Effects of urea ammonia pretreatment on the batch anaerobic fermentation efficiency of corn stovers

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Abstract: In order to enhance the biogas production and provide nitrogen sources for the growth of microorganisms, experiments on urea ammonia pretreatment of corn stovers were implemented at (35±1)°C to investigate the effects of urea ammonia pretreatment on the batch anaerobic fermentation efficiency of corn stovers. This study assessed the effects of urea ammonia contents (2%, 4%, and 6%) and moisture contents (30%, 50%, 70% and 90%) on the physical structures of lignocelluloses and the efficiency of biogas production from anaerobic fermentation of corn stovers. The results indicated that the methane production reached 230.31 mL/g VS (volatile solids) at pretreatment with 4% urea ammonia and 70% moisture contents for the batch anaerobic fermentation, which was 26.6% higher than that of the untreated group. The degradation rates of cellulose and hemicellulose were 66.34% and 75.47% after the anaerobic fermentation, respectively, which were about 22.6% and 20.9% higher than that of the untreated group, respectively. Thus, it was concluded that urea ammonia pretreatment can improve the efficiency of biogas production from anaerobic fermentation of corn stovers.
Keywords: corn stovers, urea ammonia pretreatment, batch anaerobic fermentation, methane production, degradation
DOI: 10.25165/j.ijabe.20191204.4835

Citation: Li Q, Yang F L, Zheng G X, Guan Z J. Effects of urea ammonia pretreatment on the batch anaerobic fermentation efficiency of corn stovers. Int J Agric & Biol Eng, 2019; 12(4): 169–173.

1 Introduction

China is one of the largest agricultural countries in the world. It has been estimated that approximately 800 million tons of crop stalks are generated every year[1], which mainly include corn stovers, rice straw and wheat straw. The yield of corn stovers is about 240 million t, accounting for about 30% of the total stalks. Currently, the main utilization of corn stovers (approximately 35%) is in direct combustion, which not only damages the soil structure and negatively affects agricultural income, but also causes hazy weather and endangers human health[2].

Anaerobic fermentation is widely utilized in treating corn stovers because it can produce bioenergy and organic fertilizer, meanwhile reducing environmental pollution[3]. However, corn stovers consist of polysaccharides (such as cellulose, hemicellulose and lignin), which form complex structures, consequently resulting in reduced availability of these carbohydrates for enzymatic hydrolysis and subsequent microbial conversion[4]. Therefore, the rate of hydrolysis is commonly the rate-limiting step during the anaerobic fermentation. Pretreatment of corn stovers is considered an effective method for enhancing their digestibility and utility in biogas production. The usual methods of pretreatment mainly include physical, chemical, biological and combined processes[5]. Hendriks et al.[6] found that the reduction of raw-material particle size by physical pretreatment can effectively improve the specific surface area and the accessibility of cellulose, resulting in increased efficiency in its enzymatic hydrolysis. However, during the process of physical pretreatment of corn stovers, large amounts of energy can be consumed. Zhang et al.[7] discovered that the best fermentation conditions for white-rot fungi on corn stovers were as follows: water ratio of 70%; temperature of 30°C; inoculun concentration of 0.25 g/kg corn stovers; and pH 4 in 7 days. Biological pretreatment needs low energy; however, the species are stringent, and engineering application is difficult[8,9]. Chemical pretreatments (acidification and alkalization) have been widely applied in engineering practice because of their fast effects and short pretreatment times[10]. Zheng et al.[11] pretreated corn stovers with wet-state sodium hydroxide and found that 72.9% more total biogas production, 73.4% more methane yield, and 34.6% shorter technical digestion time were obtained at a NaOH dose of 2% and a loading rate of 65 g/L for anaerobic fermentation as compared to the untreated group. Yao et al.[12] reported that the cellulose conversion ratio increased from 31.88% to 95.74% when the corn stovers were pretreated with a 1.875% acid solution at a 1:15 liquid ratio at 170° for 60 min. Although chemical pretreatment methods can greatly promote the fermentation process, they may not be applied in practical situations because of their high costs and secondary contamination. During batch anaerobic fermentation, the growth of microorganisms requires small-molecule organic matter as the fermentation material, while a lack of nitrogen can reduce the efficiency of anaerobic digestion[13-18]. Ammoniation pretreatment not only has the same alkalization effect, but also provides the nitrogen source[19-22]. Ma et al.[23] found that with 4% ammonia pretreatment, the biogas production per gram of volatile solids (VS) of rice straw increased by 34.8% over that of the untreated batch. Luo et al.[24] observed that with 4% ammonia pretreatment, the cumulative gas production

Received date: 2018-12-06 **Accepted date:** 2019-07-10
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of rice straw increased by 20.67% to 38.20% over that of untreated straw.

Urea, a well-known nitrogen fertilizer, can be applied for the pretreatment of corn stovers because it is relatively cheap and harmless to the human body. Meanwhile, the biogas slurry generated after fermentation can be used as marsh manure to increase crop yield without subsequent treatment problems. However, little information is currently available on pretreatment of corn stovers by urea ammonia. The main objective of this study was to investigate the anaerobic fermentation efficiency of corn stovers under different ammonia pretreatment conditions. Batch assays were implemented to compare the specific surface area (SSA), cumulative methane production, and degradation of lignocellulose in different ammonia pretreatment conditions, as well as determining the process parameters to provide a theoretical basis for engineering practice.

2 Materials and methods

2.1 Materials

The corn stovers used in this study were obtained from Xiangfang Farm in Harbin. The air-dried corn stovers were chopped to 1 cm in diameter for pretreatment and anaerobic fermentation. The inoculum was collected from an anaerobic continuous-fermentation digester in our laboratory. The physical and chemical characteristics of the corn stovers and inoculum are listed in Table 1.

Table 1 Characteristics of corn stovers and inoculum

	TS	VS*	TC*	TN*	C*	HC*	L*	pH
Corn stovers	95.07±0.13	88.95±0.17	42.18±0.34	0.61±0.02	35.37±0.72	29.21±0.43	7.59±0.24	ND
Inoculum	3.44±0.06	2.52±0.04	32.03±0.65	1.75±0.08	ND	ND	ND	6.95±0.03

Note:* % of TS; TS: total solids; VS: volatile solids; TC: total carbon; TN: total nitrogen; C: cellulose; HC: hemi-cellulose; L: lignin; ND: not determined.

2.2 Urea Ammonia pretreatment

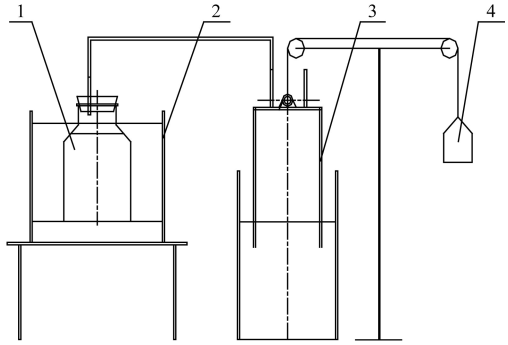
First, the chopped corn stovers (100 g) were placed in a sealed plastic bag, into which urea was added at charges of 2%, 4%, and 6% (the mass ratio of urea to corn stovers, based on dry matter), respectively. Next, water was added into the bag to regulate the moisture content (MC) of the stovers to 30%, 50%, 70%, and 90%. The moisture content was calculated according to a formula used by Li et al.[25] and Reilly et al.[26], as expressed in Equation (1):

$$MC=1-\text{dry matter weight of corn stovers}/(\text{weight of corn stovers + water added}) \quad (1)$$

Finally, the sealed plastic bag was shaken and put into the 35±1° constant-temperature incubator. When the pH of this system stabilized (pH±0.2), the pretreatment process was considered complete. The pH of the fermentation liquor during the process of pretreatment was monitored every day.

2.3 Batch anaerobic fermentation

The untreated and urea ammonia pretreated corn stovers were digested in a batch anaerobic fermentation tank. Corn stovers weighing 20 g with 600 g of inoculum were digested in 1 L anaerobic digestion reactors (with a working volume of 0.65 L)[27-29]. The initial TS and pH of the fermentation liquor were about 6.5% and 7.0, respectively. The batch experiments were performed at a mesophilic level of (35±1)°. All reactors were monitored for 20 d, during which the reactors were shaken five times daily. The generated biogas was captured by a gas-collecting bag, and the production was measured every 24 h during the experiment. The experiments for the different pretreatments were repeated three times.



1. Fermentation tank 2. Water bath 3. Gas gathering tank 4. Counter weight

Figure 1 Experimental device for batch anaerobic fermentation

2.4 Analytical methods

The TS, VS and ash contents of the corn stovers were assayed according to the protocol specified in the Standard Methods for the Examination of Water and Wastewater[30]. The pH value was measured by HI9224 (Hanna Inc., Italy); the biogas composition (hydrogen, methane, nitrogen, hydrogen sulfide and carbon dioxide) was measured via gas chromatography (GC-6890N, Agilent Inc., Santa Clara, CA, USA) by using a thermal conductivity detector (TCD); the carbon and nitrogen levels were determined by using an elemental analyser (EA 3000, Leeman Technologies Co., Ltd., Beijing, China); the specific surface area (SSA) was measured by JW-BK112 (JWGB Inc., China); the cellulose, hemicellulose and lignin contents were measured according to the National Renewable Energy Laboratory (NREL) method[31]. All of the measurements were conducted in triplicate, and the averaged data were reported.

3 Results and discussion

3.1 Urea ammonia pretreatment

The pretreatment time is an important factor because it affects the efficiency of actual production. Therefore, it was necessary to determine the requisite pretreatment time in advance. The changes in pH with different pretreatments of the corn stovers with 2%, 4%, and 6% urea ammonia contents presented a similar pattern. Hence, only the variation in pH for the 2% urea ammonia pretreatment is graphed in Figure 2.

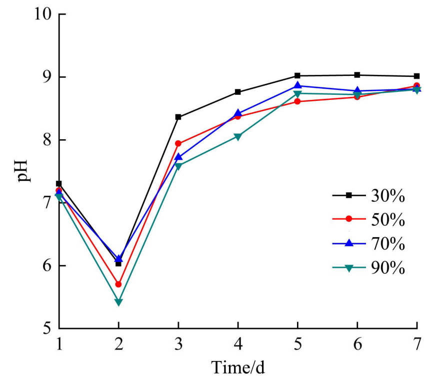
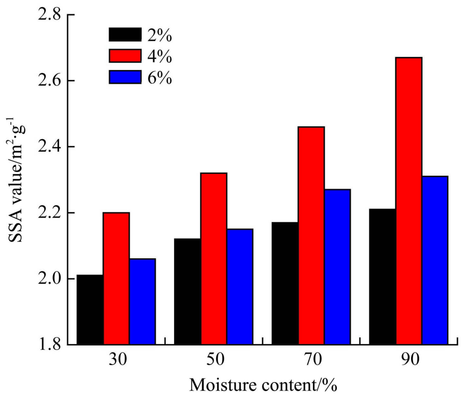


Figure 2 Variation in pH during pretreatment process

The pH values for the different moisture contents were between 7.10-7.30 at the beginning of the pretreatment. The pH value decreased rapidly during the initial pretreatment period for which the lowest pH (approximately 5.7) was obtained on day 2 for the different moisture contents. Thereafter, the pH values increased to about 8.5. For all moisture contents, negligible variations in pH were observed after the five days of pretreatment. Therefore, a five-day pretreatment time was used for later experiments in order to insure that the allotted time was more than sufficient.

3.2 SSA of corn stovers

The SSA values of the corn stovers after pretreatment at different urea ammonia and moisture contents are graphed in Figure 3. The results indicate that urea ammonia pretreatment can obviously influence the SSA of corn stovers. The SSA values increase with an increase in moisture content at the same urea contents. The highest SSA value of 2.67 m^2/g was obtained when the moisture and urea contents were at 90% and 4%, respectively, which was approximately 1.47 times that of untreated corn stovers. This finding indicates that more moisture is beneficial for facilitating the pretreatment process.



Note: % is for dry matter; SSA of untreated corn stovers is 1.81 $m^2.g^{-1}$

Figure 3 SSA of corn stovers after pretreatment

3.3 Methane production

Methane production of per gram VS is a significant parameter for measuring the biodegradable properties of corn stovers in anaerobic fermentation because this parameter can adequately represent the capacity of the pretreatment.

The cumulative methane production for pretreated corn stovers at different urea ammonia and moisture contents is listed in Table 2.

Table 2 Responses of each treatment for different factors

Treatment	Factor A	Factor B	Methane production/mL·g^{-1} VS
T1		2%	152.13
T2	30%	4%	183.73
T3		6%	153.22
T4		2%	159.44
T5	50%	4%	195.19
T6		6%	156.98
T7		2%	175.44
T8	70%	4%	230.31
T9		6%	192.72
T10		2%	163.04
T11	90%	4%	209.36
T12		6%	166.19

The ANOVA results of the batch anaerobic fermentation for cumulative methane production are listed in Table 3, indicating that the model is significant because the *F* value of 36.81 is greater than the calculated one (0.01). The *p*-value of Factor A of 0.0014 is lower than 0.01; whereas, the *p*-value of Factor B of 0.0001 is much lower than 0.01, suggesting that both factors are significant in this experiment, Factor B being more significant relative to the response values when compared to Factor A. I.e., the urea ammonia content has a greater influence on the methane production than the moisture content. Therefore, regardless of the moisture content, the maximum methane production was obtained when the urea ammonia content was 4% at different moisture content levels. There are two reasons for this phenomenon: Pretreatments both (a) destroy the covalent bond between the hemi-cellulose and lignin to increase the degradation rate of the cellulose and (b) change the C/N ratio of the substrate. It is well known that the C/N ratio of corn stovers is too high (65-85), which can result in acidification and a failure in the anaerobic digestion process. Pretreatment with urea ammonia can improve the substrate characteristics of anaerobic fermentation due to the 46% nitrogen content in the urea The C/N ratio in the substrate was 26.27 after pretreatment at urea contents of 4%, which is the closest to a suitable anaerobic fermentation C/N ratio range (20-30).

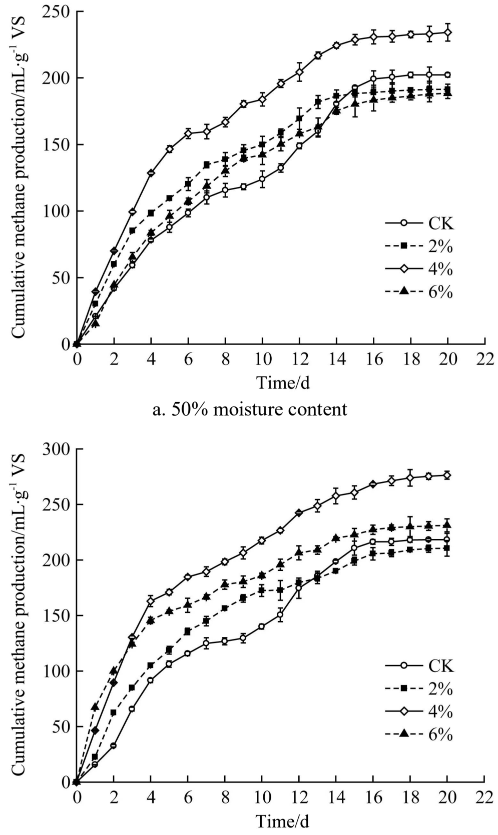
Table 3 Analysis of variance results for methane production

Source	Sum of squares	Degrees of freedom	Mean Square	*F* value	*P*-value	Significance
Model	9347.36	5	1869.47	36.65	0.0002	significant
Factor A	3214.22	3	1071.41	21.01	0.0014	
Factor B	6133.14	2	3066.57	60.12	0.0001	
Residual	306.82	6	51.00			
Cor total	9653.38	11				

Note: Factors A and B represent moisture content and urea content, respectively.

After direct urea pretreatment for anaerobic fermentation to produce methane, the cumulative production at moisture contents of 50% and 70% during the fermentation is graphed in Figure 4. When the moisture content was 50%, the cumulative production for urea contents of 2% and 4% was greater than that of the untreated group after 12 d of fermentation. The cumulative production for urea contents of 6% increased slowly, which was probably due to a small loss of hemicellulose and soluble materials caused by the urea pretreatment. Zhu et al.[17] pretreated corn stovers with sodium hydroxide. Their results indicated that the concentration of sodium hydroxide was 5.0%; the anaerobic fermentation time, 40 d; and the cumulative methane production, 372.4 mL/g VS, being a little higher than our experimental results. When the moisture content in our experiment was 70%, the methane production for urea contents of 2%, 4% and 6% increased rapidly during the first five days of fermentation. On the seventh day, the cumulative production for urea contents of 2%, 4% and 6% were 166 mL/g VS, 203 mL/g VS and 181 mL/g VS, respectively, being higher than that of the untreated group at 152 mL/g VS. The methane production increased rapidly in the initial stage of fermentation, possibly due to the pretreatment's destruction of the covalent bond between the hemicellulose and lignin, thereby increasing the number of extracellular enzymes in contact with the cellulose (wrapped by hemicellulose and lignin) during the fermentation. The SSA analysis after pretreatment also revealed that the pretreatment can significantly increase the SSA as well as the contact area between microorganisms and substrate, and lead to an increase in the metabolic rate of the microorganisms. At the end of the fermentation, for the urea ammonia content of 4%, the methane production was 230.31 mL/g VS, being 31%, 24% and 26% higher than that of the urea contents of 2%, 6% and that of the untreated group, respectively. The results of this study indicate that urea ammonia content lower than 2% is not effective in facilitating methane production; moreover, content higher than 6%

may inhibit methanogen activity, thereby leading to decreases in production efficiency. The methane content of the biogas for different urea ammonia pretreatment was above 55% after three days of anaerobic fermentation (data not shown). The results of the cumulative methane production for 50% and 70% moisture contents indicate that both the urea and the moisture contents will significantly affect the anaerobic fermentation efficiency of corn stovers.



a. 50% moisture content

b. 70% moisture content

Figure 4 Methane production of corn stovers at 50% and 70% moisture content

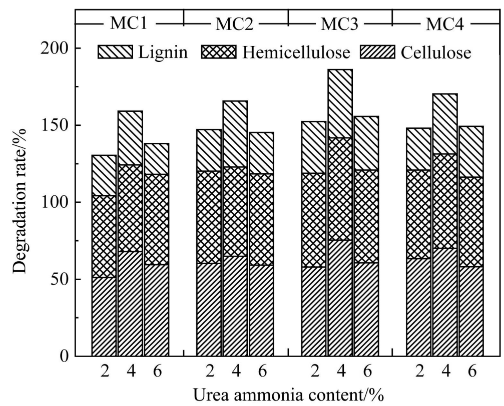
3.4 Degradation of lignocellulose

Lignocellulose is the main component of corn stovers, accounting for about 80% of the total solid content. However, the external parts of the cellulose are entangled with covalently linked hemicellulose and lignin, thus making degradation by extracellular enzymes difficult and consequently resulting in low conversion efficiencies. The purpose of pretreatment is mainly to destroy the natural barrier of lignin and hemicellulose, increase the SSA of the lignocellulose, and facilitate utilization by methanogens, thereby improving biogas production from the anaerobic fermentation. In this study, the degradation rate of the lignocellulose was used to evaluate the effect of the pretreatment on the degradation of the organic matter in the stovers. The variation in lignocellulose composition after pretreatment at different moisture and urea ammonia content levels is listed in Table 4. Thus, the results indicate that urea pretreatment of corn stovers can significantly affect the degradation rate of hemicellulose. After the urea ammonia pretreatment, the hemicellulose content of 22.15% was observed at urea ammonia and moisture contents of 6% and 90%, respectively, being 24.1% lower than that of the untreated stovers at 29.21%. A small variation in the cellulose content (about 5%) was obtained, mainly because the cellulose was wrapped by the hemicellulose and lignin, the long-chain structure of the cellulose being broken only after the outer hemicellulose had been destroyed. In this experiment, the decrement in the lignin degradation was slight. The results of the lignocellulose degradation presented here ~~were~~ are lower than those obtained by Zhang et al.[32], whose degradation rate was about 25% after 16 h of aerobic hydrolysis pretreatment.

Table 4 Changes in composition after urea ammonia pretreatment (%)

Pretreated condition		Condition after urea ammonia pretreatment		
Urea ammonia	Moisture content	Hemicellulose	Cellulose	Lignin
2	30	28.68±0.34	39.65±0.31	7.38±0.21
	50	28.03±0.15	38.94±0.12	7.22±0.14
	70	27.37±0.42	38.34±0.21	6.92±0.38
	90	26.80±0.63	37.19±0.57	6.81±0.61
4	30	27.59±0.72	37.57±0.46	7.02±0.45
	50	25.83±0.22	36.27±0.52	6.74±0.52
	70	24.95±0.54	35.43±0.71	6.42±0.36
	90	24.37±0.18	33.78±0.38	6.32±0.18
6	30	23.56±0.82	36.89±0.23	6.98±0.73
	50	22.68±0.35	35.51±0.64	6.37±0.26
	70	22.27±0.26	33.68±0.47	6.16±0.13
	90	22.15±0.08	33.12±0.72	6.03±0.55
Untreated		29.03±0.46	36.27±0.62	7.48±0.34

The respective degradation rates of the lignocellulose during the anaerobic fermentation process are depicted in Figure 5. These results indicate that the degradation increases with increasing urea ammonia contents when that content is less than 4%. The highest degradation rates of cellulose and hemicellulose at 66.34% and 75.47%, respectively, were obtained at 4% urea ammonia and 70% moisture contents after anaerobic fermentation, being about 22.6% and 20.9% higher, respectively, than that achieved for the untreated group at 54.11% and 62.42%, respectively. Such degradation may be attributed to the fact that the urea ammonia pretreatment increases the accessibility of extracellular hydrolytic enzymes of microorganisms to the cellulose, thereby promoting the cellulose degradation. This result is also consistent with the results of the highest methane production for this condition. However, when the urea ammonia content was higher than 6%, a decrease in the degradation of cellulose was observed. The hemicellulose degradation rates for all urea ammonia and moisture contents were higher than 50% after anaerobic fermentation, which



Note: MC1, MC2, MC3 and MC4 represent moisture contents of 30%, 50%, 70% and 90%, respectively.

Figure 5 Lignocellulose degradation rate of corn stovers after 20 d of anaerobic fermentation may be caused by the destruction of the chemical bond during the pretreatment stage. Moreover, this result is consistent with the results obtained by Zhu et al.[17], of which the pretreatment with sodium hydroxide revealed that the degradation of cellulose at a concentration of 7.5% was 61.3%. Whereas, in this study, a significant improvement in lignin degradation was observed with a 4% urea ammonia pretreatment.

4 Conclusions

The SSA analysis of corn stovers revealed that the SSA values increase with increasing moisture contents at the same urea ammonia contents. The highest SSA value of 2.67 m^2/g was obtained when the moisture and urea ammonia contents were 90% and 4%, respectively, being 1.47 times higher than that of the untreated group.

The ANOVA results obtained from the batch anaerobic fermentation for methane production indicated that both the urea ammonia and the moisture contents had an obvious effect on that production, the urea ammonia content being more significant relative to the production when compared to the moisture content. The maximum methane production of 230.31 mL/g VS for urea ammonia and moisture contents of 4% and 70%, respectively, at the end of the fermentation, was 26.6% higher than that of the untreated group at 181.88 mL/g VS.

Therefore, pretreatment with urea can significantly increase the degradation of lignocelluloses. The degradation rates of the cellulose and hemicellulose were 66.34% and 75.47% when the urea ammonia and moisture contents were 4% and 70%, respectively, being about 22.6% and 20.9% higher, respectively, than that of the untreated group at 54.11% and 62.42%.

Acknowledgements

This study was supported by a grant from the Southwest University Talent Introduction Project (SWU118104).

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