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Full Length Article

Effect of Ammoniation Pretreatment on the Anaerobic Digestion Characteristics of Corn Stalks Treated with Different Ensiling Methods

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Abstract

In this study, improvement of methane production efficiency, the continuity of biogas engineering during the anaerobic digestion of corn stalks, and the effects of pretreatment with 20 wt% ammonia solution on the anaerobic digestion of corn stalks treated with three different ensiling methods were investigated. The batch for anaerobic digestion experiment was carried out for 60 days at a moderate temperature of 37 ± 0.2ºC. The ammoniation pretreatment had a significant effect on those three different anaerobic digestion processes, which significantly accelerated the biogas production and shortened the anaerobic digestion time by 15 days, although, the biogas output was compromised to a certain extent. The pretreatment improved the stability of these anaerobic digestion systems of differently treated corn stalks. The pH of these systems was finally stabilized at around 7.5. This pretreatment accelerated the start-up stages of the anaerobic digestion of corn stalks, and the methane contents increased rapidly to higher than 50%. This work could be useful in providing information on efficient utilization of agricultural wastes for energy.

Keywords: Ammoniation pretreatment; Anaerobic digestion; Corn stalk ensiling; Agricultural waste

Introduction

2017, the straw output of China was estimated to be about 884 million tons, of which 736 million tons were collectible, and nearly 30% of it was contributed by corn stalk (National Bureau of Statistics of China 2014). As an important renewable biomass resource, the total utilization rate of corn stalks was less than 40%, and most of them were burned without recycling, causing environment pollution and waste of resources (Wang *et al.* 2014). In view of the environmental issue and increasing energy crisis, more attention has been paid to anaerobic digestion of cellulosic materials such as straws and the production of biogas. Ensiling of corn stalks is a pretreatment method and preservation technology. The deterioration in quality and effective components of corn stalks during storage can be inhibited by ensiling, which also prolongs the storage of biogas raw materials thus, favoring continuous biogas production (Hansen *et al.* 2004; Vervaeren *et al.* 2010). Because corn stalk is mainly composed of lignin, cellulose, and hemicellulose, the cross-linking and entangling of these components limits the degradation and utilization abilities of hydrolytic microorganisms. Therefore, the anaerobic digestion of corn stalks treated with different ensiling methods shows issues including poor stability, long start-up time and extended digestion period (Wang *et al.* 2014). Therefore, corn stalks should be pretreated reasonably before anaerobic digestion and biogas production. To date, more common effective pretreatment methods involve physical, chemical and biological techniques; among which, ammoniation is an effective chemical approach (Ma *et al.* 2011). During the ammoniation pretreatment, breakdown of fibrous structures and formation of ammonium salts occurs simultaneously. The waste liquid after the pretreatment can serve as a nitrogen fertilizer, avoiding secondary pollution, which usually occurs during chemical pretreatment processes (Luo *et al.* 2015) ammoniated rice straws with urea solutions. After this pretreatment, they reported an increase by 20.67–38.20% in the cumulative biogas output compared with the control group. The output reached a maximum under the urea content of 4 wt%. Likewise, Kim (Kim and Lee 2006) pretreated straws with an ammonia solution, and reported about 62% lignin removal rate at 60ºC, 5 wt% ammonia, a solid-liquid ratio of 1:6, and after 12 h treatment, it could significantly increase methane production.

The ammoniation pretreatment of raw corn stalks has been widely studied, although the focus was mainly on the

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palatability and nutrition for livestock. Few studies have also evaluated the effects of this pretreatment on the anaerobic digestion and biogas producing characteristics of ensiling-treated corn stalks. In this research, corn stalks, pretreated with three different ensiling approaches, were ammoniated with a 20 wt% ammonia solution. The start-up period, anaerobic digestion period, biogas production efficiency and volatile fatty acids (VFAs) content during the anaerobic digestion processes, with or without the ammoniation pretreatment, were analyzed. Based on the comparative results, the feasibility of ammoniation pretreatment for anaerobic digestion of ensiling-treated corn stalks was discussed for the improvement of efficiency and continuity of biogas production.

Materials and Methods

Experimental materials

Ensiled corn stalks were produced in our research group. The storage time was more than 10 months. The treatment methods were divided into three types according to the additive: no additive (Maize ensiled, ME), the addition of cellulose (CB), and addition of Lactobacillus and Brucella (LB), all of these biological agent purchased from Bio-Form Co., Ltd. Fresh cow manure was obtained from a farmer in the Yanjiaping Block in Qilihe District of Lanzhou City, China. The inocula were acquired from the anaerobic fermentation slurry in our laboratory. The fresh cow manure was added to the slurry (manure:slurry = 1:10; w:w). The mixture was sealed for fermentation at ambient temperature for 15 days. The fermentation conditions are mentioned in Table 1.

Experimental apparatus

A controllable thermostatic fermentation apparatus was used in the experiment, as illustrated in Fig. 1. The apparatus mainly consisted of a heating water tank, a temperature controller, a cylindrical 304 stainless steel fermenter with an effective volume of 7.5 L and the height-diameter ratio of 1:1, and a biogas collector. The fermenter was adiabatic. The temperature of the fermenter was accurately controlled through the tank by regulating the flow rate of water. The temperature was measured with a platinum electrical resistance thermometer with an accuracy of ± 0.1ºC.

Experimental methods

The anaerobic digestion experiment was carried out in a 7.5 L wide-mouth bottle (reactor) with a rubber plug. The temperature was stabilized with the thermostatic water bath at 37 ± 0.2ºC. The different types of corn stalks were separately mixed with a saturated ammonia solution and distilled water. Each 100 g of the dry corn stalk was mixed with 4.5 g of 20 wt% ammonia solution. The mixture was placed in a sealed plastic container. The reactor was filled with 4000g of substances including 1200 g of inocula. The cow manure and corn stalks were mixed in a TS ratio of 7:3, and were added to the mixture. The other component was distilled water. The mixture was then shaken and fixed. During the experiment, the reaction bottle was shaken thriceper day, and the digestion process continued until no biogas evolved. The biogas was collected using sample bags every day, and the biogas composition was determined through an analyzer. The experiment was repeated thrice in each group. The CK group contained the same amount of only biogas slurry. The composition of each group is mentioned in Table 2.

Analysis approaches

The TS and VS values were determined via the drying methods (TS was heated at 105ºC for 24 h and VS was heated at 550ºC for 4 h) as previously reported (Zhao 2006). The pH of slurry, biogas composition, and output were measured every day, and the chemical oxygen demand (COD), VFAs and ammonia nitrogen (NH_4^+-N) were measured at 5-day intervals. The biogas composition was determined with a Biogas Check device with methane and carbon dioxide measurement at ± 3.0% accuracy. The pH was determined with a PHS-3C-type portable pH meter (Oleron Company). The COD and NH_4^+-N were measured with a 5B-3C (V8) instrument (Lianhua Technologies Company) (Jiang 2001). The VFAs were determined by spectrophotometry (Wang *et al.* 2008).

Results

Effect of ammoniation pretreatment on the anaerobic digestion and biogas production of ensiled corn stalks

The biogas output data in the anaerobic digestion of ensiled corn stalks with and without ammoniation pretreatment are presented in Fig. 2. The start-up stages of three different ensiled corn stalks after ammoniation were remarkably accelerated compared to the control group, and no delay was observed. In other words, the ammoniation pretreatment can effectively accelerate the start-up processes in the anaerobic digestion of ensiled corn stalks. Throughout the reaction time, the biogas output per day increased largely and reached a maximum on days 5 to 9.In detail, the output of the ammoniated groups CB, ME and LB reached a maximum of 11.02 $L \cdot d^{-1}$ on day 5, 9.77 $L \cdot d^{-1}$ on day 7, and 9.03 $L \cdot d^{-1}$ on day 9, respectively. In contrast, the biogas output of the un-ammoniated ensiled corn stalks reached a maximum on day 20 to 23. In detail, the output of the un-ammoniated groups of LB, ME and that of CB reached a maximum of 12.19 $L \cdot d^{-1}$ on day 20, 11.59 $L \cdot d^{-1}$ on day 20, 8.21 $L \cdot d^{-1}$ on day 23, respectively. These results thus show that the ammoniation pretreatment could effectively shorten the anaerobic digestion time of ensiled corn stalks by about

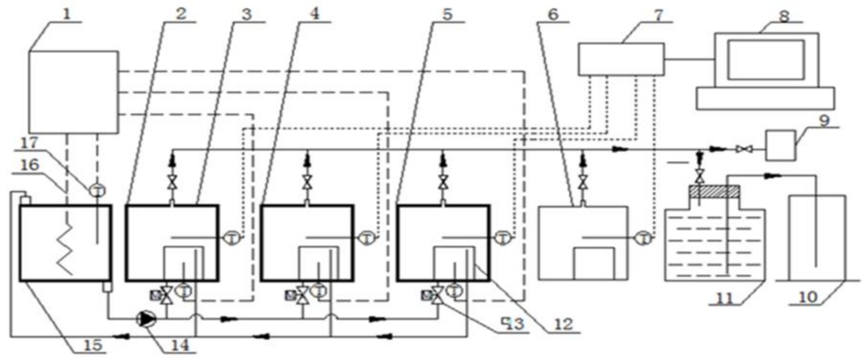

Fig. 1: Schematic illustration of the controllable thermostatic fermentation apparatus

1.Temperature controller 2. Thermal insulation 3. Fermenter① 4. Fermenter②5. Fermenter③ 6. Fermenter④ 7. Data acquisition instrument 8. Computer 9. Biogas analyzer10. Water tank 11. Biogas tank 12. Inner water tank 13. Solenoid valve 14. Hot water pump 15. Thermostatic water tank16. Heating wire 17. Temperature sensor

15 days. This may be because ammoniation can effectively destroy the crystalline structures of lignin and cellulose in ensiled corn stalks, accelerate the start-up of anaerobic digestion, thus shortening the anaerobic digestion period.

The cumulative biogas output in those anaerobic digestion processes of ensiled corn stalks, with and without ammoniation pretreatment, is presented in Fig. 3. In the start-up stages, the three kinds of ensiled corn stalks pretreated by ammonia showed a much higher output compared to the control group, and exhibited different biogas production characteristics. The pretreatment with ammonia could increase the hydrophilicity of straw surface, widen the cellular surface voids, broaden the area for microbial attachment, perturb the crystalline structure of lignin to release encapsulated cellulose, and improve the contact between hydrolytic acid producing bacteria and corn stalks (Zhou *et al.* 2012). Through the course of anaerobic digestion, methanogens are enriched continuously, and the cumulative biogas output of ensiled corn stalks increases (Li *et al.* 2009). However, we observed that due to the ammoniation pretreatment, the biogas output of ensiled corn stalks reduced by 32.54–48.27% compared to the control group in the presence of the same amount of feedstock. Among these three ammoniated ensiled groups, the biogas output of the ME corn stalks (with no additive) was the highest, at 108.97 L. On the other hand, the un-ammoniated ensiled corn stalks showed an extended start-up period but a higher cumulative biogas output. Over the reaction time, the cumulative biogas output of different ensiled corn stalks increased, and the un-ammoniated CB group presented the highest value of 167.95 L, followed by161.55 L for the ME group and 156.82 L for the LB group. Thus, compared with the control group, the ammoniation pretreatment significantly shortened the anaerobic digestion period of corn stalks that were treated with different ensiling methods, but because this pretreatment reduced the concentrations of VFAs in the slurry, the biogas output of ensiling-treated corn stalks declined to a certain extent.

Table 1: Properties of feedstock for fermentation

Feedstock	TS %)	VS (%)	pH before the treatment	pH after the treatment
AH-CB	22.56	20.13	2.4	8.4
AH-ME	28.75	26.30	3.8	8.3
AH-LB	31.10	28.47	3.1	8.3
Cow Manure	20.82	14.47	-	7.3
Inocula	9.01	5.60	-	7.6

AH: Ammonium hydroxide TS: total solid VS: volatile solid

Table 2: Composition of each group

Group	Straw (g)	Cow manure (g)	Inocula (g)	Water (g)
AH-CB	455.06	1091.95	1200.00	1252.99
AH-LB	449.03	1091.95	1200.00	1259.01
AH-ME	510.19	1091.95	1200.00	1260.60
CK	0.00	0.00	1200.00	2800.00

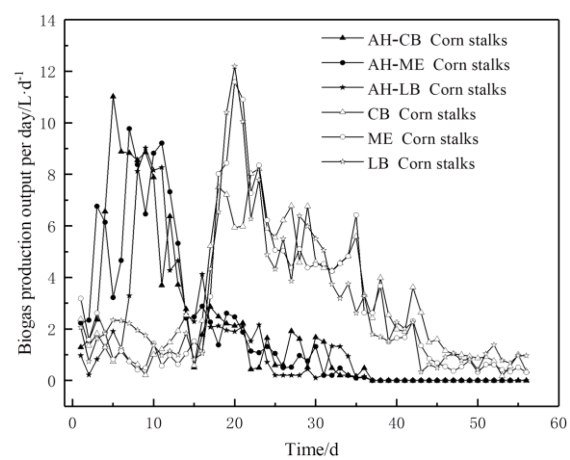

Fig. 2: Effect of ammoniation pretreatment on the biogas output of different ensiling-treated corn stalks

Effects of pretreatment with ammonia on the pH and VFA$_S$ in the anaerobic digestion of corn stalks at moderate temperatures

pH is an important index and parameter in the monitoring anaerobic digestion (Latif *et al.* 2017). The changes in pH can directly reflect the acid-base environment of an

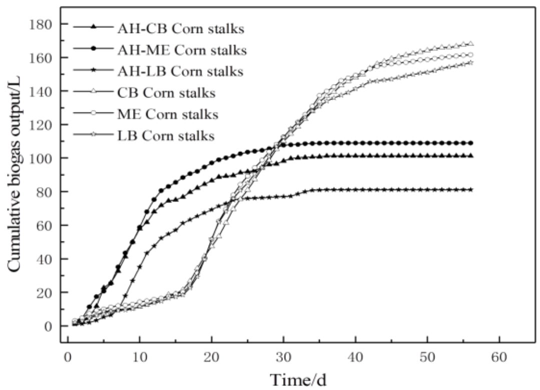

Fig. 3: Effect of ammoniation pretreatment on the cumulative biogas output ofdifferent ensiling-treated corn stalks

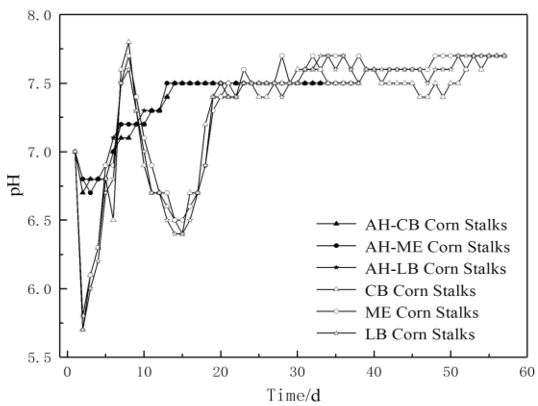

Fig. 4: Effect of ammoniation pretreatment on the pH in the anaerobic digestion of different ensiling-treated corn stalks

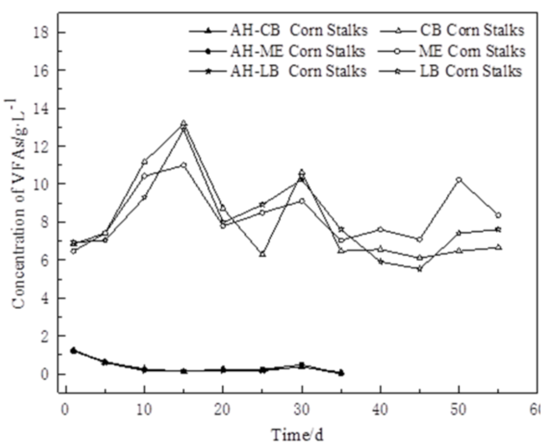

Fig. 5: Effect of ammoniation pretreatment on the concentrations of VFAs in the anaerobic digestion of different ensiling-treated corn stalks

anaerobic digestion system and the state of various biochemical reactions of microorganisms in the reactor. The pH changes in the anaerobic digestion of different ensiling-treated corn stalks with and without pretreatment are mentioned in Fig. 4. In the start-up stages, all the systems were naturally acidified and the lowest pH of 5.6 was observed in the un-ammoniated LB group on day 3. During the start-up stages of the anaerobic digestion, the microorganisms in the reactor gradually adapted to the environment. Cellulose and hemicellulose were gradually hydrolyzed into cellobiose and glucose, in the presence of hydrolytic bacteria. Acid producing bacteria rapidly converted these small organic molecules into VFAs. The production of VFAs was faster than their utilization by methanogens and thus, the C/N ratios in the slurry increased, resulting in a lack of nitrogen and insufficient nutrients for methanogens in the start-up stages. As a result, the VFAs were accumulated and pH decreased in these anaerobic digestion systems, leading to the acidification phenomena (Zhen *et al.* 2015). Along with the reaction, the methanogens in the slurry of anaerobic digestion systems utilized un-ammoniated ensiled corn stalks, gradually adapted to the environment, and the pH steadily elevated to a normal level at around 7.5. As shown in Fig. 4, the pH of systems containing ammoniated ensiled corn stalks decreased slightly in their start-up stages. These results indicate that ammoniation pretreatment can suppress the pH change in the anaerobic digestion of ensiled corn stalks, significantly improving the stability of the anaerobic digestion systems.

VFAs are important intermediates in anaerobic digestion, and they can be directly converted by methanogens (Feng *et al.* 2018). The concentrations of VFAs in the anaerobic digestion systems with and without pretreatment are mentioned in Fig. 5. The concentrations of VFAs in the ammoniated digestion systems were significantly lower than that of the control group during the entire anaerobic digestion, and the lowest concentration was 5.1 $mg \cdot L^{-1}$. The concentrations of VFAs were low from beginning to end because the residual ammoniation straws neutralized a part of VFAs in the digestion slurry, and the high concentrations of ammonia nitrogen in the slurry in the initial stages suppressed the formation of small-molecule VFAs by acid-producing bacteria (Elmashad *et al.* 2004). In the initial stages of anaerobic digestion of ammoniated corn stalks, the concentrations of VFAs decreased slightly and finally reached a stable level. A similar trend was observed in the change of VFAs concentrations in the un-ammoniated corn stalks. In the early stages of anaerobic digestion, along with the reaction, some organic macromolecules were gradually converted into VFAs, while other smaller organic molecules did so in the presence of hydrolytic acid producing bacteria (Gan *et al.* 2017). The methanogens did not completely adapt to the environment in the early stages, thus, the utilization rates of VFAs were lower than their rates of production rates acid-producing bacteria, leading to the increase in VFAs in the slurry. On day 15, the concentration of VFAs in the digestion slurry of the un-ammoniated ME group reached a maximum of 13.19 $g \cdot L^{-1}$. The biogas output of different un-ammoniated ensiling-treated corn stalks reached the maximum after

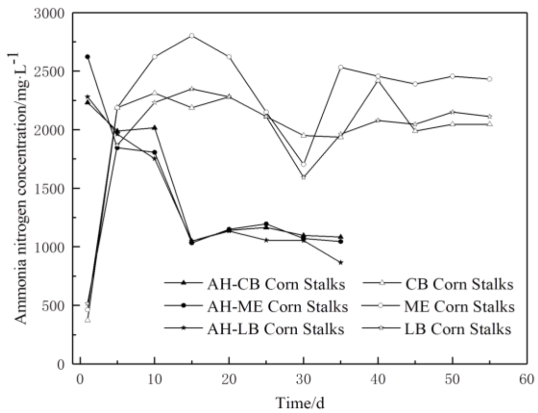

Fig. 6: Effect of ammoniation pretreatment on theammonia nitrogen concentrations in the anaerobic digestion of different ensiling-treated corn stalks

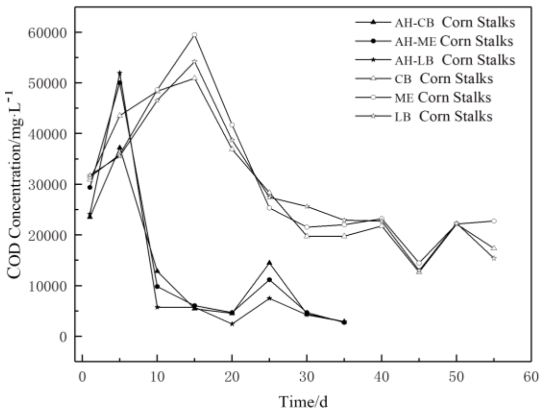

Fig. 7: Effect of ammoniation pretreatment on the COD concentrations in the anaerobic digestion of different ensiling-treated corn stalks

day 15. Throughout the reaction time, the VFAs concentrations decreased gradually and finally reached a stable level in the digestion slurry of different un-ammoniated ensiled corn stalks.

Effect of pretreatment with ammonia on the COD concentration (ammonia nitrogen) in the anaerobic digestion of corn stalks at moderate temperatures

Fig. 6 shows the ammonia nitrogen concentrations of ammoniated and un-ammoniated ensiled corn stalks. In the initial stages of the anaerobic digestion, the ammoniated corn stalks showed a very high concentration of ammonia nitrogen, which may correlate with the residual dilute ammoniain the straws after the ammoniation pretreatment. Compared with the control group, the ammoniation pretreatment reduced the biogas output of ensiled corn stalks to a certain extent. This may be because the VFAs concentrations were low in the early stages of the anaerobic digestion, and excess ammonia nitrogen consumes the hydrogen ions produced in the acidification processes, thus affecting the anaerobic digestion performance and reducing the methane output (Xu *et al.* 2012). Along with the reaction, the concentrations of ammonia nitrogen in the digestion slurry of ammoniated, ensiled corn stalks decreased gradually. With the release of nitrogen in straws, the concentrations of nitrogen in the digestion slurry was balanced dynamically with the amount of nitrogen required for the reproduction and metabolism of anaerobic microbial flora. Finally, the concentrations of ammonia nitrogen in the ammoniated groups reached a stable level of 700–1200 $mg{\cdot}L^{-1}$, and that of the digestion slurry of un-ammoniated groups increased gradually. On day 15, the concentration of ammonia nitrogen in the ME group digestion slurry reached a maximum of 2802 $mg{\cdot}L^{-1}$, and then decreased slightly. Eventually, the concentrations of ammonia nitrogen in the digestion slurry remained constant.

Fig. 7 shows the COD concentrations of ammoniated and un-ammoniated ensiling-treated corn stalks. The trends of variation in COD concentrations of the ammoniated and un-ammoniated three different ensiled corn stalks were similar, in accordance with a volcanic trend. In the early stages of the fermentation, the cellulose, hemicellulose, and other macromolecular organics in corn stalks were gradually decomposed into water-soluble, smaller organic molecules. The rates of hydrolysis of macromolecular organics were higher compared to their microbial consumption, which led to the gradual increase of COD concentrations in the digestion slurry. The reaction was accompanied by gradual enrichment and adaptation of methanogens to the environment, consuming a large amount of small water-soluble organic molecules. This led to decrease in concentrations of COD, in accordance with the fast increase of biogas output. The high COD concentrations in the initial stages of the fermentation of ammoniated corn stalks indicates that the ammoniated ensiled corn stalks can function as the feedstock for anaerobic digestion, and the maximum values were achieved in the first 5 days, proving that this feedstock is easy to decompose. Moreover, these values achieved their maximum at 60% earlier compared to the untreated materials, demonstrating that the pretreatment approach is appropriate for large-scale applications.

Effect of ammonia pretreatment on the methane concentration in the anaerobic digestion of corn stalks at moderate temperatures

The effect of ammoniation pretreatment on the methane concentration of ensiling-treated corn stalks is illustrated in Fig. 8. The substances in the fermentation flask had produced a large amount of H_2 and CO_2 in the first place, thus, the methane contents in the biogas were generally low at the initial stages of the anaerobic digestion. The start-up stages of different ammoniated ensiled corn stalks were obviously faster than those of the control group. The ammoniation pretreatment degraded the lignin on the

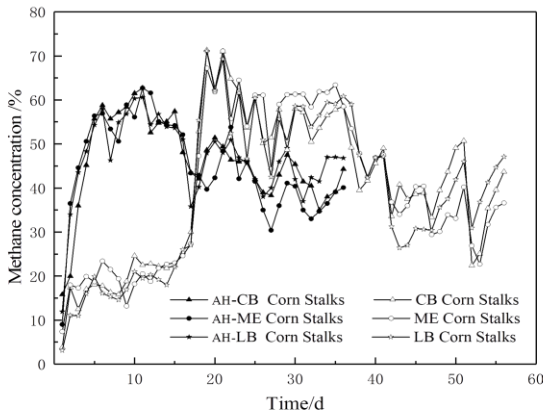

Fig. 8: Effect of ammoniation pretreatment on the methane concentrations in the anaerobic digestion of different ensiling-treated corn stalks

surface of corn stalks, and hence the hydrolytic acid producing bacteria could quickly contact the cellulose and hemicellulose inside the straws. Thus, the hydrolysis stages were no longer the rate-determining steps of the anaerobic digestion courses of corn stalks (Yuan *et al.* 2015). Along with the reaction, the number of methanogens increased gradually. These methanogens converted VFAs into methane, and led to increase in methane concentrations. The maximum methane concentrations of 62.71, 62.59 and 60.6%, respectively, occurred at day 5–8. In the final stages of the reaction, the contents of available organic matter in the digestion slurry decreased gradually, leading to decrease in methane concentrations to 45–50%. The start-up stages of un-ammoniated ensiled corn stalks were slow, and a delay was observed in the start-up stages. Over the reaction time, gradual hydrolysis of organic macromolecules such as cellulose occurred, and a steady increase was observed in the concentrations of VFAs in the digestion slurry, in the number of methanogens, and in the methane concentrations that reached the maximum values of 71.10, 71.08 and 67.2%, respectively, on day 19. On comparing, it was found that the ammoniation pretreatment accelerated the start-up stages of the anaerobic digestion and shortened the anaerobic digestion periods, but the maximum methane concentrations decreased by about 10%.

Discussion

In this experiment, the effect of ammoniation pretreatment on the methanogenic performance in anaerobic digestion of corn stalks with different ensiling methods was studied. The effect of ammoniation pretreatment was reflected by the cumulative gas production and other parameters change in anaerobic digestion of corn stalks with different ensiling methods before and after pretreatment. It can be seen from the trend of daily biogas production in Fig. 2. The ammoniation pretreatment can significantly destroy the fiber component of corn stalks, and promote the separation of cellulose and hemicellulose from lignin, which is beneficial to the utilization of easily fermentable substances by anaerobic fermentation microorganisms, and improve the start-up speed of anaerobic digestion of corn straw with different silage methods. Kim and Lee (2006) used ammonia soak to pretreat corn straw and found that at 60°C, w=15% ammonia water, the solid-liquid ratio of 1:6 soaked straw for 12 h, lignin can reach 62% removal rate, which is beneficial to improve the contact of anaerobic digestion microorganisms on corn stalks. It can be concluded from Fig. 3 that the ammoniation pretreatment can slightly reduce the cumulative yield of anaerobic fermentation biogas in different silage corn stalks to a certain extent, but it can significantly improve the anaerobic fermentation efficiency. During the 0–15 d, and the cumulative gas production of ME, CB and LB three kinds of silage corn stalks accounted for 78.48, 74.11 and 70.35% of the cumulative gas production, respectively. It can be seen from Fig. 4 and 5 that the pH undergone a process of first falling and then rising. When the pH is low, the microorganisms need more energy to maintain the neutral environment of their own cytoplasm, thus inhibiting the activity of microorganisms and reducing the biogas production of anaerobic digestion of corn stover with different silage methods. Ammoniation pretreatment significantly reduced the concentration of organic acids in the digestion broth, which may be the main reason for the reduction of biogas accumulation in anaerobic digestion of different silage corn stalks. Ammonia pretreatment can improve the stability of anaerobic digestion process, keep the pH between 7.2–7.5, which is beneficial to increase the activity of anaerobic microorganisms, increase the hydrolysis rate of anaerobic microorganisms and the rate of methanogenesis, and shorten the anaerobic Oxygen digestion cycle. It can be seen from the trend of COD in the anaerobic fermentation liquid of Fig. 7. The ammoniation pretreatment can break the ester bond between lignin and cellulose and hemicellulose, and it can damage the surface of siliceous cells and increase the release rate of organic matter in corn straw with silage methods.

At present, domestic and international methods for pretreatment of corn straw anaerobic fermentation can be divided into physical methods, chemical methods and biological methods. Ammonia pretreatment as an effective way to treat corn stover can destroy the fiber component of corn stover to some extent, and cause some functional groups to break, thus separating lignin from cellulose and hemicellulose, which is beneficial to improve the actual biogas engineering. It is beneficial to improve the utilization of digestion substrate by anaerobic digestion microorganisms in the actual biogas engineering, improve the efficiency of anaerobic fermentation, and thus improve economic benefits.

Conclusion

The anaerobic digestion start-up stages of these ensiled corn stalks were accelerated and their anaerobic digestion periods were significantly shortened. Compared to the control group, the anaerobic digestion periods were shortened by 15 days. The biogas output per day and cumulative biogas output of the ammoniated with 20 wt% CB group were the highest, was 11.02 $L{\cdot}d^{-1}$ and 167.95 L, respectively. The pH of these anaerobic digestion systems was stabilized at around 7.5. The ammoniation pretreatment significantly improved the stability of pH in the anaerobic digestion of ensiled corn stalks. The ammoniation pretreatment effectively improved the methane production of different ensiled corn stalks in the early stages of anaerobic digestion processes. Furthermore, the methane concentrations can rapidly increase to 50% in the early stages of anaerobic digestion.

Acknowledgments

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