The effects of cathodic micro-voltage combined with hydrothermal pretreatment on methane fermentation of lignocellulose substrate

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Abstract. The methane fermentation study assisted with cathodic micro-voltage was carried out to investigate the electric field effects on the fermentation of hydrothermally pretreated lignocellulose substrate. It was illustrated that a 0.25V cathode voltage and hydrothermal pretreatment could improve the biogas production, biogas quality and lignocellulose degradation ratio significantly. The cumulative biogas productions in the fermentation of hydrothermally pretreated cow dungs at 50℃, 150℃ and 200℃ with a 0.25V cathode voltage were observed in a total of 6640mL, 9218mL and 9456mL respectively over a detention time of 33 days. In comparison with the fermentation pretreated at 200℃ without any voltage, nearly doubled of cumulative biogas production was obtained in the process of cathode-assisted fermentation. It was also observed that the daily methane content greater than or equal to 70% in the biogas generated with cathode voltage were clearly greater than that without voltages. Furthermore, the fermentation applied with a 0.25V cathode voltage had resulted into significant increases of 12.64% and 9.44% in lignin and cellulose degradation ratio relative to voltage free fermentation. And in the process of fermentation applied with cathode voltage, the final lignocellulose degradation ratio increased with the hydrothermal pretreatment temperature. Thus, the hydrothermal pretreatment and assisting fermentation with low cathode voltage can effectively promote the lignocellulose degradation. All results revealed that cathodic micro-voltage combined with hydrothermal pretreatment can remarkably improve the fermentation of lignocellulosic materials, indicating that a more effective fermentation technology can be developed by applying with cathodic micro-voltage.

1. Introduction

Energy shortage is one of the most serious problems facing mankind today. Making the transition from non-renewable carbon resources to renewable bio resources is absolutely needed. Biomass energy, as a form of solar energy, is the only renewable carbon resource, which attracting more and more attention today.

The fully integrated agro-biomass ways for sustainable technologies are biofuels, heat, electricity and biomaterials [1]. However, methane fermentation is a more efficient way of energy production and promises more extensive application range in comparison to the above technologies. Production of
biogas from methane fermentation provides a versatile carrier of renewable energy, as methane can be used for replacement of fossil fuels in both heat and power generation and as a vehicle fuel [2].

Although biomass contains varying amounts of cellulose, hemicellulose, lignin and a small amount of other extractives [3], lignocellulose is the principal part of biomass [4]. The main problems encountered with the biological conversion of lignocellulose arise from its inaccessible structure as cellulose fibre that is partially arranged in a crystalline structure, integrated with hemicellulose and embedded in a matrix of lignin [5]. Therefore, it is particularly significant to improve the lignocellulose degradation rate in the utilization of biomass. The pretreatment methods of lignocellulose [6] include physical (e.g. steam treatment) [7], chemical (e.g. by acid or alkaline hydrolysis) [8], microbial [9], or a combined treatment of these methods [10].

Hydrothermal process is environment-friendly physio-chemical technology that can be conceived as the first step for the chemical utilization of lignocellulose. The effects caused by hydrothermal treatments include hemicellulose depolymerisation (to yield sugar oligomers, hemicellulosic sugars and degradation products), cellulose alteration (mainly in relation with its physicochemical features) and chemical alteration of lignin (that facilitates further delignification steps with organ solvents and/or alkaline solutions) [11]. In the comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol, liquid hot water pretreatment resulted in much better xylan recovery and ≥80% conversion by simultaneous scarification and fermentation [12]. R. Chandra et al. reported that the hydrothermally pretreated substrate had resulted into an increase of 225.6% in biogas production and 222.0% in methane production relative to untreated rice straw substrate [13]. Mark D. Redwood et al. reported that the extractive fermentation is applied to waste-derived substrates following hydrothermal pretreatment, achieving 83%-99% bio waste destruction [14]. Prasad Kaparaju et al. observed that energy output from the biogas production of hydrothermally pretreated wheat straw was the most energetically efficient and approximately 10% higher than that of untreated wheat straw [15].

After hydrothermal pretreatment, the process of methane fermentation becomes more favorable for the activity of functional microbe. Electricity has long been used to stimulate microbial metabolism [16]. In addition to direct and indirect electron transfer to microbe, other reactions may take place at the electrode surface that can directly impact microorganisms [17]. Thus, the fermentation can be improved by applying with electricity. For example, B. Tartakovsky et al. has enhanced methane production from wastewater in laboratory-scale anaerobic reactors equipped with electrodes for water electrolysis [18].

In the bipolar voltage system, oxygen generated in the anode restrained the methanogen bacteria. Thus, a single cathode may effectively create favorable conditions for methanogens. However, little research had been concentrated on applying with unipolar voltage and combining it with hydrothermal pretreatment. The objective of this work was to investigate the micro-electric field effects on the fermentation of hydrothermally pretreated lignocellulose substrate. Cow dung was considered as the model substrate in the fermentation for its high lignocellulose content. The hydrothermal pretreatment and micro-electric field regulation of fermentation were used to improve biogas production, daily methane content and lignocellulose degradation in the process of fermentation.

2. Experiment

2.1. Materials
The cow dungs and straws used as fermenting substrates were collected from Dengchuan, Yunnan Province, China. The samples were dried in oven and smashed to 60 mesh by grinder. Afterward, they were reserved at dry place for later use. The experiment was conducted under mesospheric digestion condition with the inocula expansively cultivated at 35°C which was from mesospheric fermentation installation in Dengchuan. The analysis of cow dung composition revealed that the contents of ash and volatile solids were 12.7% and 73.32% respectively. Cow dung contained 62.46% organic matter with 14780 J . g⁻¹ of caloric value.
2.2. Experimental apparatus

![Experimental setup diagram]

**Figure 1.** Schematic of experimental setup for methane fermentation.
1. Power supply; 2. Valve; 3. Thermostatic water bath cauldron; 4. Quick joints; 5. Gas collector; 6. Vacuum chamber; 7. Aspirator pump.

2.3. Experimental setup and analytical methods

The mixture of 30g feedstock which were hydrothermally pretreated in autoclave and 100mL fermentation liquor prepared with 8% total solid mass content was added into fermenter. The process of anaerobic fermentation was maintained at 35°C. The volume of biogas produced on everyday was measured according to the displacement method [19]. The main components of biogas were carbon dioxide and methane, which were analysed by gas chromatograph. VFA was determined by titrimetric and pH was measured by pH meter. Decrement method [20] was used to measure volatile solids mass removal efficiency. The analysis of cellulose and lignin were carried out by spectrophotometry and Klason method [21].
3. Results and discussion

3.1. Cumulative biogas production

As can be seen from Fig.3, in the micro-electric field of a 0.25V cathode voltage, biogas production rates of hydrothermally pretreated cow dungs at 50°C, 150°C and 200°C began to decrease after 28 days. The hydrothermally pretreated cow dungs for three temperature levels had resulted in a total biogas production of 6640mL, 9218mL and 9456mL respectively over a detention time of 33 days. With the hydrothermal temperature increased, the cumulative biogas production was raised. These results indicated that hydrothermal pretreatment can promote the cumulative biogas production and the pretreatment temperature is an important factor which can affect the methane fermentation significantly. Similar situations had been observed in other literatures. As R. Chandra described, hydrothermal pretreated substrate had resulted into an increase of 9.2% in biogas production compared to that of untreated wheat straw substrate [22].

The more astonished was that the cumulative biogas production of the hydrothermally pretreated cow dungs at 200°C was only 5105mL in the absence of micro-electric field. And yet, cow dungs with a cathode voltage of 0.25V had resulted into a total of 9456mL biogas, nearly twice as much as the one without micro-voltage. Thus, a single cathode voltage brought a remarkable improvement for biogas production of fermentation. These promotions may be caused by two reasons. On one hand, the degradation of lignocellulose was actually achieved by the enzymes generated from anaerobe in the process of fermentation. The enzymes may be stimulated by the micro-electric field, especially the hydrolases. Due to the effect of cathode voltage, the change of enzyme activity promoted the combination of reactive sites and substrates. On the other hand, the redox potential of solution which was sensitively affected by several parameters such as pH, temperature and so on, was an essential factor for anaerobe metabolism. The single cathode voltage possibly altered the redox potential to the optimal state of anaerobe, which created favorable conditions for the fermentation.

3.2. Methane content

The methane contents were grouped into three levels: the first level was $C_{CH_4}$<60%; the second was $60%≤C_{CH_4}<70%$; and the third was $C_{CH_4}≥70%$ respectively. Fig.4 showed the effect of cathode micro-voltage and hydrothermal pretreatment on daily methane content. When cow dungs were hydrothermally pretreated at 50°C and applied with 0.25V cathode voltage, the daily methane contents
were all more than 60% and the maximum days in the third level was found. As the pretreated temperature was raised, a decrease was observed in the number of days that the daily methane contents were greater than or equal to 60%. So it can be concluded that the hydrothermal pretreatment is against the methane content.

**Figure 4.** The effect of low cathode voltage and hydrothermal pretreatment on daily methane content

There was a comparison of methane content with or without micro-electric field. When applying a cathode voltage of 0.25V, an increase was observed in the number of days at the third level and the days that the daily methane content was less than 70% were decreased. These results manifested that the methane content can be improved by the applied voltage. There may be two possible reasons. Firstly, the metabolism and reproduction of hydrogenogens were improved by the stimulation of the micro-voltage, as well as the activity of methanogens. Secondly, H$_2$O, as the hydrogen donor, was more utilized by anaerobe to react with CO$_2$ in the micro-electric field, leading more generation of CH$_4$.

3.3. Hydrolysis behaviour of cow dungs

During hydrolysis of hydrothermally pretreated cow dungs over 33 days of detention period, the variations of pH were illustrated in Fig.5.

**Figure 5.** The changes in pH during the fermentation
As can be seen, the changing trends of pH through the whole hydrolysis process were similar in all experimental conditions. In 15 days, the pH kept in the range from 7.1 to 7.4. This may be due to the equilibrium between the rate of acid production and the rate of acid conversion into methane. Because of the hydrothermal treatment, part of hydrogen bonds in the lignocellulose was broken, leading to the formation of small molecules which are easily digested. Accordingly, inocula well adapted to the fermentation environment and metabolized normally at the initial stage of fermentation. The pH decrease was observed for the first time happening between 17th days to 22th day as the pH dropped to about 6.6. The possible reason was that small molecules had been almost digested and the conversion of carbohydrates and proteins into fatty acids and amino acids had taken place. Afterward, the pH returned to neutral on the 24th day. It was caused by the feedback mechanism of system. Otherwise, the processus of methanogens were inhibited when pH was lower than 6.5. Further, the pH showed a drop again. After 26th day onward, the pH started to increase, which reached to 7.5 and then did not very much. This phenomenon was due to the decomposition of fatty acids and amino acids.

3.4. *Volatile solids mass removal efficiency*

![Figure 6](image)

*Figure 6. The volatile solids mass removal efficiency of the anaerobic digestion process*

Fig.6 showed the effect of hydrothermal pretreatment and the cathode micro-voltage on volatile solids mass removal efficiency. The volatile solids are the effective representation of the content of organic substance in substrate. That is, the more volatile solids were removed, the more volatile solids were converted into methane and carbon dioxide. The hydrothermally pretreated cow dungs at 200°C with a cathode voltage of 0.25V had resulted into the maximum volatile solids mass removal efficiency as high as 48.0% after 33 days of detention time. Thus the cumulative biogas production should also be the maximum, which was consistent with the result of Fig.3. The volatile solids mass removal efficiencies of hydrothermally pretreated cow dungs at 50°C and 150°C with a cathode voltage of 0.25V were 46.8% and 46.6% respectively which were much the same. It can be concluded that the hydrothermal temperature had a slightly positive effect on volatile solids mass removal efficiency. While the volatile solids mass removal efficiency of hydrothermally pretreated cow dungs at 200°C without voltage was only 46% within 33 days, which was 2% less than that with a cathode voltage of 0.25V. Thus the cathode micro-voltage can also improve the volatile solids mass removal efficiency.
3.5. The variation of VFA

Fig. 7 illustrated the variation of VFA in the fermentation. The first drop in VFA occurred within 2 days, which indicated that there was no accumulation of fatty acids. The reason may be that the methanogen bacteria were active due to their good adaptability. As a result, the pH kept alkaline.

After that, VFA began to be cumulated from the 14th day, which inhibited the methanogens. Consequently, the pH will decline. As can be seen from Fig. 4, the pH was dropped to about 6.6 between 17th day to 22th day of detention time, which was in agreement with the speculation. By the 22th day, a second decrease was observed due to the predominance of methanogens, which was consistent with the variation of pH.

![Figure 7](image_url)

**Figure 7.** Concentrations of VFA in the fermentation of cow dungs

3.6. The degradation ratio of lignin and cellulose

Fig. 8 presented the lignin and cellulose degradation ratio for the substrates after fermentation. The electric-assisted fermentation of hydrothermally pretreated substrates at 50°C had resulted in 49.18% of lignin degradation and 46.95% of cellulose degradation. The lignin and cellulose degradation ratio of cow dungs hydrothermally pretreated at 100°C were observed as 52.50% and 54.99% in the fermentation assisted with micro-electric field. When hydrothermally pretreated at 200°C and applied with 0.25V cathode voltage in the process of fermentation, 57.32% of lignin degradation and 56.60% of cellulose degradation were found. However, for the fermentation of hydrothermally pretreated substrates at 200°C without cathodic voltage, 44.68% lignin and 47.16% cellulose degradation ratio were obtained.
The highest lignin and cellulose degradation ratio was observed for hydrothermally pretreated cow dungs at 200°C with 0.25V cathode voltage applied in the fermentation. Furthermore, the biogas production and methane content of cow dungs in the same conditions was also the highest. It was possibly caused by the following reasons. Water under high pressure can penetrate into the biomass, hydrate cellulose, and remove hemicellulose and part of lignin in the hydrothermal pretreatment. It enlarges the accessible and susceptible surface area of the cellulose and makes it more accessible to hydrolytic enzymes [23]. In addition, microbes stimulated by cathode voltage in the fermentation become more active, leading to an acceleration of fermentation. Thus, it can be speculated: The higher content of lignocellulose substrate was used for anaerobic digestion, the more bio-methane yield can be obtained, and a cathode micro-voltage and hydrothermal pretreatment can effectively improve the lignocellulose degradation ratio.

4. Conclusion
The experimental results revealed that the hydrothermal pretreatment and assisting fermentation with cathode micro-voltage showed a significant effect on methane fermentation of cow dungs, especially the cathode micro-voltage. The cumulative biogas productions in the fermentation of cow dungs assisted with a 0.25V cathode voltage were increased with the hydrothermally pretreated temperature. In the comparison with the fermentation pretreated at 200°C without voltage, nearly doubled of cumulative biogas production was obtained in the process of cathode micro-voltage assisted fermentation. The days of daily methane content which is greater than or equal to 0.7 from the fermentation of cow dungs applied with a 0.25V cathode voltage were found more than that of cow dungs applied without voltages. With the hydrothermal temperature raised, the lignocellulose degradation ratios improved. Compared to the fermentation of hydrothermally pretreated cow dungs at 200°C without voltages, 12.64% and 9.44% improvement in the lignin and cellulose degradation ratio were obtained in the fermentation assisted with micro-electric field.

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