



## Rumen bacteria convert cellulose into electricity in two-chamber microbial fuel cell

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### ABSTRACT

This paper describes the development of a microbial fuel cell (MFC) for electricity production from cellulose that is based on mixed culture of rumen bacteria *in vitro*. The fibrolytic bacteria isolated from rumen fluid were used in anode compartment of MFC, which can release glucose up to 15 g/L from carboxymethyl cellulose (CMC). At the same time, the maximum power density of  $100.60 \text{ mWm}^{-2}$  was generated when using copper sulfate as the cathodic electron acceptor. But the output voltage and current quickly fell down to 0.12 V and 0 mA after 48 h for electrode corroded and toxicity. When using methanogens isolated from the same rumen fluid instead of copper sulfate, the maximum power density about  $3090 \text{ mWm}^{-2}$  was recovered. This study demonstrates that symbiotic microorganisms could be used as catalysts and mediators effectively in the cathode for a MFC. CMC concentrations were further found to be the major factor on the output voltage and Coulombic efficiencies.

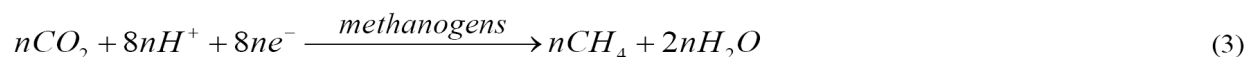
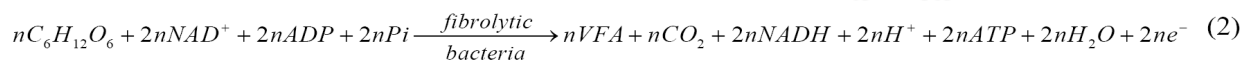
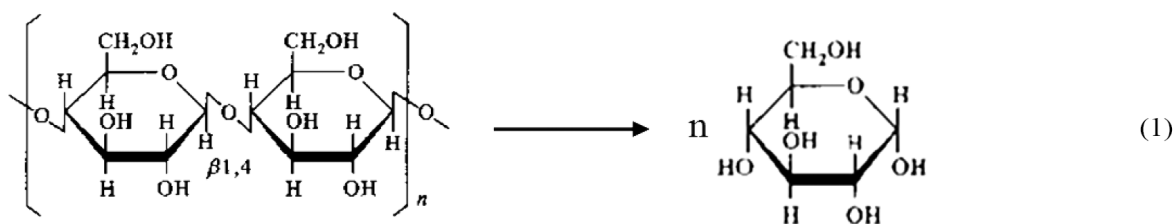
**Keywords:** Rumen microorganisms; Cellulose; Fibrolytic bacteria; Methanogens; Microbial fuel cell

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### INTRODUCTION

Waste corn straws can be used not only to manufacture ethanol, but also to generate electricity directly in our recent research using a two-chamber microbial fuel cell with rumen bacteria. MFC have been recognized to be an effective bioprocess for power generation and waste management (1,2,3). All previous works focused on organic wastewater treatment with microorganisms such as *Shewanella putrefaciens*, *Geobacteraceae sulferreducens*, *Rhodospirillum rubrum*, *Pseudomonas aeruginosa*, *Clostridium butyricum* and *Aeromonas hydrophila* (4,5). But corn solid waste about 700 million tons per year in China, comprising about 70% cellulose or hemicellulose, is neglected by MFCs researchers for their complex molecular structure which is hardly degraded by microorganisms (6). Ruminants depend on their rumen microorganisms to digest fiber-based diet, which give us some new ideas for electricity generation from cellulose (7). However to our knowledge, there is no more information except a report about this aspect from Booth (8).

The survival strategy of cattle just on fibrous diet has been testified during the last 50 years (9,10). Rumen microbial community depends on transporting electron between different groups to maintain their energy balance and microbial growth (11,12). In fact, fibrous diet is divided into oligosaccharide pieces as Eqs.(1) and then converted into volatile fatty acids (VFA) for animal, ATP for microbe growth and carbon dioxide, electron, proton for methanogens by fibrolytic bacteria. Eqs.(2) and Eqs.(3) point out a fact that methanogens play a critical role for hydrogen and electron transporting in rumen microbial community. Methane represents loss of feed energy for cattle, but for MFC, it is a fantastic redox system rather than metal oxidants like ferricyanide or permanganate. Anyway, if this bioprocess could be exhibited artificially in MFCs, electricity will be generated in the outside circuit with producing cattle-food and cooking fuel. The methods of rumen bacterial isolation and identification reported previously will make our present work feasible.



The aim of this study is to discuss the feasibility of electricity generation using methanogens and fibrolytic bacteria in a two-chamber mediator-less MFC. Two processes were discussed in this paper, one is to use fibrolytic bacteria in anode compartment solely with  $\text{CuSO}_4$  as cathodic electron acceptors, and the other is using methanogens instead of the inorganic mediators as electron acceptor. The performances of the two processes were compared including power generation, Coulombic efficiency, biomass, time of running and so on. These results will be addressed in the following sections

## EXPERIMENTAL SECTION

### 2.1 Lab-scale MFC assembly

The two-chamber MFC used in this experiment follows the design of O.Lefebvre *et al*, which has two acrylic cubic chambers of 60mm width separated with a proton exchange membrane (PEM) fixed by a sealing as shown in Fig. 1 (13). Every chamber possesses two openings on the top, one with 5mm inside diameter for substrate provision or wire connection, another is a vent. For simulating the conditions of rumen, the natural rennet was chosen to prepare PEM. Zinc-copper electrode plate (1×5cm) was used as anode and cathode respectively, which were fitted on the compartment sidewall and connected through a resistance and a digital multimeter by copper wire.

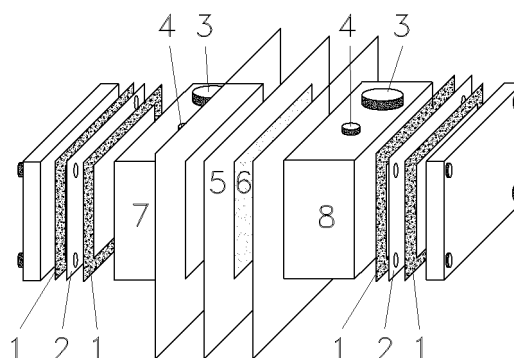


Fig. 1

Fig. 1 Design of electrode compartments

(1.Sealing 2.Electrode plate 3.Feed/wire inlet 4.Vent 5. PEM support 6. PEM 7.Anode chamber 8.Cathode chamber) Before starting up of the MFC, the microorganisms need anaerobic pre-cultivation to prepare seed suspension for 6 hours. In the anode chamber, fibrolytic bacteria isolated from rumen were used as electron donor and incubated with the seed suspension. The fibrous substrate used in the anode chamber focused on corn waste such as bran, hay and corncob, but carboxyl methyl cellulose (CMC) was used for theoretic testing analysis.

### 2.2 Microorganisms and culture media

Electricity-producing bacteria were from the same rumen fluid, Jingzhang Cattle Slaughterhouse, Tianjin, China. Isolation and culture of fibrolytic bacteria NLH-B<sub>2</sub> was done as described by Q. I. Zeng (14). CMC liquid medium of 5 mL was used to prepare seed suspension, which contains  $\text{KH}_2\text{PO}_4$  0.5 g,  $\text{NaCl}$  6.0 g,  $(\text{NH}_4)_2\text{SO}_4$  2.0 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.1 g,  $\text{K}_2\text{HPO}_4$  2.0 g,  $\text{CaCl}_2$  0.1 g, CMC 15.0 g in 1.0 L distilled water. The seed suspension of NLH-B<sub>2</sub> was cultivated for 6 hours at 30°C on an anaerobic incubator with pyrogallic acid. Before inoculation, CMC medium was purged with nitrogen for 10min in order to remove oxygen. After pre-cultivation, the seed suspension of NLH-B<sub>2</sub> was inoculated with  $2.0 \times 10^7$  cells/mL into anode compartment which was filled with

different concentration CMC ranging from 10 g/L to 50 g/L or other fibrous substrate and some salts as above. Anaerobic condition was prepared by purging nitrogen into electrode compartments for 20min before inoculation.

Isolation and culture of methanogens NLH-F<sub>5</sub> followed the same method of S. He. (15) The methane-generation medium was used to prepare seed suspension of NLH-F<sub>5</sub>, which contains KH<sub>2</sub>PO<sub>4</sub> 0.4 g, K<sub>2</sub>HPO<sub>4</sub> 0.4 g, NH<sub>4</sub>Cl 1.0 g, MgCl<sub>2</sub> 1.0 g, yeast extract 1.0 g, L-cysteine 0.5 g and VB<sub>2</sub> 75 μg in 1.0 L distilled water. NLH-F<sub>5</sub> also needs pre-cultivation for 12 hours at 37 °C on an anaerobic incubator. Anaerobic conditions were made as above. After pre-cultivation, the seed suspension of NLH-F<sub>5</sub> with OD<sub>600</sub>=0.1 was inoculated into cathode compartment which was filled with 20 mM KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> buffer and 0.2 M NH<sub>4</sub>Cl and purged with nitrogen from the vent before inoculation.

The MFC worked at room temperature about 25~30 °C for 48 hours or more long time. Cellulose was used in all tests as the sole energy source for bacteria. Output voltage, current and potential were examined at a fixed time interval.

### 2.3 Calculations

The voltage and current across an external resistance 200 Ω in the circuit of the MFC were monitored using a digital multimeter (Agilent HP 34970, US) connecting to a computer by universal serial bus interface every one minute. Measurements of the working potential of the MFC were carried out by a digital potential difference tester SDC-□A. Power density  $P$  (mWm<sup>-2</sup>) was calculated according to  $P=UI/A$ , where  $U$  (V) denotes the voltage,  $I$  (mA) the current, and  $A$  (m<sup>2</sup>) the area of the electrode. Coulombic efficiency was calculated as  $CE = C_p/C_{th} \times 100\%$ , where  $C_p$  is the total coulombs calculated by integrating the current measured every one minute over time, i.e.  $C_p = \int_{i=1}^t I \cdot dt$ , where  $t$  (s) is the total reaction time once voltage output below 0.07V.  $C_{th}$  is the theoretical amount of coulombs available and calculated as  $C_{th}=FbCV/M$ , in which  $F$  denotes the Faraday's constant (96,485 C mol<sup>-1</sup>),  $M$  relative molecular mass of substrate (242 g mol<sup>-1</sup>),  $b$  the number of moles of electrons produced per mole of substrate (4 mol),  $C$  (mg L<sup>-1</sup>) the concentration of removed substrate,  $V$  (200 mL) the electrode liquid volume.

### 2.4 Analyses

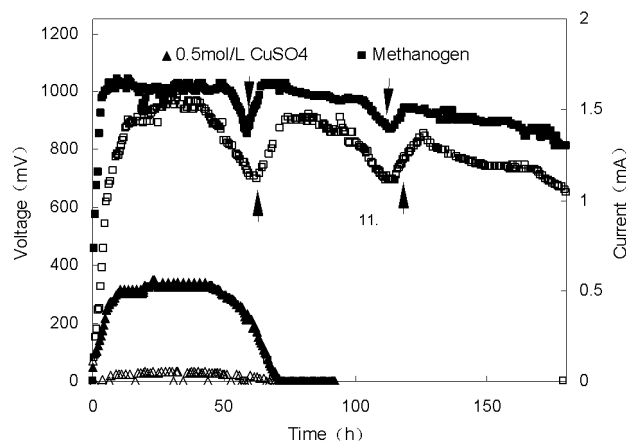
Biomass of NLH-B<sub>2</sub> was analyzed by cell counting using CMC solid medium containing 2% agar. The sample of 100 μL from anode compartment was diluted to fixed multiples at interval of 4 hours, and then 100 μL diluent was coated evenly on CMC plate and cultivated for 48 hours at 30°C. The biomass was calculated as  $S=100aX$  in which  $S$  (cells/mL) is cell concentration,  $a$  the dilution multiples,  $X$  the number of single-colony in the plate. Biomass of NLH-F<sub>5</sub> was assayed at ultraviolet 600 nm by Spectrophotometer TU-1901 every 2 hours. CMC was analyzed by acid hydrolysis method using 2 M sulfuric acid. A polarimeter was used to detect glucose concentration of the hydrolysis reaction. The conductivity of anode was assayed every 12 hours using Conductivity Detector DDS-307.

## RESULTS AND DISCUSSION

### 3.1 Electricity generation using methanogens and copper sulfate

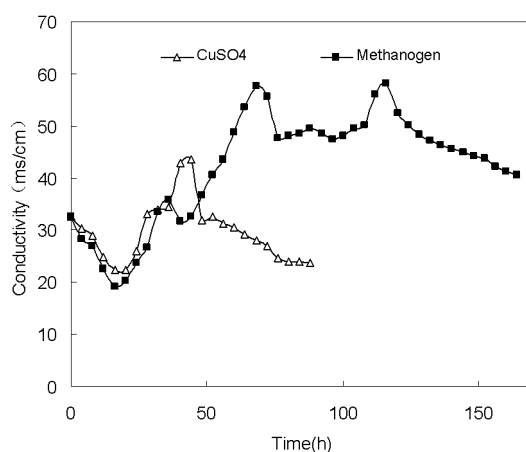
In our previous work, the highest maximum power density (100.60 mWm<sup>-2</sup>) and OCP (630 mV) were achieved in MFC when using 0.5mol/L CuSO<sub>4</sub> in cathode. But, as we all known excess Cu<sup>2+</sup> is potentially toxic to the anodic microorganisms, which can diffuse through the membrane over long-term operation and reduce the overall performance. So, we want to use microorganisms to instead of copper sulfate in cathode compartment. In this experiment, Methanogens NLH-F<sub>5</sub> isolated from the same rumen sample as NLH-B<sub>2</sub> was used as electron acceptor. Comparative experiments including biomass, long-term performance and Coulombic efficiency were performed at the same conditions in the two-chamber MFC. For long-time running test, the experiment was operated in batch mode by adding CMC into anode according to the results of analysis. The corresponding curves were obtained by observing output voltage and current continuously for 180 hours. As can be seen from Fig. 2, the digital multimeter can't monitor voltage and current after 60 hours when using copper sulfate in cathode although replenishing CMC. The highest maximum voltage and current about 1.03 V and 1.5 mA were achieved at 15 hours using methanogens instead of copper sulfate. This steady-state lasted 180 hours through adding CMC twice which power density is about 3090 mWm<sup>-2</sup>, while the MFC using copper sulfate only produced maximum voltage 330 mV and current 0.53 mA respectively.

In this electricity generation test, the external resistance was 200Ω. CE was calculated as 29.9% and 45.1% using CuSO<sub>4</sub> and methanogens. That means when using CuSO<sub>4</sub> as acceptor the capability of cellulose degradation in anode becomes weaker with the internal resistance increases. All the phenomena indicate cell metabolism changed in anode compartment for electron acceptor changing in cathode.



**Fig. 2** Voltage and current generation using copper sulfate (0.5 mol/L) and methanogens NLH-F<sub>5</sub> as electron acceptor in the cathode of a two-chamber MFC (open symbols indicated current and filled symbols indicated voltage, the arrows point the time of adding CMC into anode)

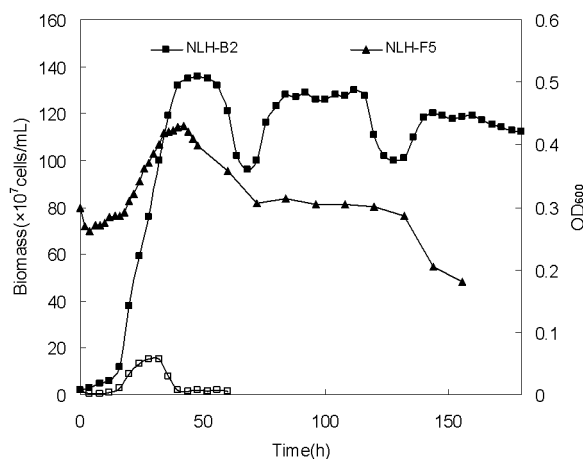
The MFC using methanogens in cathode is considered to have less internal resistance than using CuSO<sub>4</sub> as electron acceptor, which was deduce the main reason for the performance improving. The results shown in Fig. 3 revealed that the maximum conductivity was 49.5 ms/cm when using methanogens which accounted for more than two times of that as using CuSO<sub>4</sub>. Moreover, The MFC can maintain about 45~49 ms/cm of conductivity for 76 hours as using methanogens in cathode, which hints the metabolism of NLH-B<sub>2</sub> in anode was normal and the internal resistance reduced due to the accumulation of VFA. On the contrary, the conductivity can reach 43.59 ms/cm at the initial time when using CuSO<sub>4</sub> in cathode, but with the diffusion of Cu<sup>2+</sup> the conductivity fall down to 31.85 ms/cm, although adding fresh CMC the situation was not improved.



**Fig. 3** Comparison of conductivity in anode as using copper sulfate (open symbols) and methanogens (filled symbols) in cathode

### 3.2 Comparison of microorganism growing in the two-chamber MFC

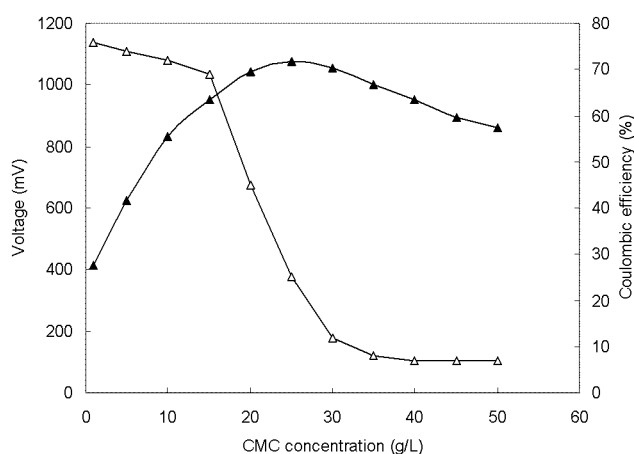
The aim of this phase is further to demonstrate the speculation about the negative effects on cell metabolism caused by Cu<sup>2+</sup> diffusion. The biomass about  $2 \times 10^7$  incubated in anode was same at the beginning, but which only increased 7 folds at the stationary phase when using CuSO<sub>4</sub> in cathode and declined after 8 hours although adding fresh substrate. As can be seen from Fig. 4, microorganisms in anode show a healthy growing trend as using methanogens in cathode. NLH-B<sub>2</sub> in anode entered the logarithmic growth phase after incubation 12 hours and reached  $1.35 \times 10^9$  cells/mL, which kept 16 hours and maintained a longer time by replenishing CMC. Methanogens NLH-F<sub>5</sub> in cathode is obviously different with NLH-B<sub>2</sub>, which biomass decreased at the beginning and lagged about 18 hours until NLH-B<sub>2</sub> growing rapidly. The highest maximum OD<sub>600</sub> was about 0.43 when NLH-B<sub>2</sub> reached stationary phase, but which can't last a longer time and declined slowly during the latter 100 hours. The decline of NLH-F<sub>5</sub> may be related to the dissolved oxygen or product accumulation in anode which need further investigation.



**Fig. 4** Comparison of biomass when using copper sulfate and methanogens in cathode

### 3.3 Effects of initial CMC concentration on electricity generation

When using NLH-B<sub>2</sub> and NLH-F<sub>5</sub> to generate electricity, CMC added in anode is the common carbon source for cell metabolism in anode and cathode. CMC concentration is an important factor for voltage output and coulombic efficiency in a two-chamber MFC. The voltage output in the MFC was monitored at CMC concentrations ranging from 1 g/L to 50 g/L with an external resistance of 200  $\Omega$ . It can be seen from Fig. 5 the maximum voltage output climbed from 413 mV to 1075 mV with the CMC concentration up to 25 g/L and then decreased when CMC concentration was above 25 g/L. The overall Coulombic efficiency decreased with the increase of CMC concentration. When initial CMC concentration increased from 1 g/L to 15 g/L the Coulombic efficiency decreased slightly from 76% to 69%. Further increase in CMC concentration to 20 g/L led to the sharp reduction of coulombic efficiency to 45%. A lower coulombic efficiency ranging from 7% to 25% was obtained under a higher CMC concentration between 50 g/L and 25 g/L.



**Fig. 5** Relationship between CMC concentration voltage (filled symbols) and coulombic efficiency

The curve of Coulombic efficiency in Fig. 5 suggested that electrons generation or transport have been plausible repressed in some degree at higher CMC concentration. The reducing sugar test indicated the concentration of residual glucose in anode increased from zero to 10.46 g/L with CMC concentration up to 35 g/L, at the same time the Coulombic efficiency decreased sharply. The residual glucose reduced slightly when CMC concentration was above 35 g/L, but Coulombic efficiency still at a low level. It is plausible that the cell metabolism is repressed by the presence of glucose in anode and this phenomenon does exist in other bacteria which was called carbon catabolic repression (16,17,18). So, higher residual glucose in anode may be the important negative impact on electrons generation. Of course, there is another reason electrons maybe were consumed by oxygen diffused from the feed inlets.

## CONCLUSION

The current studies on electricity generation using symbiotic fibrobacteres and methanogens in a MFC provided a

potentially novel possibility for renewable bio-energy production with corn waste. A better result was achieved when using methanogens instead of copper sulfate, the voltage output, current and power density ranging from 330 mV to 1.03 V, 0.53 mA to 1.5 mA and 100.06 mWm<sup>-2</sup> to 3090 mWm<sup>-2</sup> respectively. However, the celluloses bioconversion affected by the CCR occurring only at relatively low CMC concentration levels, where fabric digestion maintains a low reaction rate, may make an obstacle for exoelectrogens in MFC. In any case, compared with the current, the higher power density was reported about 3.9 Wm<sup>-2</sup> using permanganate as the electron acceptor which is slightly more than the results utilizing the symbiotic microbial communities in MFC (19,20). That is to say there maybe exists a new world of exploration in the natural microbial communities for survival in a limited resource and space, which contribute in unknown ways to electron or hydrogen transport. The mechanistic complexities in current MFC will be the subject of further studies, which might also open a new door for renewable bio-energy production.

#### Acknowledgement

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