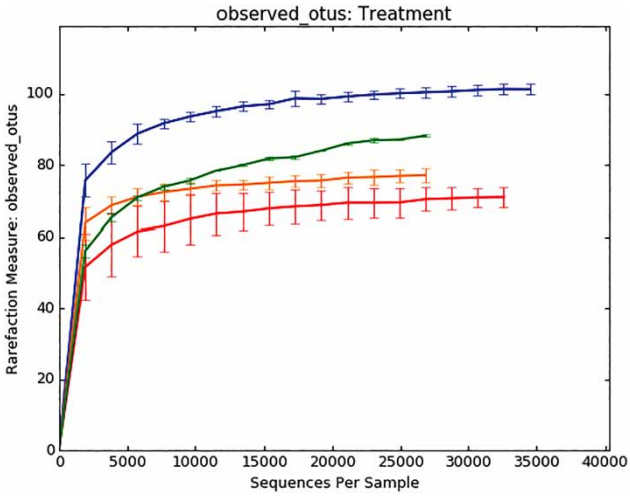

Figure 4 | OTU dilution curve.

and glucose and starch as substrates. The microbial enrichment of anode biofilm cultured with glucose and starch as substrate is relatively less, which is consistent with the hydrogen production capacity of the reaction system. Also, the experimental group with microbial enrichment has a relatively good ability to produce hydrogen by electricity, and the hydrogen production capacity of the experimental group with little microbial enrichment is relatively poor.

Results of microorganism community diversity

Sequencing quality evaluation

Using the method of random sampling of the sequencing sequence, a dilution curve was plotted with the number of sequences extracted and what they can represent (Figure 4). The dilution curves of the five samples are shown to be gradually flat at the level of 97% similarity, but they are still not saturated, indicating that most of the sample information was obtained for the five samples and basically reflected the microbial community group structure of the system.

OTU cluster analysis

A total of 3,695 operational taxonomic units (OTUs) were generated for the five samples. The numbers of OTUs included in MYSN, MBSN, MDSN, MPTT, and MDF (see Table 3 for explanation of abbreviations) samples were 464, 728, 636, 784, and 1,083, respectively. The number of OTUs of the MDF sample was the highest at 1,083; similar and unique OTU numbers can be obtained by comparing the culture conditions similarly as a group (Figure 5).

As can be seen from Figure 5, among the three MYSN, MBSN, and MDSN samples, there were 214 OTUs (18%

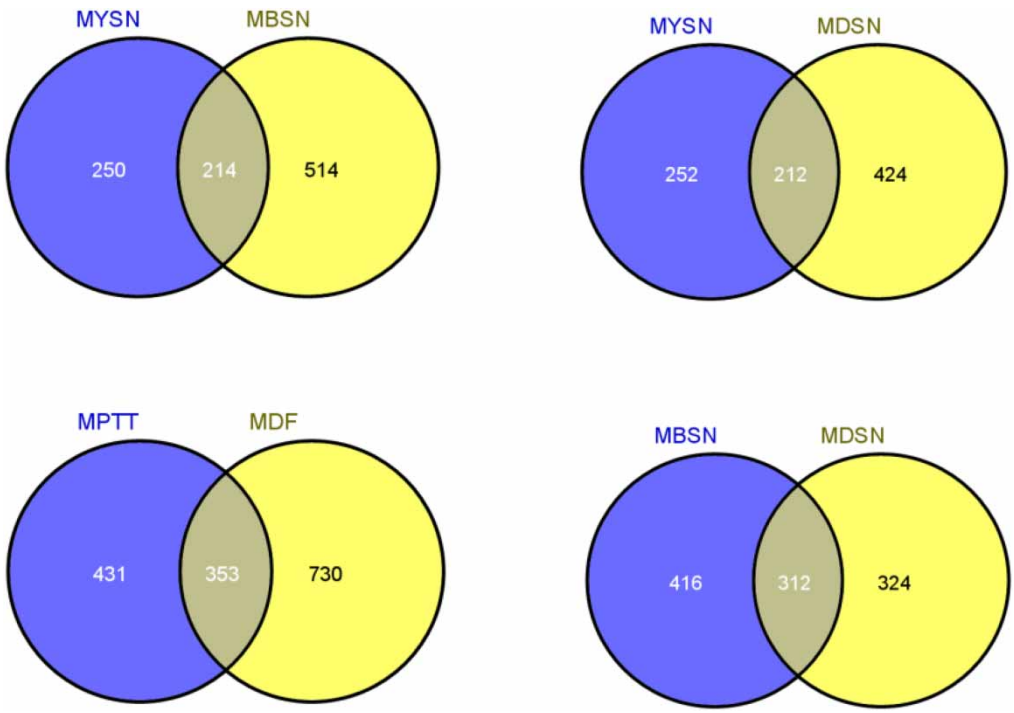

Figure 5 | Comparison of microbial OTU in different experimental groups. MYSN – anodic film of sodium acetate culture; MBSN – anodic film of sodium propionate culture; MDSN – anodic film of sodium butyrate culture; MPTT – anodic film of glucose culture; MDF – anodic film of starch culture.

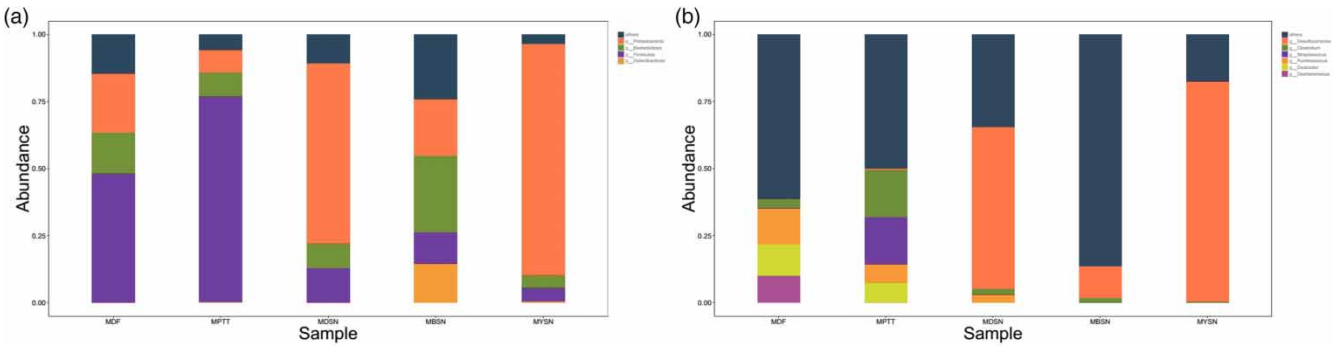

Figure 6 | The relative abundance of dominant flora in anodic film stack: (a) level of phylum classification; (b) genus classification level.

of the total load) of MYSN and MBSN. MYSN and MDSN had 212 OTUs (19.3% of their total load); the number of OTUs shared by MBSN and MDSN was 312 (25.9% of the total load); the common microorganisms belonged to the phylum *Proteobacteria*. The other two samples shared 353 OTUs (18.9% of the total), and the common microorganisms mainly belonged to *Firmicutes*.

Analysis of dominant bacteria and community structure

Four dominant phyla (*Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Deferribacteres*) and six dominating genera (*Desulfuromonas*, *Clostridium*, *Streptococcus*, *Ruminococcus*, *Oxobacter*, *Dechloromonas*) were screened with the relative abundance greater than 0.1. When using sodium acetate, sodium propionate and sodium butyrate as substrates, the main species of *Desulfuromonas* in the *Proteobacteria* phylum (relative abundance >0.1) had relative abundances of 0.82, 0.12, and 0.60, respectively. The relative abundances of *Desulfuromonas* in the *Proteobacteria* phylum with glucose and starch as substrates were only 0.006 and 0.001, respectively. When glucose was used as the substrate, the genera *Streptococcus* and *Clostridium* in the *Firmicutes* were predominant, with relative abundances of 0.18 and 0.18, respectively.; When starch was used as a substrate, species of *Ruminococcus* and *Oxobacter* in the *Firmicutes* and *Dechloromonas* in the *Proteobacteria* were predominant, with relative abundances of 0.14, 0.12, and 0.10, respectively. The relative abundances of these four dominant phyla and six dominant genera in each experimental group were screened for relative abundance stacking (Figure 6).

As shown in Figure 6, at the level of phylum classification, the abundance >0.1 serves as a threshold. Among the five samples, the relative abundance of *Proteobacteria* is ranked as MYSN > MDSN > MBSN > MDF > MPTT; *Bacteroidetes* is MBSN > MDF > MDSN > MPTT > MYSN; *Firmicutes* is ranked as MPTT > MDF > MDSN > MBSN > MYSN and *Deferribacteres* phylum is ranked as MBSN > MYSN > MPTT > MDSN > MDF. However, most of the known electroactive bacteria belong to the *Proteobacteria* phylum, suggesting that the anodic membrane cultured with sodium acetate as the substrate predominates in electroactive microorganisms, i.e. among the five substrates, sodium acetate is the most suitable for the growth of electroactive microorganisms.

For the level of genus classification, with the threshold of the abundance >0.1, the relative abundance of the genus *Desulfuromonas* of the five samples is ranked as: MYSN > MDSN > MBSN > MPTT > MDF. The relative abundance of *Clostridium* genus is MPTT > MDF > MDSN > MBSN > MYSN. *Streptococcus* genus is only available in sample MPTT, with a relative abundance of 0.18. None of the other samples showed the presence of this genus. The relative abundance of *Ruminococcus* is MDF > MPTT > MDSN > MYSN > MBSN. The *Oxobacter* genus was detected in only MPTT and MDF, and the relative abundance of *Dechloromonas* was MDF > MDSN > MBSN > MYSN > MPPT. Of the six genera, only the *Desulfuromonas* genus is known to have electroactive bacteria. Once again, the subordinate level shows that sodium acetate is the most suitable for the growth of electroactive microorganisms. The glucose- and starch-based experimental groups all had their own unique dominant genus, and the relative abundance of *Desulfuromonas* was minimal, which was directly associated with the acidification during the experiment.

CONCLUSION

(1) Sodium acetate, sodium propionate, and sodium butyrate are good raw materials for hydrogen production. Glucose and starch can be used to produce hydrogen, but the system environment is difficult to be controlled and easily acidified.

(2) According to the results of the five experimental groups, the hydrogen production capacity of the experimental group with sodium acetate as the substrate is the best, and the hydrogen content and current reached 25.85% and 23.4 mA, respectively. The electrochemically active oxidation peak of this group is also the highest.

(3) According to the results of the five experimental groups, when sodium acetate was selected as the substrate, the membrane biomass reaches the highest, and the microbial enrichment on the membrane surface is the highest. When glucose and starch were selected as substrates, the biodiversity is improved, yet the amount of electrochemically active microorganisms is reduced. When sodium acetate, sodium propionate and sodium butyrate were selected as substrates, the biological diversity is not as good as that of glucose and starch, whereas the electroactive microorganisms are relatively abundant. Among these microorganisms, *Desulfuromonas* belonging to the *Proteobacteria* phylum contributes most to improvement in the hydrogen production performance of MEC, yet the relative abundance of this genus reaches the largest in the experimental group by selecting sodium acetate as the substrate, 0.82. Furthermore, the electrochemical activity is positively related to the hydrogen production capacity.

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