Biogas production from microbial-alkali pretreated corn stover by solid-state anaerobic digestion

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Abstract: Corn stover pretreated by *Ceriporiopsis subvermispora* was used as substrate to enhance biogas production via solid state anaerobic digestion (SS-AD). Effects of temperature, inoculum volume and alkaline concentration on the biogas production profermance were investigated. The results showed that pretreatment of corn stover by *C. subvermispora* benefited the biogas production. The optimal conditions were temperature 35°C, inoculum 10:1 (w/w) and adding 5.0% of alkali, under which the maximum productions were obtained for daily and cumulative biogas of 14.07 L/kg VS and 211.09 L/kg VS. During the SS-AD process, the total solids (TS) and volatile solid (VS) were significantly degraded. The volatile fatty acids (VFA) concentration increased to 106 mmol/L and alkalinity decreased by about 23%. The results collectively suggested that microbial-alkali pretreatment of corn stove combining mesophilic condition could be a promising way to produce biogas in SS-AD.

Keywords: solid-state anaerobic digestion, corn stover, *Ceriporiopsis subvermispora*, microbial-alkali pretreatment, biogas production

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1 Introduction

In China, fossil fuels currently are still the main energy sources[1]. With increased concerns on energy security, climate change and environmental pollution, development of renewable energies attracts the increasing interests[2]. Lignocellulosic biomass, such as agricultural residue and forest waste, is abundant and widely available as renewable resources. The estimated annual yield of these biomass materials is more than 0.7 billion tons in China[2]. Corn stover is a renewable, cheap and widely available resource which usually used as animal feeds, fuels for cooking, and raw materials for papermaking or production of biodiesel, bioethanol, biogas, biohydrogen, and other chemicals[3]. Nowadays, open burning of corn stover is still increasing due to the Chinese peasants living patterns changes, which releases the pollutants and increases the PM_{10} and $PM_{2.5}$ values (Emissions from field burning of agricultural wastes, NFR 4F)[4].

Anaerobic digestion (AD) is the engineered methanogenic decomposition of organic matter under oxygen-free conditions and involves a mixed consortium of different species of anaerobic microorganisms that transform organic matter into biogas[5,6]. The anaerobic digestion process is runned at the liquid or solid manner. Liquid AD is usually operated with a total solid

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concentration of 0.5% to 13%, while solid state AD (solid-state anaerobic digestion, SS-AD) generally contains 15%-60% total solids (TS), which is suitable for treatment of residues like manure, plant residues and other organic wastes. Compared to Liquid AD, SS-AD requires less capacity of the reactor, less energy for heating, no processing energy for stirring and reduced material transportation costs[7-9]. Currently, increasing studies have been investigated to improve the biogasification by using SS-AD[10,11].

Lignocellulosic biomass is an ideal carbon substrate for SS-AD[12,13]. However, the complex three-dimensional structures including cellulose, hemicellulose and lignin create their recalcitrance to the cellulolytic enzymes, which inhibits the hydrolysis process of anaerobic digestion[14]. Fungi have the distinct deconstruction characteristics on lignocellulosic biomass. However, most white rot fungi, such as *Phanerochaete chrysosporium*, simultaneously degrade holocellulose (cellulose and hemicellulose) and lignin, resulting in a low cellulose recovery[15]. Some species preferentially degrade lignin and part of the hemicelluloses, leaving a cellulose-rich residue[15,16]. *Ceriporiopsis subvermispora* has high lignin-degrading selectivity, especially for removal of aromatics like ester-linked phenolic acids[17]. In our previous study, the parameters for pretreating corn stover by *Ceriporiopsis subvermispora* had been previously optimized to increase the cellulose release with the lignin degradation ratio of 34.82%, cellulose degradation ratio of 10.03% and selectivity coefficient of 3.47 under the optimized conditions[18].

Hence, the present study will aim to (1) compare the biogas production performance with *C. subvermispora* pretreated or unpretreated corn stover as the substrate; (2) optimize the SS-AD process parameters, such as temperature, inoculum amount and alkali concentration for maximizing the biogas production.

2 Materials and methods

2.1 Materials

Corn stover was collected from the Xuzhou region of Jiangsu province during the harvest season. It was air-dried to the moisture content of less than 10%, ground to pass through a 5 mm screen and stored at room temperature (22°C) prior to use. All other chemicals were of reagent grade.

Sludge effluent from mesophilic liquid anaerobic digester fed with biological waste (operated by Yixing-Union Biochemical Co., Ltd. Wuxi, Jiangsu Province, China) was used as the inoculum for the SS-AD. The effluent was dewatered by centrifugation to increase its TS content. The effluents were incubated at (35±1)°C and (50±1)°C for two weeks for being acclimated to mesophilic and thermophilic conditions before inculcation into SS-AD reactors, respectively.

2.2 White rot fungus pretreatment of corn stover

The white rot fungus *C. subvermispora* (ATCC 90466) was obtained from American Type Culture Collection (Manassas, VA, USA) and maintained on 3% (w/v) malt-extract agar (MEA) plates at 4°C. For pretreatment, 20 g of corn stover (dry basis) were autoclaved in 250 mL Erlenmeyer flasks at 121°C for 15 min, and then inoculated with *C. subvermispora* culture at 28°C for 18 d.

2.3 SS-AD system

The solid state anaerobic digestion system used the sludge effluent as inoculum. The untreated and/or microbial-treated corn stover was mixed with the acclimated sludge at the varying C/N ratio and TS content for optimizing the ratios of the biogas production. The inoculated corn stover was placed into 2 L reactors and digested at 35°C for 15 d.

2.4 Concurrent NaOH pretreatment and anaerobic digestion

Two hundred grams of untreated and/or microbial-treated corn stover (dry basis) were mixed evenly with 200 mL NaOH solution and 2 000 g sludge. The mixture was immediately fed into the SS-AD reactor for digestion tests. The digestion conditions and sampling procedures were the same as those described above.

2.5 Analytical methods

2.5.1 Determination of cellulose, hemicellulose and Klason lignin contents

Dried corn stover was weighted to determine weight losses. Compositions of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ash in corn stover were determined according to procedures of van Soest et al[19]. The amount of hemicellulose, cellulose and Klason lignin in corn stover were calculated as the difference between ADF and NDF, ADF and ADL, ADL and ash, respectively.

2.5.2 Biogas production analysis

Biogas production was recorded every 2 d by the method of water displacement, and the total biogas volume calculated after anaerobic digestion.

2.5.3 TS, VS, pH and alkalinity analysis

TS, VS, pH, alkalinity and volatile fatty acids (VFA) were analyzed at the end of the digestion tests. TS, VS, pH and alkalinity were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The samples for pH measurement were prepared by suspending 5 g wet digestate into 50 mL distilled water. The samples for VFA measurement were prepared by suspending 10 g wet digestate in 30 mL distilled water followed by centrifugation (8000 r/min, 5 min). One fraction of supernatant was determined by using the spectrophotometric methods described by the Standing Committee of Analysts (1979) and Siedlecka, et al.[20]. The calculation equation was as follows:

$$VFA\ (\text{mg/L}) = \frac{C \times V_1}{V} \times 10^3$$

where, C represents the VFA concentration on the standard curves; V represents the sample volume, mL; V_1 represents the dilution ratio.

2.6 Statistical analysis

Each experiment was repeated three times using duplicate samples. The results were expressed as means ± standard deviations. Statistical comparisons were made by one-way analysis of variance (ANOVA), followed by Duncan's multiple-comparison test. Differences were considered significant when the p-values were <0.05.

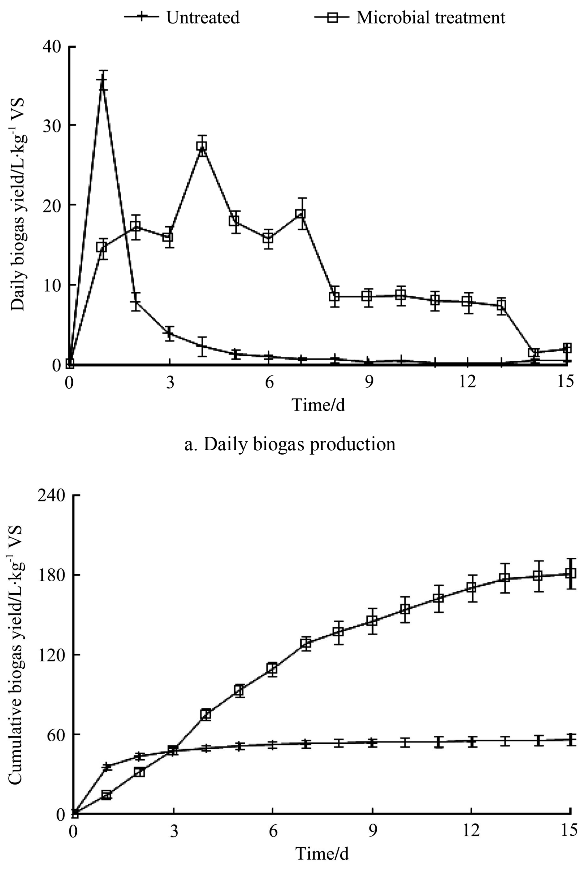
3 Results and discussion

3.1 Comparison of biogas production with *C. subvermispora* pretreated or unpretreated corn stover as the substrate

The corn stover pretreated by *C. subvermispor* was mixed with the effluent and thee characteristics are shown in Table 1. As shown in Figure 1, corn stover pretreated by *C. subvermispor* contributed to the higher average daily biogas production of 11.42 L/kg VS and the cumulative biogas production of 181.09 L/kg VS, which indicated that *C. subvermispora* pretreatment might expose the degradable cellulose in corn stover for further biogas metablism.

Table 1 Characterization of corn stover pretreated by *C. subvermispora* and inoculum

Parameter	*C. subvermispor* pretreated corn stover	Inoculum
TS/%	95.01±7.54	85.00±1.45
VS/%	88.60±5.32	15.08±3.16
pH	--	7.66±1.61
VFA/mmol·L^{-1}	--	30.85±2.72
Alkalinity/mmol·L^{-1}	--	45.77±4.83
Lignin/%	9.52±0.30	--
Cellulose/%	55.73±3.71	--
Hemicellulose/%	22.80±0.92	--
Protein/%	2.48±0.31	10.97±1.62
Lipid/%	0.00	3.82±0.53
Ash/%	4.55±0.71	42.82±3.59



a. Daily biogas production

b. Cumulative biogas production

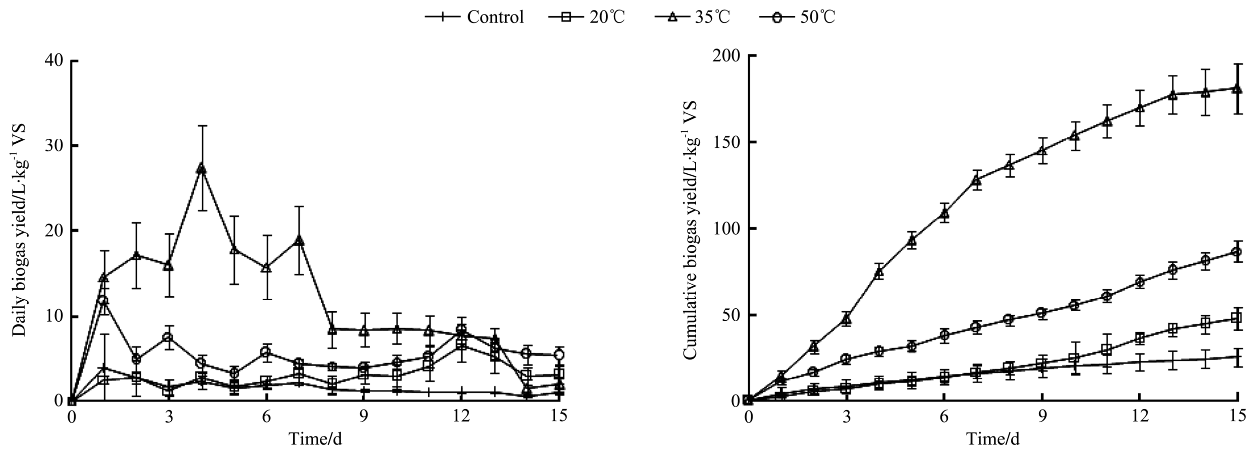
Figure 1 Effects of microbial pretreatment on biogas production by solid state anaerobic digestion at 35°C

3.2 Effect of temperature on the solid-state anaerobic digestion

Temperature is one of the key parameters affecting the anaerobic digestion process[6,21]. Anaerobic digestion can be carried out under ambient (<25°C), mesophilic (25-45°C) and thermophilic (>45°C) conditions[22]. Generally, the thermophilic conditions lead to higher metabolic rates and higher death rates of bacteria compared to those under the mesophilic conditions[23,24]. To evaluate the temperature on the anaerobic digestion of *C. subvermispora* pretreated corn stover, three temperatures of ambient (20°C), mesophilic (35°C) and thermophilic (50°C) conditions were used for assessing the differences of the digestion process.

As shown in Figure 2, the control group (untreated corn stover mixed with the inoculum) reached to the maximum daily biogas production of 4.07 L/kg VS at the beginning of the first 24 h, and then kept to decrease with the increase of AD process. When the *C. subvermispora* pretreated corn stover mixed with inoculum was cultured at 20°C, the average daily biogas production was 3 L/kg VS. Thermophilic (50°C) condition seemed to be benefit for the biogas production with 11.85 L/kg VS, 7.67 L/kg VS, 5.82 L/kg VS and 8.46 L/kg VS at the 1st, the 3rd, the 6th and the 12th day. Mesophilic (35°C) temperature was optimal for average daily biogas production with the highest level of 27.51 L/kg VS at the 4th day.

Figure 2b depicted that the mesophilic (35°C) condition gave the highest cumulative biogas production of 181.52 L/kg VS, followed by 87.03 L/kg VS at the temperature of 50°C. Lower temperature (20°C) possibly was unfavorable for biogas production with the approximately 29.48% of cumulative biogas yield at 50°C. These results proved that enzymes and microorganisms at the appropriate temperature could reach their highest activities in the digestion process and favor for the following degradation of organic matter and production of biogas[25].



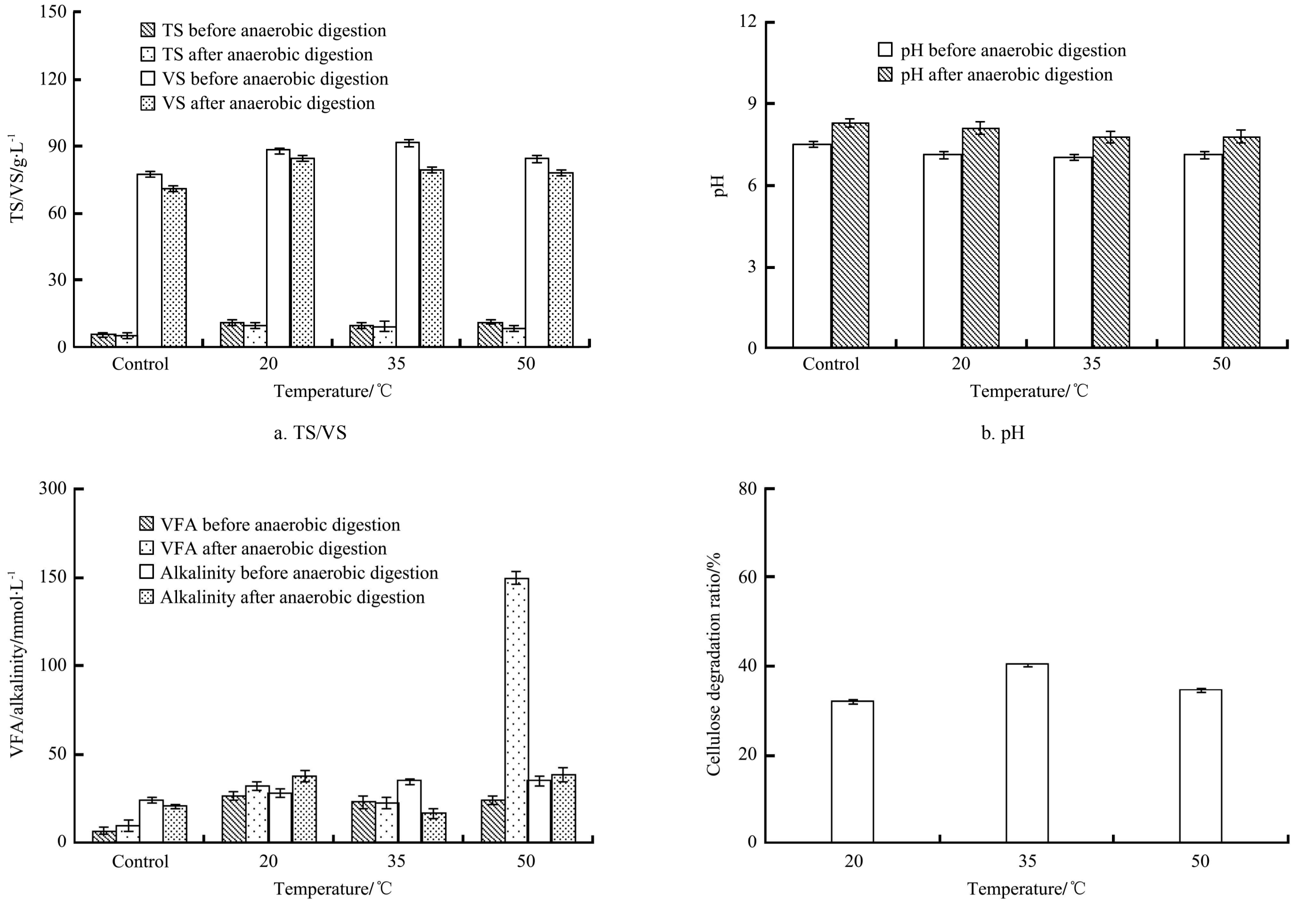
a. Daily biogas yield

b. Cumulative biogas yield

Figure 2 Effects of temperature on biogas yield of anaerobic digestion

Figure 3a presented the TS and VS changes at three conditions after 15 d SS-AD. The mesophilic (35°C) condition consumed highest VS by decrease of 13%, which meant that the utilization ratio of substrate was highest to produce biogas. Anaerobic digestion is generally operated under neutral pH conditions (pH 6.5-7.6). The observed toxicity under low pH conditions is associated with the presence of un-dissociated VFA[26]. The methanogenic activity will slow considerably at a pH value less than 6.3 and higher than 7.8 and this will inhibit biogas production[27,28]. As shown in Figure 3b, increase of pH values could be observed in these four experimental groups, which would guarantee the biogas production smoothly.

Volatile fatty acids (VFA) are weak acids that are released as intermediates during the breakdown of organic matter in anaerobic digestion of sludge[29]. The presence of high VFA has been reported to have damaging effects on methanogenic activity. From Figure 3c, the VFAs were higher in 20°C, 50°C and control group than that in 35°C. Figure 3d showed that the ratios of cellulose reduction during SS-AD process under three temperature conditions. The highest cellulose reduction of 40.36% was observed at 35°C condition which is in agreement with the highest biogas production at this condition. The lowest cellulose reduction of 32.09% was observed with 20°C cultivation. This indicated that the biogas production was possibly closely relevant to the cellulose degradation.



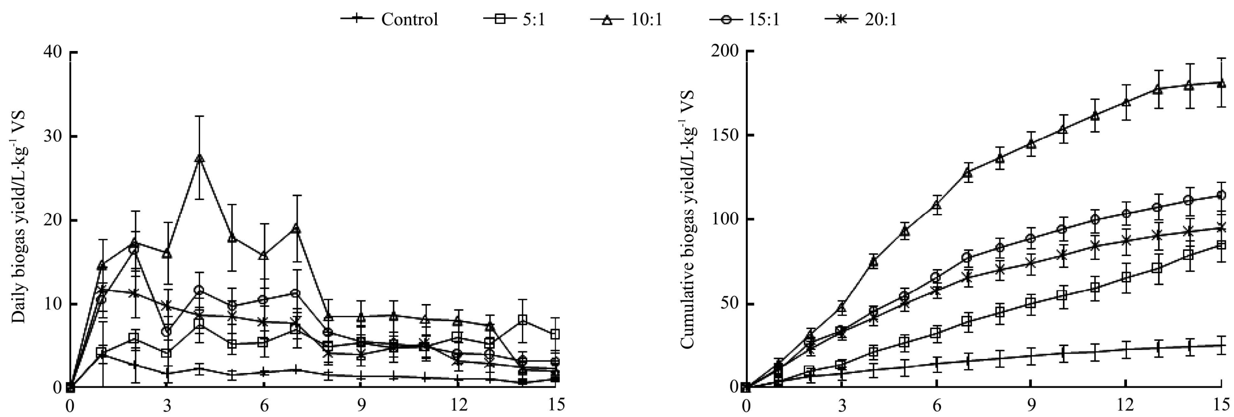
a. TS/VS

b. pH

c. VFA/alkalinity

d. Cellulose reduction

Figure 3 Effects of temperature on TS/VS, pH, VFA/alkalinity and cellulose reduction before and after solid state anaerobic digestion



a. Daily biogas yield

b. Cumulative biogas yield

Figure 4 Effects of inoculation on biogas yield during anaerobic digestion

3.3 Effect of inoculum volume on the solid-state anaerobic digestion

Various amounts of effluent (inoculum) were mixed evenly with *C. subvermispora* pretreated corn stover (dry basis) to the ratio of 5:1, 10:1, 15:1 and 20:1 (w/w). As shown in Figure 4a, the change of inoculum load led to the significant daily biogas production ($P<0.05$). When the ratio of inoculum to substrate was 5:1, daily biogas production was at the stable level of 5 L/kg VS, and no peaks were observed. Ratio of 10:1 reached to three peak values and highest level of 27.51 L/kg VS appeared on the 4th day. When ratio increased to 15:1, maximum daily biogas production appeared to be 16.40 L/kg VS on 2nd day, and other two small peaks of 11.64 L/kg VS and 11.37 L/kg VS were observed on the 4th and the 7th day, respectively. When inoculum volume was 20:1, the highest daily biogas production appeared to be 11.85 L/kg VS on the 2nd day. As shown in Figure 4b, the inoculum load affected the cumulative biogas production significantly (P<0.05). The maximum cumulative biogas production was 182.04 L/kg VS at the ratio of 10:1, and the minimum level was 85.39 L/kg VS at the ratio of 5:1.

Sludge effluent typically contains high amounts of microbial communities, ammonium, phosphate, suspended solids and dissolved solids. From Table 1, the effluent used in the present study contained the higher protein of (10.97±1.62)% and lipid of (3.82±0.53)%. The increase of inoculum load provided more nutrients including protein/lipid and microorganism populations, and accelerated the SS-AD process and biogas production. Figure 5a presented the TS and VS changes in four experimental groups after 15 d SS-AD. VS reduced as effluent loading increased from 5:1 to 20:1. 10:1 of effluent to substrate caused the highest VS reduction (13%) during SS-AD. The TS reduction followed a similar pattern as that of the VS. The higher utilization of VS in the substrate would lead to the higher biogas production. The pH values of four groups increased at a certain extent after SS-AD Figure 5b. The ratio of 10:1 and 20:1, slight pH changes could be observed which was favorable for biogas production.

Variations of VFAs and alkalinity during anaerobic digestion were presented in Figure 5c. There was no significant accumulation of VFA probably because of the sufficient alkalinity in the inoculum which could rapidly balance the produced total short chain VFAs. Cellulose reduction of *C. subvermispora* pretreated corn stover could be found in Figure 5d. The highest cellulose reduction of 40.36% was observed with the ratio of 10:1, which is in agreement with the highest biogas production under this condition.

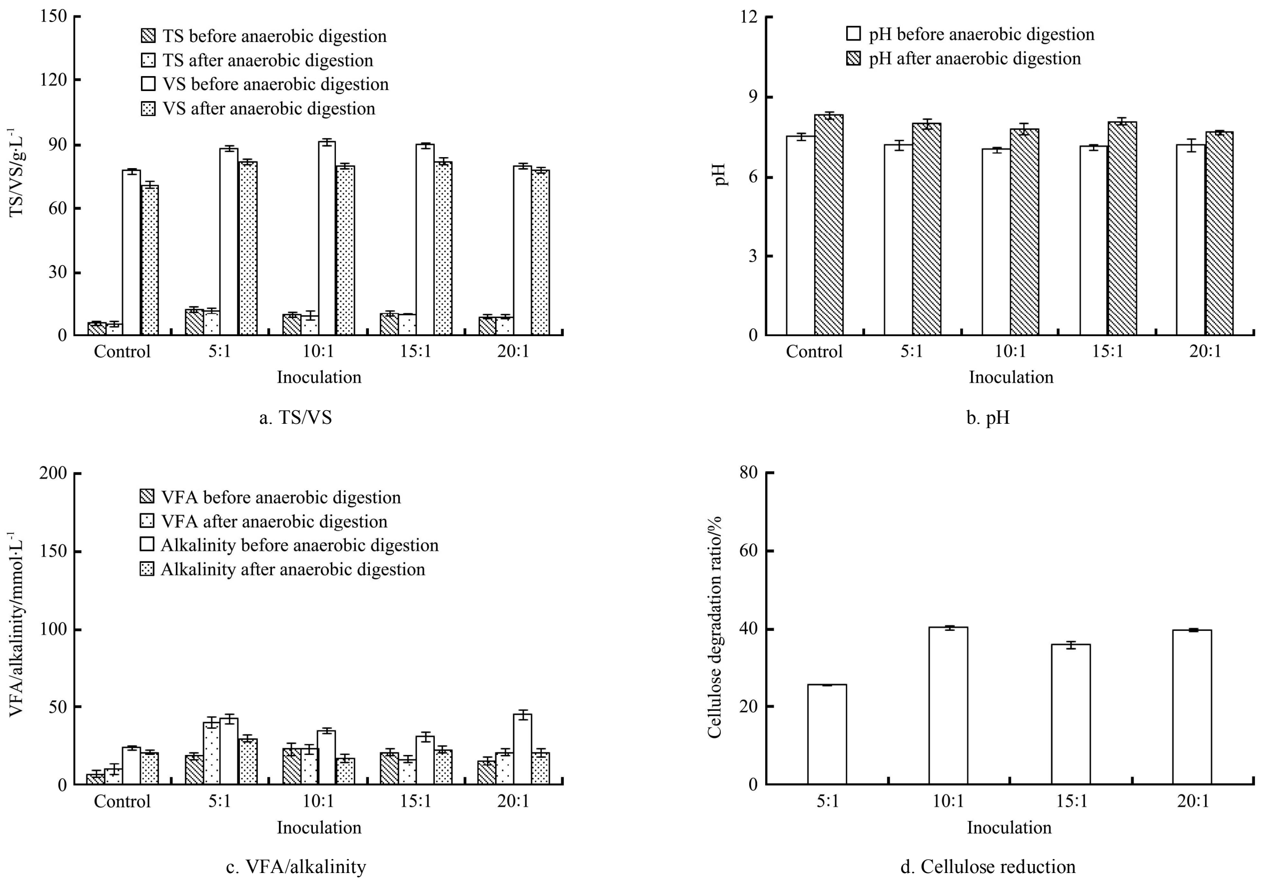
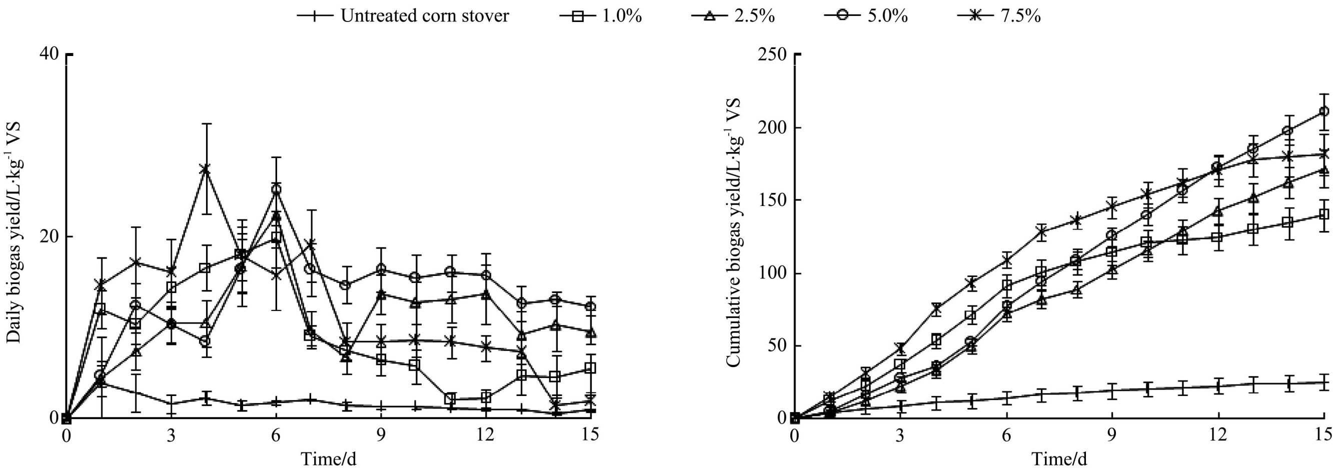


Figure 5 Effects of inoculation on TS/VS, pH, VFA/alkalinity and cellulose reduction before and after solid state anaerobic digestion

3.4 Effect of alkaline concentration on the solid-state anaerobic digestion

In the present study, NaOH concentrations of 1.0%, 2.5%, 5.0% and 7.5% was used to investigate the effect on solid-state anaerobic digestion. From Figure 6a, the daily biogas productions reached to 13, 15 and 28 L/kg VS with the NaOH additions of 2.5%, 5.0% and 7.5%, respectively. After 7 d AD process, the daily biogas production decreased to 7-8 L/kg VS in the 7.5% group, which was lower than those of other two experiments. As shown in Figure 6b, the highest cumulative biogas yield of 211.09 L/kg VS was achieved at the NaOH concentration of 5.0%, followed by 180.88 L/kg VS at 7.5%, and 172.05 L/kg VS at 2.5%. Generally, simultaneous alkaline treatment and digestion simplifies the operation by eliminating a separate reactor required for alkaline pretreatment and reducing material handling. Additionally, the increase in alkalinity may help prevent a drop in pH during acidogenesis, which can create a more stable environment for the methanogenic bacteria[30]. However, excessive NaOH loading caused the browning reaction in the inoculum, reduced the biodegradability of sludge activity and finally affected the biogas production of anaerobic digestion[31,32].



a. Daily biogas yield
b. Cumulative biogas yield

Figure 6 Effects of alkali concentration on biogas yield during anaerobic digestion

TS, VS, pH and VFA changes in the process of SS-AD with different concentration of alkaline treatment were shown in Figure 7. From Figure 7a, TS and VS values in each group decreased which indicated that the degradation and utilization of substrates happened in a certain degree. The highest VS degradation rate of 20% was observed with 5% NaOH treatment, which corresponded to the highest cumulative biogas production value. The pH values were at the stable level of about 8.0 (Figure 7b), which was within the range for microbial suitable growth.

With the increase of NaOH concentration, VFA and corresponding alkalinity changed significantly (P<0.05). After hydrolysis and acidification steps, solid organic matter was decomposed into water-soluble volatile organic substances and small molecules of fatty acids which usually led to the increasing VFA values, and excessive acidosis would be observed. Addition of alkaline would effectively alleviate this phenomenon. When NaOH concentrations were set as 2.5% and 5.0%, VFA and alkalinity changed slightly and the biogas production rate was relatively stable. With the increase of the alkaline concentration to 7.5%, VFA and alkalinity of SS-AD changed significantly (p<0.05).

Cellulose was the main effective component to the biogas production[33]. Addition of 5% of NaOH caused the cellulose degradation of 45.03%. With the alkaline concentration of 1%, 2.5% and 7.5%, the cellulose degradation rate was 36.36%, 43.20% and 44.27%, respectively (Figure 7d). The cellulose degradation rate was in agreement with the highest methane production at these conditions, and 3.88% and 17.05% of hemicellulose degradations were also observed after loading 3.5% and 5.0% NaOH, respectively.

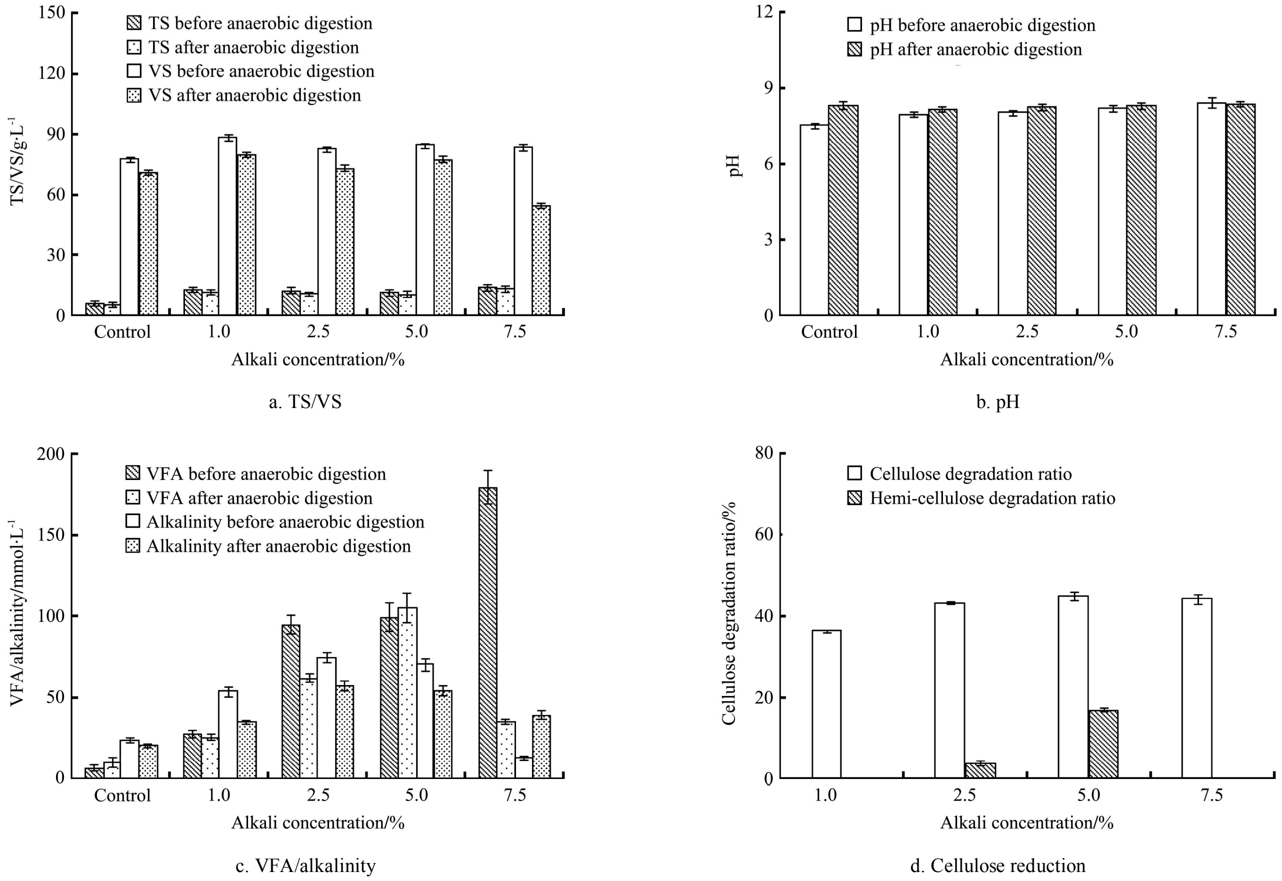


Figure 7 Effects of alkali concentration on TS/VS, pH, VFA/alkalinity and cellulose reduction before and after solid state anaerobic digestion

4 Conclusions

The mesophilic condition (35°C) was optimum for biogas production of corn stover in solid-state anaerobic digestion after pretreatment of *C. subvermispora*. The inoculum of 10:1 (w/w) and addition of 5% NaOH favored the biogas production performance. Under the optimal conditions, the degradation rate of VS was 20% with little changes in pH, VFA and alkalinity, and the maximum degradation rate of cellulose and hemicellulose. The changes of VFAs and microorganism populations/ communities at the optimized SS-AD conditions are investigating in our laboratory to elucidate the metabolic mechanism of VFAs/methane formations and further apply for controlling the biogas production process.

Acknowledgments

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