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# Effects of Different Substrates on MEC Anode Film Formation and Extracellular Polymer

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**Abstract.** In this paper, a single-cell microbial electrolysis cell (MEC) reactor is used, with activated sludge as the inoculum, glucose, sodium acetate, sodium propionate and sodium butyrate as substrates. The MEC anode film formation and the different nutritional conditions are studied for the influence of extracellular polymers. Experimental research shows that MECs running on different substrates show obvious current density and anode membrane electrochemical activity. MEC anode membrane current density of sodium acetate substrate culture is the highest (12.78 A/m<sup>2</sup>), followed by glucose and butyl Sodium and sodium propionate. MEC anode electroactive microorganisms can use a wide range of substrates. The energy efficiency of different substrates is different, and the energy efficiency of acetic acid is the highest. The electrochemical activity and hydrogen yield of the anode biofilm are the same as those of the MEC current density. Increasing the inoculum within a certain range is conducive to improving the electrochemical activity of the anode biofilm and the hydrogen yield of the anode film. Further analysis of the composition of the anode membrane biomass and extracellular polymer (EPS) showed that increasing the inoculation amount is beneficial to the increase of the anode membrane biomass. A large number of anode-attached bacteria can produce higher current density and EPS content. The protein in the anode membrane EPS The content is significantly higher than polysaccharides, and as the protein content increases, the electrical density also increases.

## 1. Introduction

With the deepening of research in recent years, the microbial electrolysis cell (MEC) technology has also made some major developments, but large-scale industrialization is still very difficult due to the low hydrogen yield. In order to further improve the MEC operating performance, scholars have optimized a series of operating parameters such as water retention time, PH, nutritional conditions, temperature, etc. [2-6]. The growth and enrichment on the anode surface is also of great significance to the performance of MEC. The results of the study confirm that the adjustment of operating parameters directly affects the anode potential of the MEC, and the anode potential directly represents the life activity of electroactive microorganisms [7]. In the MEC reaction system, electroactive microorganisms form anode biofilms efficiently, which can generate current more quickly and can also obtain better hydrogen production performance [8].

MEC anode biofilm is the accumulation of electroactive microorganisms on the anode surface, also called biomass matrix [9]. The formation of the biofilm matrix is closely related to the extracellular polymer (EPS). In addition, EPS is also related to the microbial surface microbial physical properties



and chemical characteristics, which is helpful for the connection between microbial single cells and their adsorption and deposition on the anode surface [10]. The extracellular polymer (EPS) is mainly composed of polysaccharides, proteins, nucleic acids, and lipids. It not only promotes the growth and fixation of microorganisms on the anode surface, but also participates in extracellular electron transfer [11].

In this paper, a single-cell microbial electrolysis cell is used as an experimental device to study the effect of different substrates (glucose, acetic acid, propionic acid, butyric acid) on MEC anode biofilm formation at a temperature of 35 °C and a constant voltage of 0.8V, and by analyzing the anode Biofilm extracellular polymer (EPS) polysaccharide and protein content changes, so as to further understand the mechanism of extracellular polymer on MEC electricity and hydrogen production.

## 2. Materials and Methods

### 2.1. Materials

*2.1.1. Inoculum.* The inoculum used in the experiment came from the anaerobic activated sludge obtained by the pig manure cultivation and acclimation in the Biomass Energy Laboratory, School of Energy and Environmental Sciences, Yunnan Normal University. Its pH was 7.7, TS was 13.04%, VS was 7.03%, The contents of volatile organic acids were: acetic acid 22.5, propionic acid 17.4, butyric acid 16.4, valeric acid 9.7.

*2.1.2. Raw Materials for Hydrogen Production.* The raw materials for the experiment were anhydrous glucose, anhydrous sodium acetate, anhydrous sodium propionate and anhydrous sodium butyrate, all from Xilong Chemical Co., Ltd.

*2.1.3. Buffer Solution.* The buffer solution is prepared as follows:  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 5.54g/L;  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 23.088g/L; KCl, 0.26g/L;  $\text{NH}_4\text{Cl}$ , 0.62g/L.

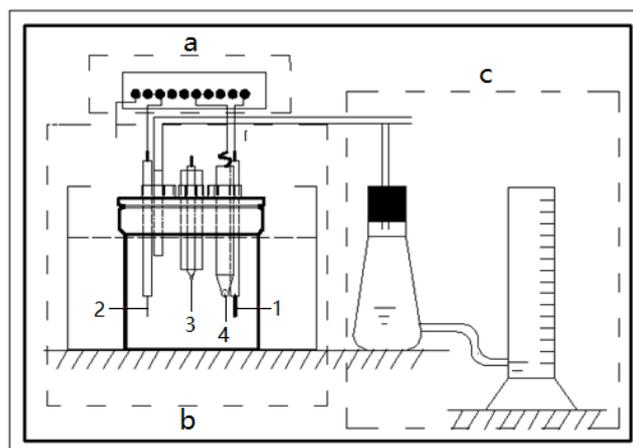
*2.1.4. Trace Element Nutrient Solution.* Traceelement:  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ , 0.025g/L; NaCl, 1g/L;  $\text{FeCl}_2 \cdot 7\text{H}_2\text{O}$ , 0.072g/L;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.01g/L;  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.024g/L; Ammoniatriaceticacid, 1.5g/L;  $\text{MgSO}_4$ , 3g/L;  $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ , 0.01g/L;  $\text{ZnCl}_2$ , 0.13g/L;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.1g/L;  $\text{H}_3\text{BO}_3$ , 0.01g/L;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.6g/L;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.1g/L;  $\text{Na}_2\text{MoO}_4$ , 0.025g/L and a multivitamin tablet.

*2.1.5. Experimental Device.* The experimental device is a single-cell MEC reactor, which is mainly divided into three parts: record detection, electrolytic fermentation and gas collection. As shown in figure 1, the fermentation tank structure of the single-cell MEC reaction device is cylindrical, and the sealing cover is made of polytetrafluoroethylene; the electrode is immersed in the fermentation liquid and connected to the online recorder (SIN-R7000A, China) by wire; the gas is collected by Drainage gas gathering method. The inner diameter of the MEC fermentation tank is 46 cm and the height is 80 cm. In order to ensure that the electrolytic fermentation hydrogen production is in an anaerobic environment, the sealing cover and the sealing base are equipped with corresponding sealing gaskets. The anode uses a self-made graphite electrode (30×30×2mm), the cathode uses a platinum electrode (30×30mm), and the electrode spacing can be adjusted. The reference electrode uses a saturated Ag/AgCl reference electrode. During the entire experiment, the single-cell MEC reactor is always placed in a constant temperature stirring control system.

### 2.2. Experimental Method

In this experiment, the enrichment of the anode was done under MEC operation. Then separately add 120g of activated sludge inoculum, respectively add 6g of glucose, sodium acetate, sodium propionate and sodium butyrate to different reactors, and finally adjust with distilled water so that the content of each reactor is 400g, and adjust Culture medium PH = 7. The control voltage is 0.8V and the

temperature is 35°C. After nitrogen is blown out to discharge excess air, it is quickly connected to the gas collecting device.



**Figure 1.** MEC reaction device. a-electrolytic recording unit, b-mec single cell fermentation tank, c-drainage collection unit, 1-graphite anode, 2-cathode platinum electrode, 3-ag /AgCl reference electrode, 4-ph electrode.

MEC anode biofilm hydrogen production performance verification method: During the MEC anode biofilm cultivation process, after the reactor runs smoothly and the current value is stable, the reactor is removed to take out the anode biofilm that has been cultured and placed in the same as the cultivation process. In the glucose medium, the temperature is controlled at 35°C and the voltage is controlled at 0.8V. No additional inoculum is required. Other external conditions are consistent with the MEC anode biofilm cultivation process, thereby verifying the hydrogen production of the MEC anode biofilm characteristic.

### 2.3. Analysis Method

The electrolysis voltage and current are obtained after reading the data through a wide-screen paperless recorder, and after certain processing; the electrode potential is measured using an Ag/AgCl reference electrode; the gas production is read by the drainage gas collection method; the gas composition is measured by the meteorological chromatograph; analysis and analysis of organic acid content using meteorological chromatograph ; anode biofilm bioelectrochemical activity using cyclic voltammetry (CV) test by electrochemical workstation. The EPS in the anode biofilm is extracted by ultrasonic method. After drying and burning, the concentration of volatile suspended solids (MLVSS) is determined according to the weighing method; the Coomassie brilliant blue method is used to determine the protein content in EPS.

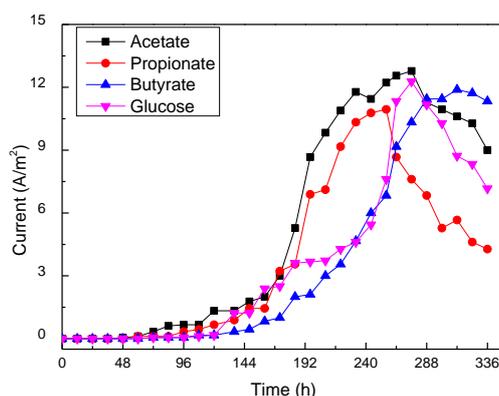
## 3. Results and Analysis

### 3.1. Analysis of Electricity and Gas Produced by Anode Biofilm

**3.1.1. Current Analysis during Anode Biofilm Cultivation.** Figure 2 shows the electricity generation during the MEC anode membrane formation process under different fermentation parameters. No electrolysis current was generated in the initial stage of all MEC, but the electro-producing bacteria began to accumulate on the surface of the anode under the effect of applied voltage, and different fermentation substrate experimental groups began to generate current one after another. The peak of electricity generation is the sodium acetate substrate before the highest, the highest current density is 12.78 A/m<sup>2</sup>; followed by the sodium propionate substrate, the highest current density has dropped to

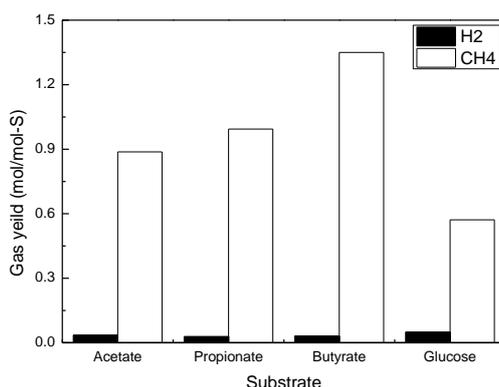
10.78 A/m<sup>2</sup>; the current density of sodium butyrate and glucose substrate. The change trends are similar, with the highest current densities being 11.72 and 12.28 A/m<sup>2</sup>, respectively.

The current generated during the MEC anode membrane cultivation showed that the electroactive microorganisms in the reaction system began to adhere to the anode surface and oxidize the substrate to generate electron flow [12]. However, from the perspective of the highest current density of MEC: HAc > Glucose > HBu > HPr, this result is consistent with Yang [12].



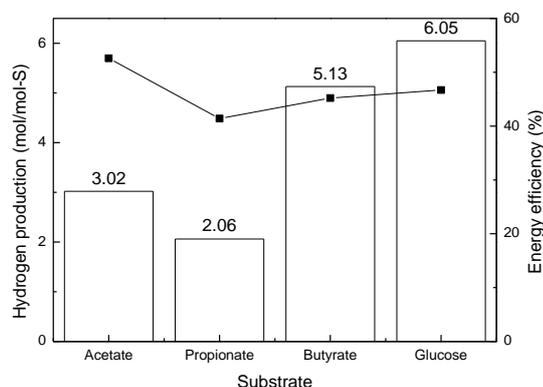
**Figure 2.** Effect of different substrates on MEC current density.

**3.1.2. Changes in Gas Production During Anode Membrane Culture.** Figure 3 shows the gas generation during the formation of MEC anode film by different substrates. The yields of hydrogen in MECs with different substrates were acetic acid 0.0355, propionic acid 0.0286, butyric acid 0.0315 and glucose 0.0498 mol/mol-substrate. In addition to glucose, the hydrogen yield of the volatile organic acid substrate conforms to the law of current change. Glucose is an excellent electron and proton donor and there is dark fermentation to produce hydrogen, so the MEC hydrogen yield of glucose substrate is the highest. In addition, the experiment used a single-cell MEC reactor and the inoculum contained a large amount of methanogens. A large amount of methane was detected in all experimental groups (0.57-1.35 mol/mol-substrate), which also caused hydrogen production at the low rate [13].



**Figure 3.** Effect of different substrates on MEC gas production.

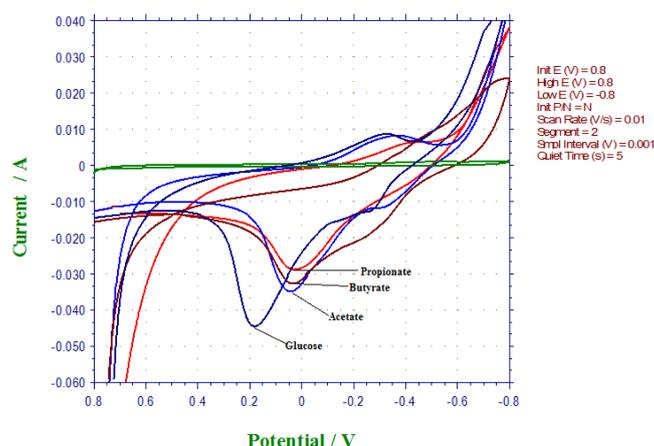
*3.1.3. Analysis of Hydrogen Production Characteristics of Anode Biofilm.* After the current value is stable during the MEC anode biofilm cultivation process, the cultured anode biofilm is removed and transferred to the same substrate concentration medium quality, but there is no inoculum, the other external conditions is as same as the MEC anode biofilm cultivation process, and the hydrogen production performance of the MEC anode membrane cultured under different substrates is verified. Figure 4 shows the hydrogen production potential of the anode film formed under different fermentation substrates. For the MEC of different substrates, considering the different electron donors of the substrate, the energy efficiency of different substrates is calculated, and it can be seen that the energy efficiency There are differences, the highest efficiency of acetic acid is followed by glucose, and the lowest energy efficiency of propionic acid. Acetic acid can be directly used by electro-producing bacteria, so when it is used as a substrate, there is less mutual nutrition between bacteria. The hydrogen yield of different anode biofilms is similar to the corresponding current density and the change trend of electrochemical activity. Appropriate environmental parameters are conducive to the rapid enrichment of electroactive microorganisms on the surface of the anode and accelerate the life metabolism activities. Increasing the oxidation substrate produces electron flow, which in turn increases the hydrogen yield.



**Figure 4.** Hydrogen production potential of anodic membrane formed by different substrate culture.

### *3.2. Analysis of Electrochemical Characteristics of Anode Biofilm (CV Curve Analysis)*

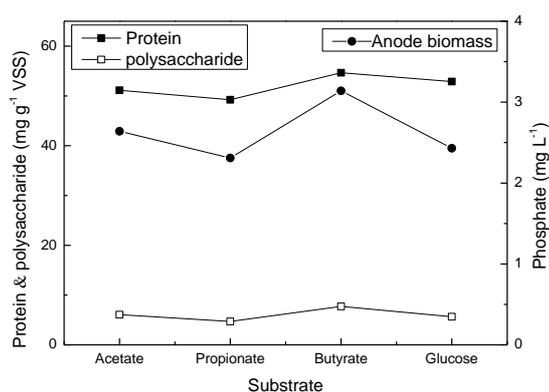
As shown in figure 5, the anode biofilms formed by different fermentation substrates cultured at a scan rate of 10mV/s have a clear redox peak compared to the blank electrode, which indicates that the microorganisms attached to the anode surface have electron transport capabilities. The oxidation peak of the electroactive microorganisms showed a stable value around -0.1~0.1V. Studies have shown that the CV characteristics of the MEC anode biofilm are consistent with the equilibrium potential of the iron-reducing bacteria *G. Sulfurreducens* cells, which is about -0.2~+0.1V [14]. *G. Sulfurreducens* is a typical electro-producing bacteria that transports electrons to iron oxides or electrode surfaces over long distances. For different substrates, the oxidation peak positions of glucose and organic acids in MEC anode biofilms are different, which indicates that the dominant colonies of domesticated anode biofilms under different substrate conditions may be inconsistent or at least the substances involved in electron transfer are different.



**Figure 5.** Anode CV curve under different substrates.

### 3.3. Analysis of EPS Content of Anode Biofilm

EPS is an important component of microbial membranes, which can protect biofilms from environmental factors [14]. Figure 6 shows the changes in protein and polysaccharide content in the anode membrane EPS under different fermentation parameters. It can be seen from the figure that the protein content of the EPS component of the MEC anode membrane is significantly higher than that of polysaccharides, and protein is the main component of EPS. The EPS protein content of the MEC anode film formed under different substrates is relatively close. The protein and polysaccharide content are: 51.13 and 6.06 (acetic acid), 49.23 and 4.69 (propionic acid), 54.65 and 7.72 (butyric acid), 52.89 and 5.65  $\text{mg}\cdot\text{g}^{-1}$  VSS (glucose). The main functional microorganisms in the anode membrane are electroactive microorganisms. Efficient attachment of electroactive microorganisms at the anode is a prerequisite for good electricity and hydrogen production capacity. Some studies have shown that under the stimulation of higher current density, bacteria will secrete more EPS components to maintain the stability of the cell structure [15]; another explanation is that the conductive nanofibers are included in the EPS extraction process of the MEC anode membrane, and the presence of bacterial nanofibers is of great significance for the attachment of electron-producing microorganisms and electron transfer to the anode [16].



**Figure 6.** The membrane biomass and EPS composition of anode biofilms formed by different fermentation parameters.

## 4. Conclusion

(1) The MEC running on different substrates showed obvious current density and anode membrane electrochemical activity, and there was little difference between different experimental groups, among

which the MEC anode membrane current density of MEC cultured with sodium acetate substrate was the highest (12.78 A/m<sup>2</sup>).

(2) MEC anode electroactive microorganisms can use a wide range of substrates. Different substrates have different energy efficiency, and acetic acid has the highest energy efficiency.

(3) The protein content of the anode biofilm EPS formed by different substrates culture is significantly higher than that of polysaccharide. Membrane biomass and EPS composition showed that: MEC current density was positively correlated with anode membrane biomass, EPS protein content was similar to anode membrane biomass change trend, and EPS protein composition obviously increased with electric density.

## References

- [1] Wang B, Gao G, Li F, Zhou Q, Song X, Zhai H and Li Y 2017 Research progress of microbial electrolysis cell application *Progress in Chemical Industry* **36** (03) 1084-1092.
- [2] Yang N, Hafez H and Nakhla G 2015 Impact of volatile fatty acids on microbial electrolysis cell performance *Bioresource Technology* **193**.
- [3] Liu H, Ramnarayanan R and Logan B E 2004 Production of electricity during wastewater treatment using a single chamber microbial fuel cell *Environmental Science & Technology* **38** (7).
- [4] You S, Zhao Q, Zhang J, Jiang J, Wan C, Du M and Zhao S 2007A graphite-granule membrane-less tubular air-cathode microbial fuel cell for power generation under continuously operational conditions *Journal of Power Sources* **173** (1).
- [5] Jadhav G S and Ghangrekar M M 2009 Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration *Bioresource Technology* **100** (2).
- [6] Catal T, Kavanagh P, O'Flaherty V and Leech D 2011 Generation of electricity in microbial fuel cells at sub-ambient temperatures *Journal of Power Sources* **196** (5).
- [7] Wagner R C, Call D F and Logan B E 2010 Optimal set anode potentials vary in bioelectrochemical systems *Environmental Science & Technology* **44** (16).
- [8] Finkelstein D A, Tender L M and Zeikus J G 2006 Effect of electrode potential on electrode-reducing microbiota *Environmental Science & Technology* **40** (22).
- [9] Rittmann B E and McCarty P L 2001 *Environmental Biotechnology: Principles and Applications* (New York: McGraw-Hill Book Co.).
- [10] Yan L, Liu Y and Ren Y 2013 Research progress of the effect of extracellular polymers on aerobic granular sludge *Progress in Chemical Engineering* **32** (11) 2744-2748+2756.
- [11] Nam J Y, Kim H W, Lim K H, et al. 2010 Electricity generation from MFCs using differently grown anode-attached *Bacteria*. **15** (2) 71-78.
- [12] Yang N, Hafez H and Nakhla G 2015 Impact of volatile fatty acids on microbial electrolysis cell performance *Bioresour. Technol.* **193** (6) 449-455.
- [13] Kato S, Hashimoto K and Watanabe K 2012 Methanogenesis facilitated by electric syntrophy via (semi) conductive iron-oxide minerals *Environ. Microbiol.* **14** (7) 1646-1654.
- [14] Vu B, Chen M, Crawford R J, et al. 2009 Bacterial extracellular polysaccharides involved in biofilm formation *Molecules* **14** (7) 2535-2554.
- [15] Zhang L, Zhu X, Li J, et al. 2011 Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances *J. Power Sources* **196** (15) 6029-6035.
- [16] Reguera G, Mccarthy K D, Mehta T, et al. 2005 Extracellular electron transfer via microbial nanowires *Nature* **435** (7045) 1098-1101.