Improving biomethane yield by strengthening acidification of maize stover in two-phase anaerobic digestion

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Abstract: In this study, the acidification and two-phase anaerobic digestion (AD) were conducted in batch and continuous stirred tank reactors, respectively, to determine the effect of acidification on methane production in AD. The results showed that two-phase AD achieved an observable enhancement in the methane production under optimal acidification conditions (organic loading rate of 60 g TS/L, the ratio of raw material to inoculum (based on dry weight) of 2:1, the temperature of 45 ℃, urea concentration of 4%, and time of 6 d). Under these conditions, the daily biogas and biomethane productions were 0.48 L/g TS and 0.30 L/g TS, respectively, which were 26.32% and 57.89% higher than those of the untreated group, respectively. The ammonia nitrogen (AN), alkalinity, and pH value of the methanogenic phase of C4 continued to increase up to 956 mg/L, 5680 mg/L, and 7.41, respectively, after 60 d, which might have destroyed the stability of the system. Therefore, for the purpose of reusing the nitrogen source, reducing AN, and maintaining the stability of the reaction system, another set of acidification and two-phase AD with water pretreatment using the discharge of the methanogenic phase of C4 as the inoculum was subsequently conducted. The results showed that the daily biogas productions of single-phase and two-phase AD were 5.26% and 15.79% higher than that of the untreated group, respectively; similarly, their daily methane yields were 10.42% and 21.05% higher than that of the untreated group.

Keywords: alkaline pretreatment, two-phase anaerobic digestion, strengthening acidification, maize stover, reactor, biogas, biomethane production

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1 Introduction

Maize crop is one of the highly produced cereals in China; averagely 2.2×10^8 t of maize stover are produced in 2016[1]. Developing practical approaches to reutilize maize stover (MS) in order to minimize the environmental problems associated with inappropriate waste management has become a big challenge facing to local farmers as well as governments[2]. Due to the increasing demand for energy and environmental pollution, China has put forward a Mid- and Long-Term Plan for Renewable Energy Development, which emphasized that renewable energy should account for more than 20% of total energy consumption by 2020[3,4]. Anaerobic digestion (AD) could generate sustainable bioenergy while reducing and stabilizing solid degradable organic wastes[5]; as a result, crop stover could represent a good substrate for renewable energy production by AD[6,7].

Most reports about the fermentation of MS were on single-phase AD. Two-phase AD separates acidification and methanation, thereby providing a suitable metabolic environment for both acidogenic and methanogenic bacteria[8], which could increase the system stability and improve the biogas yield[9-12]. Single-phase and two-phase AD of food waste were compared under mesophilic conditions, and significantly higher methane production occurred during two-phase mesophilic digestion compared with that in the single-phase operation (methane yield of 380 L CH_4/kg volatile solids (VS) *vs*. 446 L CH_4/kg VS)[13]. Similarly, the two-phase AD of unscreened dairy manure at a sludge retention time (SRT)/hydraulic retention time (HRT) of 10 d (2 d acidogenic and 8 d methanogenic) for AD resulted in 50% and 67% higher biogas production at organic loading rates (OLRs) of 5 g VS/(L d) and 6 g VS/(L d), respectively, relative to that of the single-phase configuration with an SRT/HRT of 20 d[14]. The biogas production data of two-phase fermentation showed a 37% higher methane yield compared with that of single-phase fermentation[15]. However, few studies have focused on acidification and two-phase AD of MS; thus, the reports on batch acidification combined with continuously stirred tank reactor (CSTR) methanation of two-phase AD are insufficient.

Volatile fatty acids (VFAs) are produced at the acidogenic phase; VFAs mainly contain acetic acid, propionic acid, and butyric acid, which are crucial intermediates of the AD process. Liu et al.[16] proved that the acetic acid can be directly used by methanogens; however, propionic and butyric acid need to be initially oxidized into acetate, hydrogen, and carbon dioxide before

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the action of methanogens. Most studies on acidogenic fermentation have reported that when the major products are butyric and acetic acid, the process is called butyric acid-type fermentation and is conducive to microbial digestion[17,18]. This shows that it is important to obtain the optimum concentration and components of VFAs for effective conversion during AD. Temperature, pH, C/N ratio, and HRT have been reported as the key factors for controlling the production of VFAs during the fermentation stage[19-22].

In this study, a novel method of two-phase AD, namely batch acidification combined with CSTR methanation, was adopted. Firstly, five-factor, four-level orthogonal experiment was conducted for strengthening acidification under 4% sodium hydroxide coupled with 2%-5% urea pretreatment on MS, and the optimal acidified condition was determined by biomethane production of two-phase AD. However, the ammonia nitrogen (AN), alkalinity, and pH increased constantly with an increase in the feeding time. Thus, afterward, another set of acidification and two-phase AD with water pretreatment using the discharge of the methanogenic phase as inoculum was conducted to reuse the nitrogen source, reduce AN, and maintain the stability of the reaction system. Therefore, this study primarily aims to intensify the acidification of MS for achieving the final purpose of improving the biomethane yield of two-phase AD.

2 Materials and methods

2.1 Substrate and inoculum

The MS and inoculum used in this study were collected from a farmland and an AD plant treating pig manure, respectively, in a rural area of Shunyi, Beijing, China. After collection, the MS was rolled out on a rooftop and dried by air, and then chopped with a knife and smashed into 20 mesh size by a pulverizer (SW-180, Yanshan Masanori Co., Beijing, China). The inoculum was preserved in a plastic bucket at 4℃ in a refrigerator. The characteristics of the MS and inoculum are listed in Table 1. Before AD, the powdered MS was pretreated with a solution prepared from 4% (relative to the dry weight of MS) sodium hydroxide combined with 2%-5% urea concentration and 6 times water (w/w) at 35℃ for 1 d.

Table 1 Characteristics of MS and inoculum

Characteristics	MS	Inoculum
Total Solids (TS)/%	91.75±0.23	8.51±0.07
Volatile Solids (VS)/%	83.45±0.15	4.52±0.05
VS/TS	0.91±0.01	0.53±0.01
Total Carbon (C)/%	43.21±0.54	29.2±1.01
Total Nitrogen (N)/%	1.23±0.08	2.6±0.21
C/N	35.13±0.29	11.23±0.36
Cellulose/%[b]	38.31±0.68	--
Hemicellulose/%[b]	20.05±0.42	--
Lignin /%[b]	3.37±0.11	--

Note: a. Fresh matter; b. Dry matter.

2.2 Experimental design

2.2.1 Acidification experiment of MS

The acidification experiment was conducted in a 1.0 L blue-cap bottle with a 0.5 L working volume. A five-factor, four-level orthogonal experiment was conducted herein. The specific orthogonal design is presented in Table 2. After acidification, the optimum condition was obtained and a confirmatory test was conducted to determine the optimal acidification conditions.

Table 2 Orthogonal experiment design

Test No.	Loading /g TS L^{-1}	R: I (dry weight)	Temperature /℃	Urea /%	Time /d
1	50	2:1	35	2	3
2	50	3:1	45	3	4
3	50	4:1	55	4	5
4	50	5:1	65	5	6
5	60	2:1	45	4	6
6	60	3:1	35	5	5
7	60	4:1	65	2	4
8	60	5:1	55	3	3
9	70	2:1	55	5	4
10	70	3:1	65	4	3
11	70	4:1	35	3	6
12	70	5:1	45	2	5
13	80	2:1	65	3	5
14	80	3:1	55	2	6
15	80	4:1	45	5	3
16	80	5:1	35	4	4

2.2.2 Two-phase anaerobic digestion

The acidification process was conducted in a batch reactor, and the next methanogenesis reaction occurred in six identically operated CSTRs under mesophilic conditions (35℃±1℃) with a once-per-day feeding regime. Every reactor had a 10 L total volume and 8 L effective working volume. The reactors were started at an OLR of 50 g TS/L and inoculum concentration of 20 g TS/L. After 30 d of start-up time, the OLRs of the six CSTRs were increased to 60 g TS/L and the feeding continued for an HRT of 40 d. Based on the optimal conditions from the orthogonal experiment, CSTR1 (C1) was fed with MS without pretreatment; CSTR2 (C2) was fed with MS pretreated with 4% sodium hydroxide; CSTR3 (C3) was fed with MS pretreated with 4% sodium hydroxide and 4% urea; CSTR4 (C4) was fed with the discharge of the acidified phase, whose acidification condition was optimal for VFAs and ethanol production from the orthogonal experiment; CSTR5 (C5) was fed with MS pretreated with 4% sodium hydroxide and 2% urea; and CSTR6 (C6) was fed with the discharge of the acidified phase, whose acidification condition was optimal for the acetic acid and acetic plus butyric acid concentration orthogonal experiment. The daily biogas and biomethane production of one-phase and two-phase AD were measured and compared with each other. To calculate the biogas production performance and system stability, parameters such as the total solids (TS), VS, VFAs, AN, and alkalinity of each reactor were measured every 5 d after feeding at the increased OLR of 60 g TS/L.

2.2.3 Utilization of the effluent of methanogenic phase

The characteristics of the discharge of the methane phase are listed in Table 3.

The pretreatment and feeding methods resulted in the constant increase in AN and alkalinity; therefore, utilization of the effluent of the methanogenic phase should be considered. In this study, the effluent of the methanogenic phase was used as inoculum for acidification and two-phase AD and the pretreatment solvent was adjusted from sodium hydroxide to water. The specific acidification experimental design is shown in Table 4.

Single-phase and two-phase AD were conducted under optimum acidification conditions. The feeding method, OLR, and HRT were the same as those in Section 2.2.2. S1 was fed with MS without pretreatment, S2 was fed with MS treated with water, and S3 was fed with discharge of the acidified phase.

Table 3 Characteristics of the effluent of methanogenic phase (%)

Characteristics	TS	VS	C	N	Cellulose	Hemicellulose	Lignin
Effluent of methanogenic phase	4.37±0.05	2.96±0.03	28.32±0.78	3.12±0.31	11.82±0.42	13.74±0.38	5.18±0.09

Table 4 Acidification experimental design

Levels	Factors			
	OLR /g TS·L^{-1}	R:I (TS:TS)	Temperature /℃	Time /d
1	60	6:1	35	5
2			45	6
3			55	7

2.3 Analytical methods

TS, VS and pH were determined according to the standard methods (APHA, 1998). The elemental composition, such as C, N, H, and S of the MS and inoculum were analyzed by a Vario EL/microcube elemental analyzer (Elementar, Germany). Biogas production of batch acidification was measured and the CSTRs were measured using 1 L gas collecting bottles and wet gas flowmeter (LMF-1, Changchun Automobile Filter, China), respectively at room temperature. The H_2, N_2, CH_4 and CO_2 content in the biogas were analyzed by a gas chromatograph (SP2100A, Beifen-Ruili) equipped with a thermal conductivity detector (TCD). The concentration of VFAs, mainly acetic acid, propionic acid, butyric acid and ethanol were determined by a gas chromatograph (SHIMADZU, GC2014) equipped with a flame ionization detector (FID). The AN concentration was measured by an auto analyzer (KT260, Foss, China). The lignin, cellulose and hemicellulose contents of MS were determined by a fiber analyzer (A2000I, ANKOM, USA).

3 Results and discussion

3.1 VFAs analysis in batch acidification phase

3.1.1 Variations of VFAs and pH in acidification phase

In this study, the VFAs production (from every g TS of MS), acetic acid concentration, and acetic plus butyric acid concentration were chosen to measure the effect of acidification. During the process of acidification, the decrease in pH was clearly associated with the accumulation of VFAs. As shown in Figure 1, the pH of all groups were around 7.3 before acidification, and were all reduced after acidification, except for experiment No.10. On the other side, the components of VFAs were mainly acetic acid, propionic acid, and butyric acid. At this stage, the acidification types of all groups were mostly acetic acid-type. In experiment No.5, the maximum VFAs production was 1387.62 mg/L and a relatively high acetic acid content (76%) was achieved. In addition, experiment No.4 and No.7 produced the highest acetic acid content (89%), and condition No.7 produced the highest acetic plus butyric acid content (94%).

3.1.2 Range analysis

After range analysis conducted by SPSS21 software, the results indicated that the optimal levels of each factor for VFAs production were 50 g TS/L, 4:1, 45 ℃, 4%, and 6 d; however, the optimal levels of each factor for the acetic acid content were 50 g TS/L, 4:1, 65 ℃, 5%, and 3 d. In addition, the optimal levels of each factor for the acetic plus butyric acid content were 80 g TS/L, 5:1, 55 ℃, 4%, and 4 d. These optimal conditions were proved to be different from the results in the orthogonal test; thus, a verification test must be considered to determine the optimal conditions.

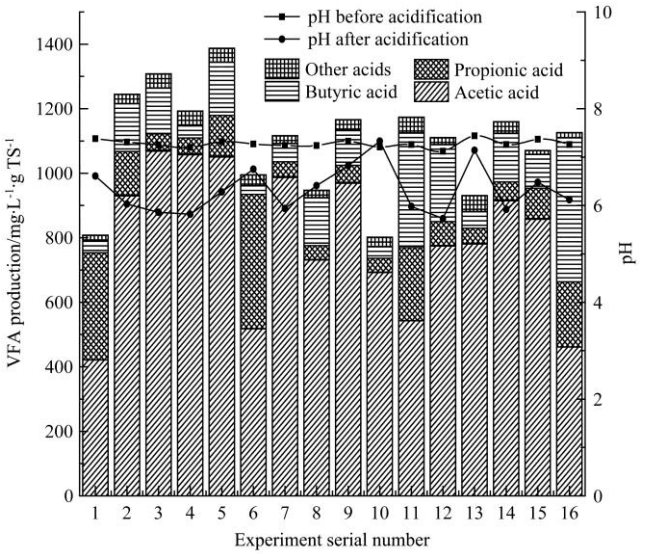

Figure 1 Production and proportion of VFAs components

3.1.3 Results of validation test

Validation experiments were conducted to confirm optimum VFA production conditions. The results of the validation test are presented in Table 5. The optimum group of each index obtained by the range analysis was compared with the optimal set in the orthogonal table. The results showed that the maximum VFAs production (g TS of MS) was obtained in experiment No.5, while the highest acetic acid content and acetic plus butyric acid content were obtained in experiment No.7. The optimal conditions corresponding to all the analytical indicators are shown in the orthogonal table. The two optimal conditions of the orthogonal acidification experiment and validation test were used for single-phase and two-phase AD to determine the optimal conditions for producing biomethane.

3.2 Single phase and two-phase AD

3.2.1 Biomethane production performance

As shown in Figure 2, the daily biogas and biomethane production were stabilized after 20 d of feeding. The daily biogas productions of C1-C6 were 0.38, 0.44, 0.45, 0.48, 0.45, and 0.44 L/g TS, respectively, and those of C2-C6 were 15.79%-26.32% higher than that of the untreated group (C1). There was no significant difference between the daily biogas production of C2, C3, C5, and C6, and that of C4 increased the most. At this point, the advantages of two-phase AD are not fully represented. The daily biomethane productions of C1-C6 were 0.19, 0.22, 0.23, 0.30, 0.24, and 0.26 L/g TS, and those of C2-C6 were 15.79%-57.89% higher than that of the untreated group (C1). Among them, the improvement rates of single-phase AD groups (C2, C3, and C5) were 15.79%-26.32%, and those of the two-phase AD groups (C4 and C6, respectively) were 57.89% and 36.84% compared with that of C1.

Based on the daily biomethane production, two-phase AD greatly improved the biomethane production performance, which was mainly attributed to the separation of acidogenic and methanogenic phases. In two-phase AD, methanogenic bacteria can directly use the VFAs produced in the acidogenic phase to produce biomethane. More specifically, for C4, the biogas methane content reached 63%, which was 14% and 12% higher than that of the untreated group (C1) and single-phase group (C3),

respectively. In addition, the comparison of the biogas yield of C4 and C6 indicated that among the three analytical indexes selected to measure the effect of acidification in orthogonal experiments, VFAs production was more valuable. This might have been because the contents of propionic and valeric acids produced in the acidification process were difficult for methanogens to utilize and did not affect the utilization of VFAs by methanogens. Therefore, in the butyric acid-type fermentation, the acetic and butyric acid contents did not directly determine the performance of the subsequent methane production, and instead the total yields of VFAs and ethanol had a significant influence on methane production in the methanogenic phase.

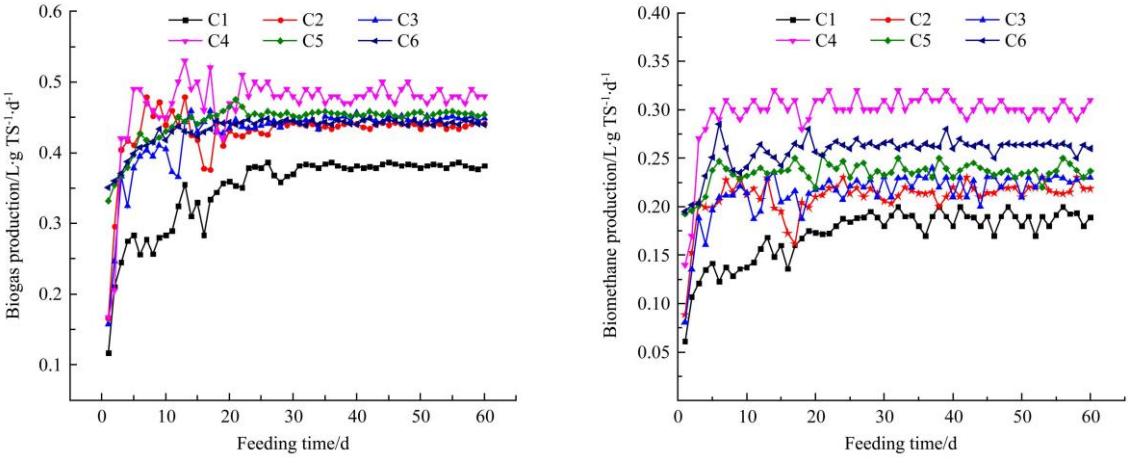

Figure 2 Daily biogas and methane productions of unit TS of C1-C6

3.2.2 Substance transformation

The changes in TS and VS of six CSTRs are shown in Figure 3. The TS and VS of all the reactors gradually increased with the increase in feeding time, especially in C4 and C6. After 60 d of digestion, the contents of TS in C4 and C6 were 4.48% and 4.47%, respectively, and the amount of VS in both reached 2.40%. This was because the feeds of C4 and C6 were both the discharge of the acidification phase, which contained a large amount of inoculum. Without many components for AD, the inoculum will accumulate in C4 and C6, and thus cause a constant increase in the contents of TS and VS.

3.2.3 System stability

The changes in AN, pH, and alkalinity among the six reactors are shown in Figure 4. Owing to the presence of urea in the pretreatment process, the AN concentration in C3-C6 continuously increased. After 60 d of AD, the AN concentrations of C1-C6 were 158, 215, 802, 956, 663, and 886 mg/L, respectively, which were lower than the inhibition limit reported in previous works[23]. Furthermore, the pH and alkalinity of C2-C6 were increased continuously, which were attributed to the alkaline pretreatment. As shown in Figure 4, at the end of the HRT, the pH of C1-C6 were 7.16, 7.42, 7.38, 7.41, 7.43, and 7.45, respectively, and the alkalinity values of C1-C6 were 1360, 4035, 4310, 5680, 4545, and 5015 mg/L, respectively, which were not in the range of inhibition[24].

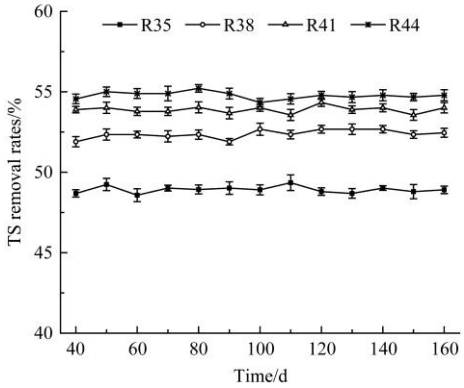

Figure 3 Changes in the discharge of TS and VS of C1-C6

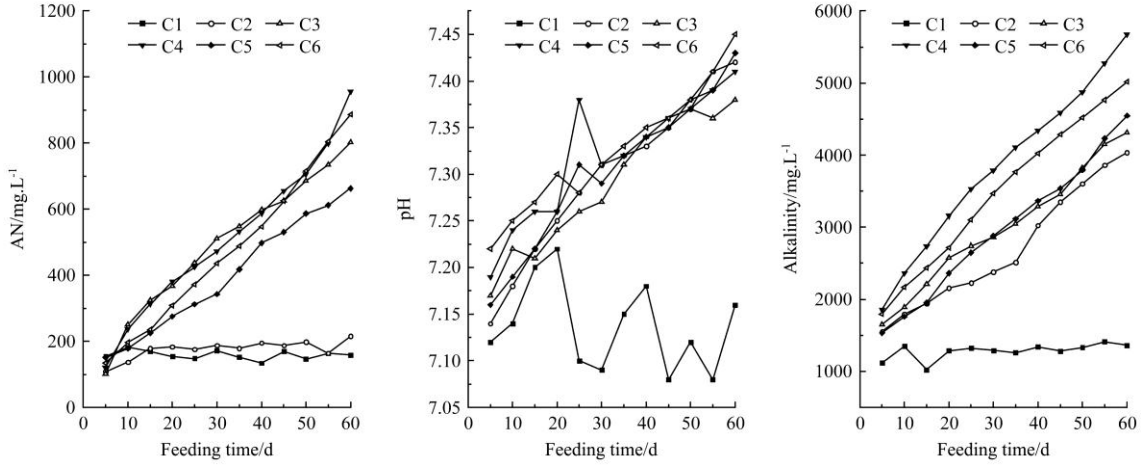

Figure 4 Changes of AN, pH and alkalinity of C1-C6

3.3 Treating discharge of methane phase as inoculum

3.3.1 VFAs and ethanol production

The results of the acidification of MS when treating the discharge of the methanogenic phase in two-phase AD as inoculum are shown in Figure 5. The VFAs and ethanol yields increased gradually with increasing acidification time under the same

reaction temperature. In general, the optimal acidification effect was obtained on day 7 under 55 ℃, where the total sum of the VFAs and ethanol yield was 9380 mg/L; however, at 35 ℃, it was 8944 mg/L. Thus, there was no clear difference between 55 ℃ and 35 ℃; therefore, the energy consumption on day 7 at 35 ℃ was taken as the optimal acidification condition and was applied to single-phase and two-phase AD.

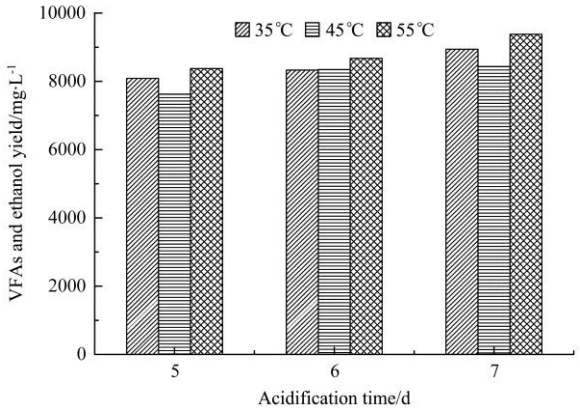

Figure 5 VFAs and ethanol concentration of acidification experiment

3.3.2 Biogas and biomethane production

The daily biogas and biomethane yields of water-pretreated MS were higher than those of the untreated group under both single-phase and two-phase AD (Figure 6). More specifically, the daily biogas productions of single-phase and two-phase AD were 5.26% and 15.79% higher than that of the untreated group; similarly, the daily biomethane yields were 10.42% and 21.05% higher than that of the untreated group, respectively. The methane content of the methanogenic phase in the two-phase AD of water pretreatment reached 55%, which was 5%-6% higher than that of the unpretreated group. These data further reflected the advantages of batch acidification combined with CSTR methanation.

3.3.3 System stability

The changes in AN, alkalinity, and pH of the three reactors with feeding time are shown in Figure 7. The AD of S1, S2, and S3 were conducted in C1, C2, and C3, respectively, after chapter 3.2, so the initial values of AN, alkalinity, and pH in S2 and S3 were relatively high. As shown in Figure 7, with the increase in feeding time, the AN, alkalinity, and pH of S1 remained stable and those of S2 and S3 showed a gradual decrease. In addition, the R:I of the acidogenic phase of S3 was 6, which showed that the amount of total nitrogen in the methanogenic phase decreased, which eventually led to a gradual decrease in the AN concentration. After 60 d, the AN, alkalinity, and pH of S2 were 286 mg/L, 3230 mg/L, and 7.42, respectively, and those of S3 were 738 mg/L, 3280 mg/L, and 7.35, respectively. Therefore, using the discharge of the methanogenic phase as inoculum for acidification and two-phase AD can effectively achieve the purpose of reusing the nitrogen source, reducing AN, maintaining the stability of the reaction system, and protecting the environment. At the same time, water pretreatment is environmentally friendly, and there would be no effect on increasing AN and alkalinity of the system.

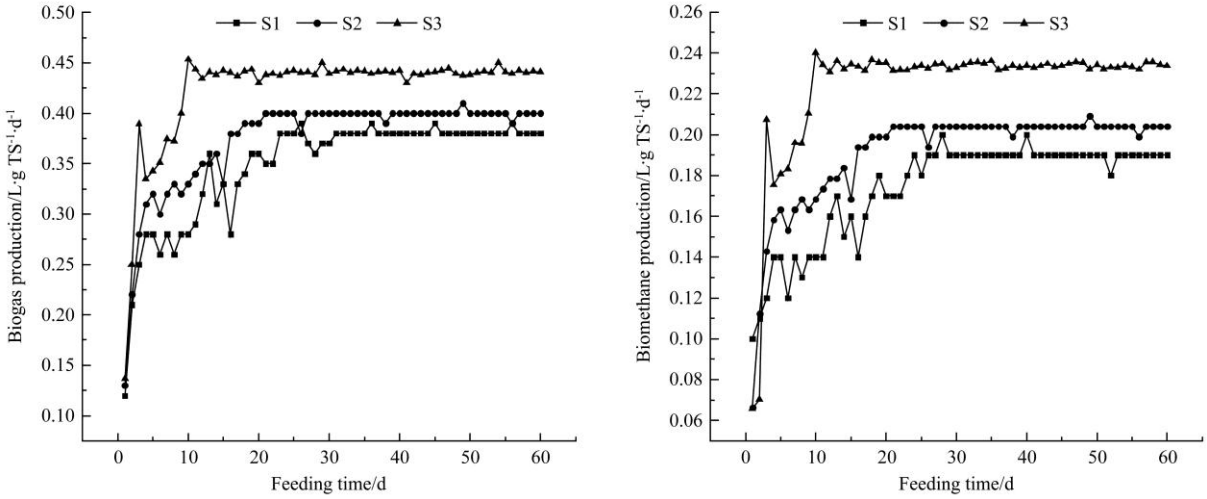

Figure 6 Daily biogas and methane production under water pretreatment

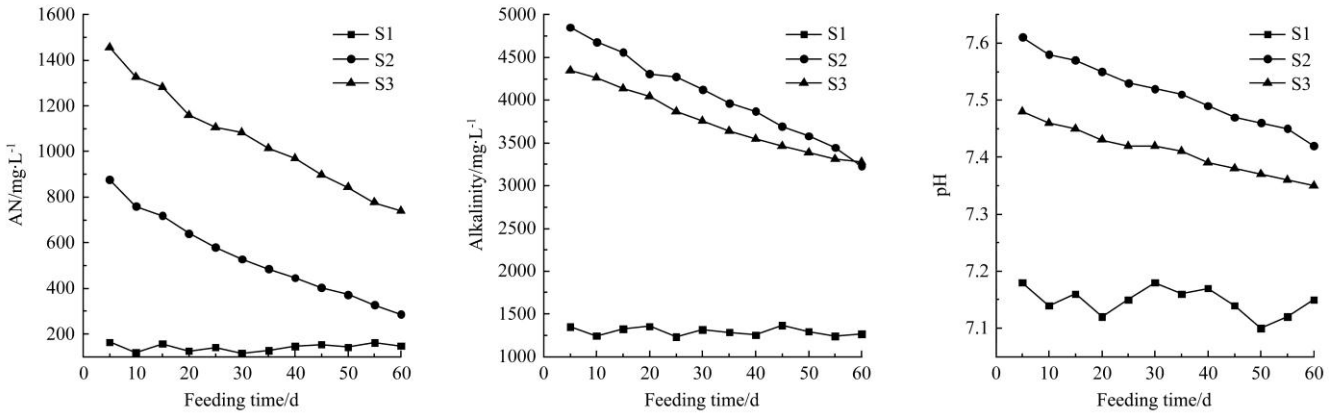

Figure 7 Changes in AN, alkalinity and pH of S1, S2, S3

4 Conclusions

Biogas and biomethane productions indicated that enhancing acidification could significantly improve the methane production performance of maize stover in two-phase anaerobic digestion and the methane content in the methanogenic phase. This could be attributed to the separation of acidification and methanation, which provided a suitable metabolic environment for both acidogenic and methanogenic bacteria. In addition, using the effluent of the methanogenic phase as inoculum for acidification and two-phase anaerobic digestion under water pretreatment also enhanced the biomethane yield and methane content to achieve the purpose of reusing the nitrogen source, reducing ammonia nitrogen, maintaining the stability of the reaction system, and protecting the environment.

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