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Influence of Temperature on Biogas Production Efficiency and Microbial Community in a Two-Phase Anaerobic Digestion System

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Abstract: In this study, the influence of temperature on biogas production efficiency and the microbial community structure was investigated in a two-phase anaerobic digestion reactor for co-digestion of cow manure and corn straw. The results illustrated that the contents of solluted chemical oxygen demand (SCOD) and volatile fatty acid (VFA) in the acidogenic phase and biogas production in the methanogenic phase maintained relatively higher levels at temperatures ranging from 35–25 °C. The methane content of biogas production could be maintained higher than 50% at temperatures above 25 °C. The microbial community structure analysis indicated that the dominant functional bacteria were *Acinetobacter*, *Acetitomaculum,* and *Bacillus* in the acidogenic phase and *Cenarchaeum* in the methanogenic phase at 35–25 °C. However, the performances of the acidogenic phase and the methanogenic phase could be significantly decreased at a lower temperature of 20 °C, and microbial activity was inhibited obviously. Accordingly, a low temperature was adverse for the performance of the acidogenic and methanogenic phases, while moderate temperatures above 25 °C were more conducive to high biogas production efficiency.

Keywords: two-phase anaerobic digestion; temperature; biogas production; microbial community

1. Introduction

Currently, global energy consumption and environmental pollution have become global issues. Oil, as one of the most important economic energy developments, has been excessively exploited and consumed. Therefore, the development and utilization of primary energy and the exploration of alternative renewable clean energy have become a focus of global concern [1,2]. As a large agricultural country, China produced large amounts of agricultural organic waste such as straw and livestock manure every year, which was an important source of biomass energy [3]. Straw and livestock manure can be converted into clean energy such as methane, thereby realizing the reuse of agricultural organic waste [4]. However, the technology for the treatment of straw and livestock manure is not sufficiently advanced. At present, the utilization rate of straw waste in China is insufficient and a large amount of straw is incinerated, resulting in large amounts of soot and bad weather. In addition, a large amount of livestock manure was discharged without treatment, causing adverse effects for humans and livestock, including the spread of the plague [5]. Therefore, innovative and effective processes for the resourceful utilization of agricultural organic waste were urgently developed.

Pretreatment proved to be one simple but effective method for improving the biodegradability of crop residues. Our previous research showed that NaOH pretreatment could significantly improve biodegradability and enhance biogas production of corn straw [6]. This could develop

new feedstock without affecting biogas production efficiency. Generally, an anaerobic biological treatment method was also widely applied for agricultural organic waste treatment. It not only solved the excessive accumulation of agricultural organic waste but also produced clean energy, thereby realizing the recycling and harmless treatment of organic waste [7–9]. According to the widely accepted theory of four stage anaerobic fermentation, the anaerobic digestion process was composed of hydrolysis, acidogenic fermentation, H_2-producing acetogenesis, and methanogenesis [10]. However, the traditional single anaerobic digestion process could not meet the requirements for the simultaneous growth of acidogenic bacteria and methanogenic bacteria and also led to competition between the two groups of bacteria, resulting in low operating efficiency in the reaction unit. In order to solve this problem, the concept of two-phase anaerobic digestion was introduced in the 1970s [11]. The two-phase anaerobic digestion process could achieve the separation of the acidogenic phase and the methanogenic phase via a regulation of operating parameters [12,13]. Furthermore, the acidogenic bacteria and methanogenic bacteria in the two-phase anaerobic digestion process could maintain the optimal growth conditions to achieve the efficiency and stability of the anaerobic fermentation system.

In fact, the two-phase anaerobic digestion process is affected by various factors, such as pH, C/N ratio, inoculum, and temperature [14–17], which directly determine the composition of fermentation products and the methane production efficiency. As one of the main abiotic factors in determining anaerobic digestion efficiency, temperature plays an important role in the performance of the two-phase anaerobic digestion system. In practice, sudden environmental changes, e.g., dramatic increases or drops in temperature, may cause severe disturbances in all parameters of the process, and the system requires a long period of time to adapt to a stable state. Furthermore, the temperature has a significant impact on the growth and metabolism of microorganisms and the interactions between the microbial groups [18–21]. In the process of anaerobic digestion, the temperature can regulate microbial intracellular enzyme activity, thus affecting the metabolic activity of microorganisms and the anaerobic fermentation efficiency. In addition, changes in the microbial metabolism or the community dynamics affect the operation of the anaerobic digestion system [22–26]. It was reported that mesophilic conditions (30–40 °C) have been generally adopted for the anaerobic digestion of agricultural organic waste and show good performance in biogas production [27,28]. The low temperature condition has become the main reason for the limited application of anaerobic digestion technology for the treatment of agricultural organic waste in North China. It was found that low temperatures resulted in a low biogas production and unstable operational performance in the anaerobic digestion system [29]. The effects of increasing or decreasing temperatures followed by the re-establishment of the initial temperature have been assessed in some previous studies. These studies show that a decrease in temperature typically causes a lower solluted chemical oxygen demand (SCOD) removal efficiency, a lower biogas production, and a lower volatile fatty acids (VFA) accumulation. However, the studies on the effect of daily temperature fluctuations on the biogas production and the microbial community of the two-phase anaerobic digestion process were rarely reported. Therefore, it is important to study the influence of temperature on the two-phase anaerobic digestion process.

In the present study, a modified two-phase anaerobic digestion reactor was studied to assess the effects of daily temperature variations on the semi-continuous anaerobic digestion by treating a cow manure and corn straw mixture. The daily temperature variations were simulated by a forced square-wave oscillation of the reactor temperature of an anaerobic digester. Meanwhile, the effect of temperature on the efficiency of volatile fatty acid and biogas production and the microbial community structure was also investigated. Examining the effects of daily temperature variations and microbial composition on the performance of anaerobic digestion will aid in providing the necessary regulation to derive the optimal fermentation liquor for efficient biogas production.

2. Methods and Materials

2.1. Substrate and Inoculum

The cow manure (CM) and corn straw (CS) used in this study were collected from the Hailin Farm in Mudanjiang, Heilongjiang province, China. Actively digested dairy biogas slurry (BS) was gathered from a 1000 m^3 size biogas plant at the farm. It was filtered and used as inoculum to prepare for substrate/inoculum with 1:3. In order to improve the degradation efficiency of straw in the anaerobic digestion process, the corn straw needed to be NaOH-pretreated to destroy the fiber structure in the straw. The corn straw was pretreated by crushing and sieving it to a particle size range of 2–3 cm, then soaking it with alkali liquor (5% NaOH) for 24 h, and finally washing it with deionized water and drying it in an oven at 65 °C for 12 h. The properties of the corn straw and cow manure are shown in Table 1.

Table 1. The basic characteristics of corn straw (CS), cow manure (CM), and inoculum.

Parameters	CS	CS (5% NaOH)	CM	BS
pH	7.18	-	7.12	7.02
Total solid (TS, %)	86.3	30.8	17.2	5.4
Volatile solid (VS, %)	61.4	24.6	12.45	4.2
Total carbon (TC, %)	36.6	26.3	35.4	22.5
Total nitrogen (TN, %)	0.89	0.47	2.02	1.28
Carbon to nitrogen ratio (C/N)	41.1	56.2	17.6	17.3

The parameters, mean, and standard deviation comprised three samples of substrate and inoculum.

2.2. Two-Phase Anaerobic Digestion Reactor Configuration and Operation

An anaerobic digestion process for treating a corn straw and cow manure mixture was carried out in a lab-scale two-phase anaerobic digestion reactor, as shown in Figure 1. It consisted of two continuously stirred tank reactors. One was an acidogenic reactor with an effective volume of 6 L, and the other was a methanogenic reactor with an effective volume of 4.5 L.

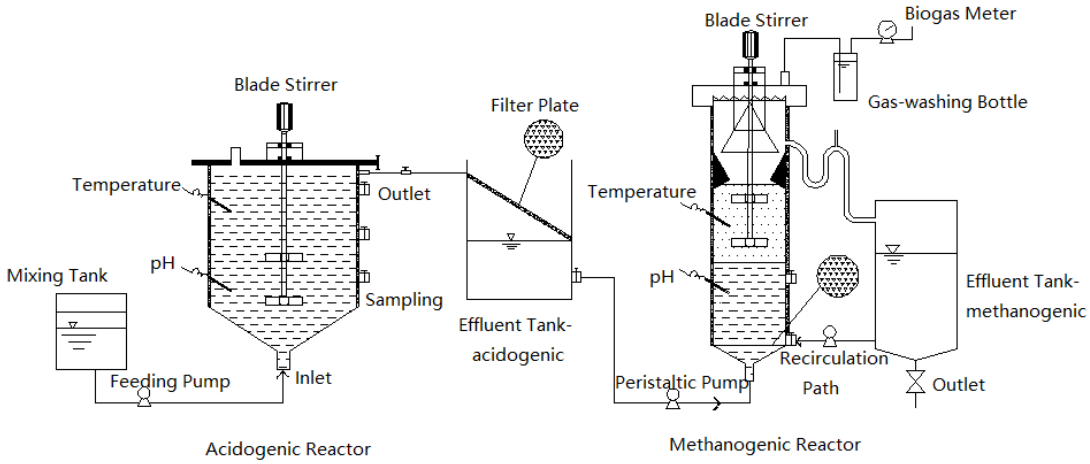

Figure 1. The configuration of the lab-scale two-phase anaerobic digestion reactor.

Initially, the acidogenic reactor was fed with 0.5 g VS/L/day of the mixture of corn straw and cow manure. It was then gradually increased to a final organic loading rate of 2.7 g VS/L/day. The operational conditions of the acidogenic reactor were as follows: initial pH of 7.2 using 1 mol/L sodium hydroxide solution or hydrochloric acid solution and a hydraulic retention time (HRT) of 25 days. Once the operation of the acidogenic reactor was stable, the acidizing mixture produced in the acidogenic reactor was used as the substrate for the start-up of the methanogenic reactor. Once

the operation of the acidogenic reactor and the methanogenic reactor was stable, both reactors were simultaneously operated at the different temperatures. The temperature first decreased from 35 °C to 30 °C, then fell to 25 °C after reactor stability until it cooled to 20 °C. The VFA and SCOD concentrations in the acidogenic reactor and biogas content and the methane production in the methanogenic reactor were determined at the different temperature conditions.

2.3. Microbial Community Analysis

The fermentation liquors taken from the acidogenic reactor and the methanogenic reactor under different temperatures were collected to analyze the microbial community via high-throughput sequencing. Total DNA were extracted according to the instructions of the DNA extraction kit (E.Z.N.ATM Mag-Bind Soil DNA Kit). The 16S rRNA gene of the extracted DNA was amplified with the broadly conserved primer sets 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), 340F (5′-CCCTAYGGGGYGCASCAG-3′) for Eubacteria, and 1000R (5′-GGCCATGCACYWCYTCTC-3′) for Archaea, targeting the V1-V3 regions. The PCR was performed with an initial denaturation step at 98 °C for 30 s followed by 30 (Bacteria) or 35 (Archaea) cycles consisting of 98 °C for 10 s, 58 °C for 30 s, and 72 °C for 45 s. It was completed by a final elongation step at 72 °C for 7 s. The obtained PCR products were sequenced using an Illumina MiSeq by Meiji Biological Medicine Technology (Shanghai, China) Co., Ltd. The resultant sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity threshold.

2.4. Analytical Methods

The SCOD was determined using Standard Methods for the Examination of Water and Wastewater [30]. The pH was recorded using a pH analyzer (pHS-3C, Lei ci, China). Total solids (TS) and volatile solids (VS) were determined based on the weighing method after being dried at 103–105 °C and burned to ash at 550 °C. The nitrogen elemental analyses were determined using the various MICRO cube (Elementar, Germany). Biogas collected from the reactors was measured with a wet type gas meter (LMF-2, Changchun, China), and its concentration and composition were analyzed by a gas chromatography (SHIMADZU GC-8A). The operation condition was illustrated in previous literature [31]. VFA concentration and composition (acetic acid, propionic acid, and butyric acid) were determined using a gas chromatograph (7890GC-FID, Agilent, Japan) according to the study reported [32].

3. Results and Discussion

3.1. Performance of the Two-Phase Digestion Reactor under Different Temperatures

3.1.1. SCOD and VFAs Contents in the Acidogenic Phase

The temperature in the acidogenic reactor was controlled at 35 °C, 30 °C, 25 °C, and 20 °C at the stable operation stage, respectively. The concentrations of SCOD and VFA under different temperatures are shown in Figure 2. To some extent, the variety of SCOD concentration reflects the hydrolysis and acidification efficiency of the anaerobic digestion process [33]. Therefore, the SCOD concentration under different temperatures could monitor the fermentation characteristics of the acidogenic phase. As shown in Figure 2a, SCOD concentration gradually increased in the first 20 days at 35 °C. Similarly, SCOD concentration also increased in the 23 days in the acidogenic phase at 30 °C. This indicated that the organic matter catabolism presented a relatively high level in the temperature range. A large amount of organic matter was degraded into cellulose, carbohydrates, amino acids, and other small molecular organic compounds [34]. However, the concentration of SCOD slightly increased at 25–20 °C because low temperatures inhibited the metabolic activity of the microorganisms, resulting in a decrease in the organic matter degradation efficiency. In addition, the activities of functional

bacteria with the ability to degrade organic compounds, such as cellulose in corn straw and cow manure, were inhibited at the low temperature conditions.

Furthermore, VFA is an important factor in measuring the anaerobic biodegradability in the anaerobic digestion process. As an intermediate of anaerobic digestion, VFA content can represent the metabolic activity of fermentative bacteria to a certain extent. As a continuation of the hydrolysis step, the hydrolyzed small molecules were further utilized by fermentative bacteria to produce VFAs and alcohols. Subsequently, the acetogenic bacteria transformed the intermediate into acetate, carbon dioxide, and hydrogen [35], and the acetate finally transformed into methane by methanogenic bacteria [36]. As shown in Figure 2b, the VFA content included acetic acid, propionic acid, butyric acid, and valeric acid at the stable stage of the acidogenic phase under different temperature conditions. Acetic acid and propionic acid were the main volatile organic acids at different temperatures, while butyric acid and valeric acid contents maintained at very low levels. The acetic acid was a necessary substrate for the methanogenic phases, and it had a certain impact on the growth and metabolism of methane bacteria. With the decrease of fermentation temperature, the total VFA and acetic acid content decreased gradually. At 35 °C, the total VFA content was 4403 mg/L and the content of acetic acid reached 3420 mg/L, which accounted for approximately 77.7% of the total VFAs. Meanwhile, the total VFA content was 3867 mg/L and 2913 mg/L, and the content of acetic acid was 2635 mg/L and 1805 mg/L at the temperature of 30 and 25 °C, respectively. However, the VFA content rapidly descended to 1270 mg/L at 20 °C, and the content of acetic acid accounted for only 65.3% of the total VFAs. It indicated that low temperatures had a negative effect on VFA production efficiency in the acidogenic phase. In addition, the contents of butyric acid and valeric acid at different temperature conditions stably maintained at 190~300 mg/L and 105~130 mg/L. Butyric acid and valeric acid performed a slight change with the various temperature conditions. It is worth noting that the propionic acid content was less than 20% of the total VFAs under different temperatures, thus it might not inhibit methane production in the methanogenic reactor.

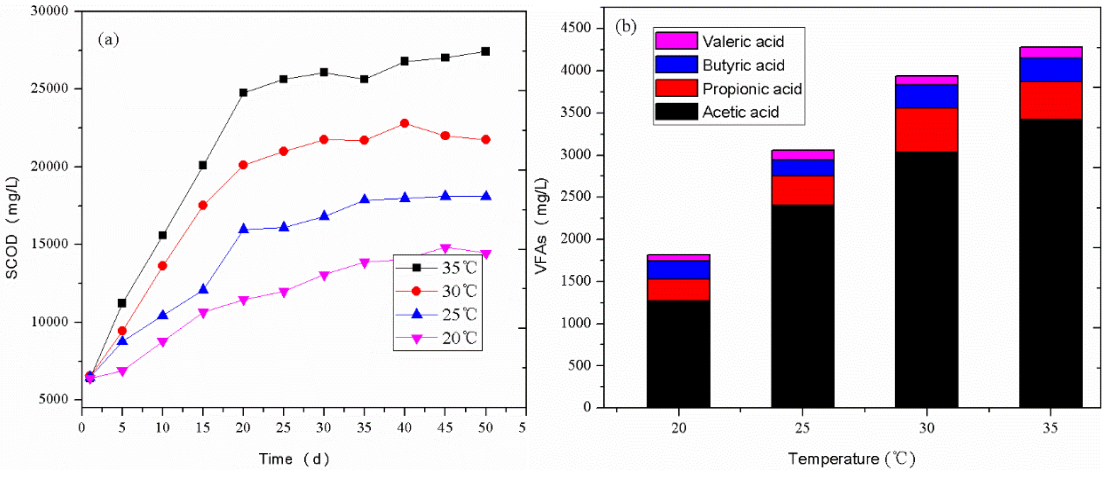

Figure 2. The concentration of solluted chemical oxygen demand (SCOD) (**a**) and volatile fatty acids (VFAs) (**b**) at different temperatures in the acidogenic phase.

3.1.2. Biogas Production Efficiency in the Methanogenic Phase

According to the characteristics of the methanogenic phase, the cow manure and corn straw mixture acidification liquid produced from the acidogenic phase at 35 °C was used as the substrate for the start-up of the methanogenic phase. The basic properties of mixture acidification liquid are shown in Table 2, indicating that the acidified substrate provided sufficient nutrients for the methanogenic bacteria in the methanogenic phase.

Table 2. The basic properties of acidification liquid in the acidogenic phase at 45 days.

Properties	Data
TS (%)	12.8
VS (% TS)	9.1
Acetic Acid (mg/L)	3295
Propionic Acid (mg/L)	371
Butyric Acid (mg/L)	265
SCOD (mg/L)	26039
pH	6.3

Methanogens were more sensitive to the temperature changes because temperature significantly influences the growth and metabolic activity of methanogens [37]. Therefore, it is important to regulate the temperature to improve biogas production in the methanogenic phase. The methanogenic reactor was operated in semi-continuous fermentation, and the temperature was gradually decreased from 35 °C to 20 °C. Figure 3 shows a variety of biogas production with the gradient decreasing of temperature. Obviously, the biogas production significantly decreased with the decreasing of temperature. It was found that biogas production gradually increased with the increase of operation time at the temperature range of 35–30 °C, and it maintained at relatively high levels. As shown in Figure 3a, the maximum biogas production reached 2450 mL at 35 °C. By comparison, biogas production decreased by about 22.4%, 36.2%, and 70.4% at temperatures of 30 °C, 25 °C, and 20 °C, respectively. When the temperature dropped to 20 °C, the biogas production decreased sharply after 6 days. Accordingly, when the temperature was above 25 °C, the methanogens had certain metabolic activity, which could maintain biogas production efficiency and operation stability in the methanogenic phase. However, when the temperature was too low, the metabolic activity of methanogenic bacteria was significantly inhibited, which directly resulted in the decrease of biogas production efficiency in the reactor.

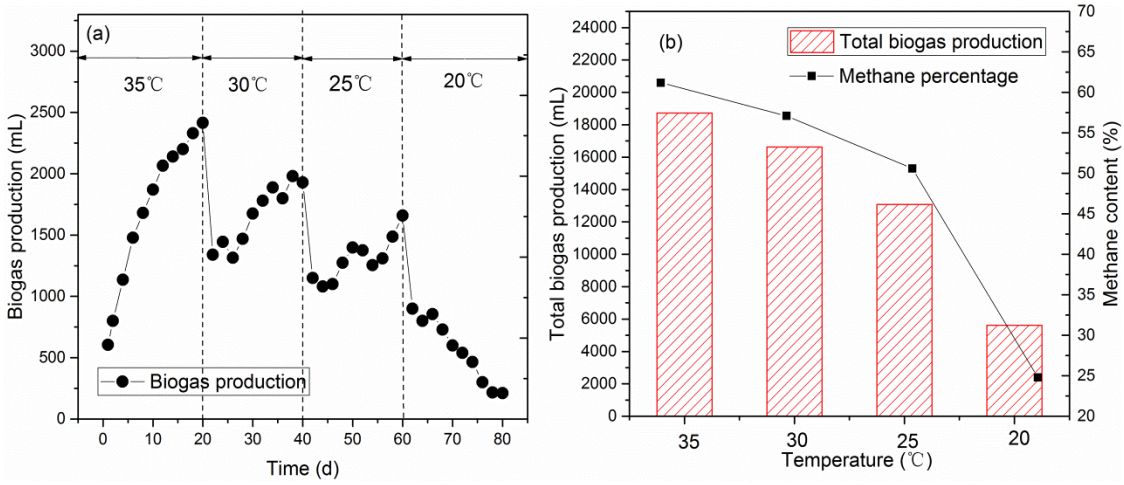

Figure 3. The variety of biogas production at the different temperatures in the methanogenic phase (**a**), and the total biogas production and methane content percentage at different temperatures in the methanogenic phase (**b**).

The total biogas accumulation and the methane content percentage in the methanogenic phase are shown in Figure 3b. When the temperature maintained at 35 °C, the total biogas production was 18075 mL. With the decrease of temperature, the total biogas production decreased sharply, and the total biogas production decreased by 10.7%, 28.2%, and 70.3% at temperatures of 30–20 °C, respectively. Additionally, the methane content accounted for 57.5% of total biogas production at 35 °C, and the average methane content decreased slightly with the decrease of temperature.

The methane content could be maintained at more than 50% at temperatures above 25 °C. Apparently, the methanogenic phase could perform great methane production efficiency in the temperature range of 35–25 °C. However, when the temperature dropped from 25 °C to 20 °C, the methane content decreased obviously with a methane content of only 25.2%. These results indicated that the activity of methanogenic bacteria was more sensitive to low temperature disturbance.

3.2. Characteristics of the Microbial Community at Different Temperatures

Anaerobic digestion is a complex ecosystem with a variety of microorganism interactions. The structure and diversity of microbial communities reflect the ecosystem function. The main functional bacteria in the anaerobic digestion process consisted of non-methanogenic bacteria and methanogenic bacteria [38–40]. On the one hand, non-methanogenic bacteria provided the essential substrate and suitable redox potential for the growth and reproduction of methanogenic bacteria. On the other hand, methanogenic bacteria mitigated feedback inhibition caused by hydrogen and organic acid accumulation for non-methanogenic bacteria. Therefore, methanogenic bacteria and non-methanogenic bacteria presented synergistic effects and mutual restraint to regulate the process of anaerobic digestion.

3.2.1. Microbial Community Structure in the Acidogenic Phase

As shown in Table 3, the richness index (ACE and Chao1) and the diversity index (Shannon and Simpson) of the samples were analyzed. The richness index and diversity index maintained at relatively high levels at 35–25 °C, indicating that abundances and diversity in the microbial community in the acidogenic phase with different temperature conditions were relatively high. However, at the temperature of 20 °C, the richness index (ACE and Chao1) was only 954 and 932, respectively, and the diversity index (Shannon and Simpson) was only 4.51 and 0.031, respectively. This indicated that the acidogenic phase performed a great microbial diversity at the temperature range of 35–25 °C.

The relative abundances at the class level at different temperatures are shown in Figure 4b. The dominant bacteria at 35 °C were Clostridia (with the relative abundance of 39.17%) and Bacteroidia (with the relative abundance of 25.62%). The dominant bacteria at 30 °C were Clostridia (31.35%), Bacteroidia (17.48%), and Beta-proteobacteria (10.8%). Similarly, the dominant bacteria at 25 °C were Clostridia and Bacteroidia. However, the relative abundance of Clostridia and Bacteroidia significantly reduced at 20 °C; the corresponding relative abundance accounted for less than 30%. Compared with the abundance of 35 °C, the relative abundance of Synergistia (15.01%) and Gamma-proteobacteria (18.32%) increased significantly at 20 °C. This indicated that Synergistia and Gamma-proteobacteria presented a certain resistance to the low temperature environment, thus it was inferred that the temperature had a certain response relationship with the microbial community structure. In addition, some other non-dominant bacteria also existed in the acidogenic phase at different temperatures, which mainly included the Spirochaetes, Anaerolineae, Mollicutes, Acidimicrobiia, and Sphingobacteriia.

As shown in Figure 4c, the dominant bacteria at the genus level were similar at 35 °C, 30 °C, and 25 °C. *Acinetobacter*, *Acetitomaculum,* and *Bacillus* were the dominant genera, which were the main functional microorganisms for the decomposition and metabolism of organic compounds in the acidogenic phase. It was reported that *Acinetobacter* and *Bacillus* were anaerobic or facultative anaerobic bacteria with the ability of cellulose degradation, which could quickly degrade the cellulose and other organic compounds for providing sufficient metabolic substrates for methanogenic bacteria in the methanogenic phase. Therefore, it was proven that the degradation efficiency of cellulose and other refractory organic compounds was higher in the acidogenic phase at these temperature ranges. These results were consistent with the above studies on the characteristics of SCOD and VFAs produced in the acidogenic phase (Figure 2). In contrast, the microbial community structure changed significantly at 20 °C. The relative abundances of *Acinetobacter* and *Acetitomaculum* obviously decreased due to the influence of low temperature, while the relative abundances of *Cryptanaerobacter* and *Solibacillus* slightly increased. It has been reported that these bacteria were not the functional microorganisms in

an acid-producing fermentation process, and they even formed a competitive relationship with other fermentative microbial groups, thereby resulting in the decrease of hydrolysis acidification efficiency and operational stability in the acidogenic phase.

Table 3. Alpha-diversity in the acidogenic phase at different temperatures.

Temperature (°C)	OTU	ACE	Chao1	Shannon	Simpson
35	1078	1210	1214	6.23	0.012
30	1009	1145	1132	6.46	0.023
25	1032	1021	1025	5.61	0.025
20	890	954	932	4.51	0.031

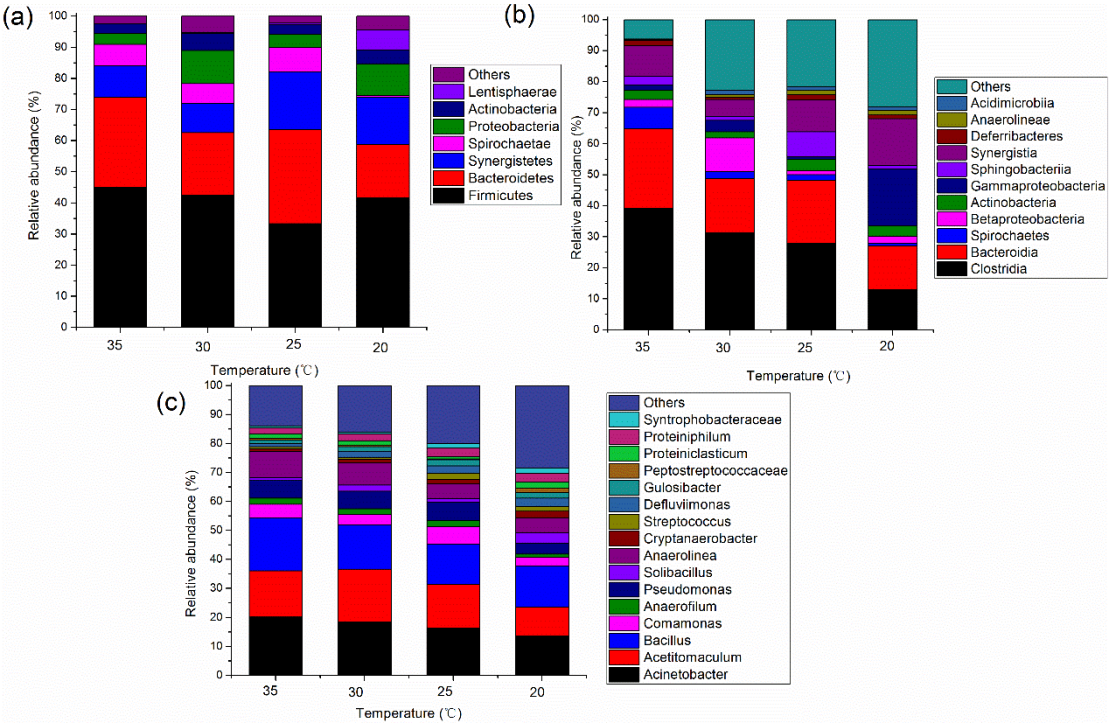

Figure 4. Microbial community structure in the acidogenic phase at different temperatures: (**a**) phylum level; (**b**) class level; (**c**) genus level.

3.2.2. Microbial Community Structure in the Methanogenic Phase

As shown in Table 4, the OTUs were 980, 976, 982, and 789 at temperatures of 35–20 °C in the methanogenic phase. Furthermore, the richness index (ACE and Chao1) and the diversity index (Shannon and Simpson) of the samples were analyzed. The microbial diversity during stable operation at temperatures of 35–25 °C was significantly higher than that at a low temperature of 20 °C. The richness indexes (ACE and Chao1) were 854 and 867, and the diversity indexes (Shannon and Simpson) were 5.01 and 0.017 at 35 °C, respectively. Obviously, the richness and diversity gradually decreased as the temperature gradient decreased. The Shannon value (4.03) and the Simpson value (0.038) showed lower levels at 20 °C. This indicated that the low temperature resulted in a significant decrease in the diversity and richness of methanogenic bacteria.

Table 4. Alpha-diversity in the methanogenic phase at different temperatures.

Temperature (°C)	OTU	ACE	Chao1	Shannon	Simpson
35	980	854	867	5.01	0.017
30	976	856	894	5.09	0.021
25	982	845	800	4.47	0.025
20	789	765	696	4.03	0.038

As shown in Figure 5a, at the phylum level, Thaumarchaeota accounted for the largest proportion in the methanogenic phase at different temperatures. The relative abundances of Thaumarchaeota reached 87%, 83%, 76%, and 61%, respectively. As shown in Figure 5b, the dominant bacteria at the class level were unclassified bacteria at different temperatures. In addition, Methanomicrobia and Methanobacteria maintained at relatively high levels at temperatures of 35–25 °C. The relative abundances of Methanomicrobia were 21%, 13%, and 10% at temperatures of 35–25 °C, respectively. The relative abundances of Methanobacteria reached 13%, 10%, and 14%, respectively. As shown in Figure 5b, the relative abundance at 20 °C presented a lower level, and the corresponding abundances of Methanomicrobia and Methanobacteria were only 5.3% and 6.7%, respectively. All of the methanogenic bacteria obtained in this study were common groups in anaerobic digestion, and they all produced methane utilizing H_2, acetic acid, butyric acid, and ethanol as substrates that were produced by Firmicutes, Bacteroides, or Clostridium in the acidogenic phase. It was reported that Methanobacteria is a strictly anaerobic bacteria that mainly used H_2, formic acid, acetic acid, and butyric acid as a carbon source and an energy source to produce methane. Methanomicrobia is known to be a multifunctional methanogen that can produce methane via three different metabolic pathways using H_2/CO_2, acetate, and methylated one-carbon compounds [41,42].

The predominant bacteria at a genus level at different temperatures in the methanogenic phase are shown in Figure 5c. Obviously, the microbial community structure of the methanogenic phase varied significantly with the decreasing of temperatures. The largest genus was *Cenarchaeum* at the different temperatures with relative abundances of 80%, 75%, 67%, and 42%, respectively. In addition, *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, and *Methanospirillum* constituted the main methanogenic microorganisms at temperatures of 35–25 °C. These results illustrated that the microbial community structure in the methanogenic phase was rich and diverse in the temperature range of 35–25 °C, which also resulted in high methane production. However, the microbial community structure of the methanogenic bacteria changed significantly when the temperature dropped to 20 °C. Methanogen diversity significantly decreased, and the relative abundances of *Methanobrevibacter*, *Methanoculleus*, and *Methanospirillum* were less than 3%. These results indicated that low temperatures had a negative effect on the methanogenic bacteria community structure in the methanogenic phase. The methane content showed a sharp decline at 20 °C (Figure 3), which may have been related to a change in the metabolic pathways of the functional microorganisms in the methanogenic phase. The above results demonstrated that the microbial community structure in the two-phase anaerobic digestion system was sensitive to temperature, and low temperatures significantly inhibited the efficiency of the acidogenic phase and the methanogenic phase. This resulted in the disorder of methane production in the reaction system and even the failure of the two-phase anaerobic digestion system operation.

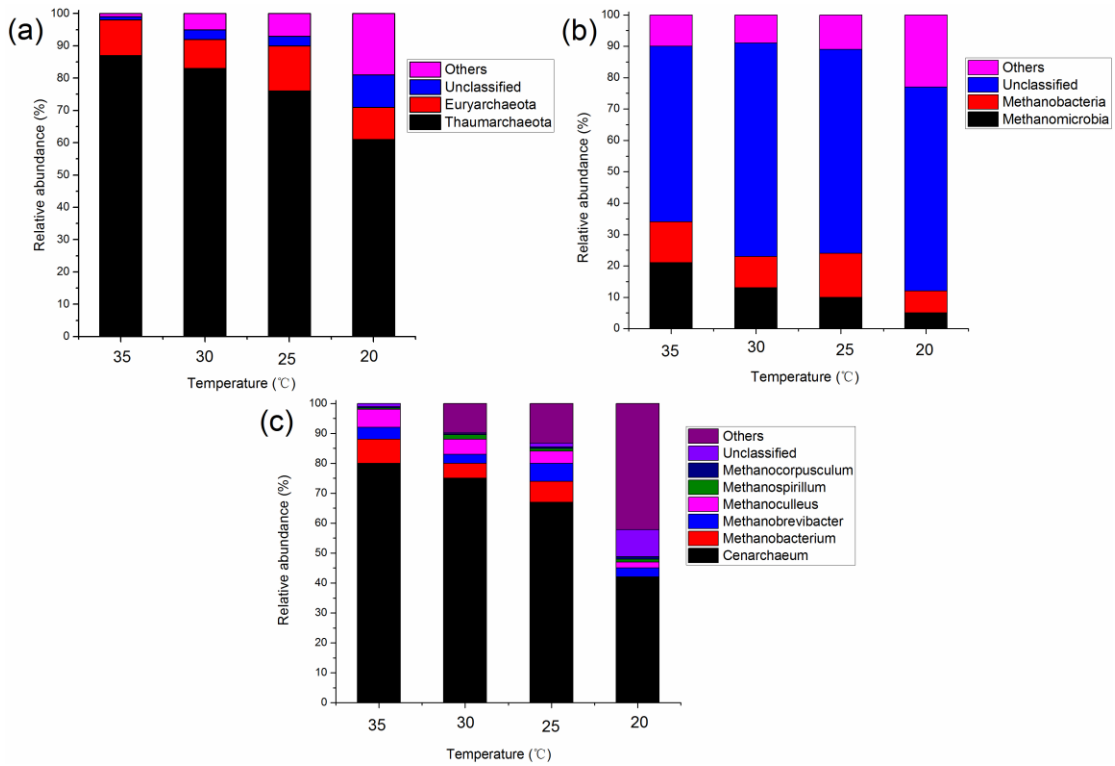

Figure 5. Microbial community structure in the methanogenic phase at different temperatures: (**a**) phylum level; (**b**) class level; (**c**) genus level.

3.3. Correlation of Community Microorganisms with Environmental Variables (VFAs and Temperature)

Redundancy analysis (RDA) illustrated the correlation of community composition with the environmental variables (temperature and VFAs) (Figure 6). It was obvious that temperature and VFAs played a cooperative role in affecting the system community microbes with an acute angle between them. Temperature variation was closely correlated to Acinetobacter, Acetitomaculum, Anaerolinea, and Bacillus, all of which were essential for complex substrate degradation and recycling. These microbes could be affected positively by the changing environmental temperature in the acidogenic reactor. This viewpoint could be supported by the following fact: the corresponding relative abundances of Acinetobacter, Acetitomaculum, and Bacillus increased obviously with the rising temperature (from 20 to 35 °C) (Figure 4).

Moreover, increasing the temperature could produce high VFA and SCOD levels in the two-stage system. It was reported that VFA concentration could be considered one of the main factors affecting the biogas production process and the performance of the methanogenic reactor. A positive relationship emerged between VFAs and Cenarchaeum, Methanoculleus, and Methanobacterium, all of which were associated with the nutrient removal processes. Moreover, the relationship of Proteiniphilum with environmental variables (temperature and VFAs) was relatively irrelevant, as was that of Comamonas, suggesting that the environmental variables of temperature and VFAs displayed no significant influence on the dynamics of Proteiniphilum and Proteiniphilum in the acidogenic system.

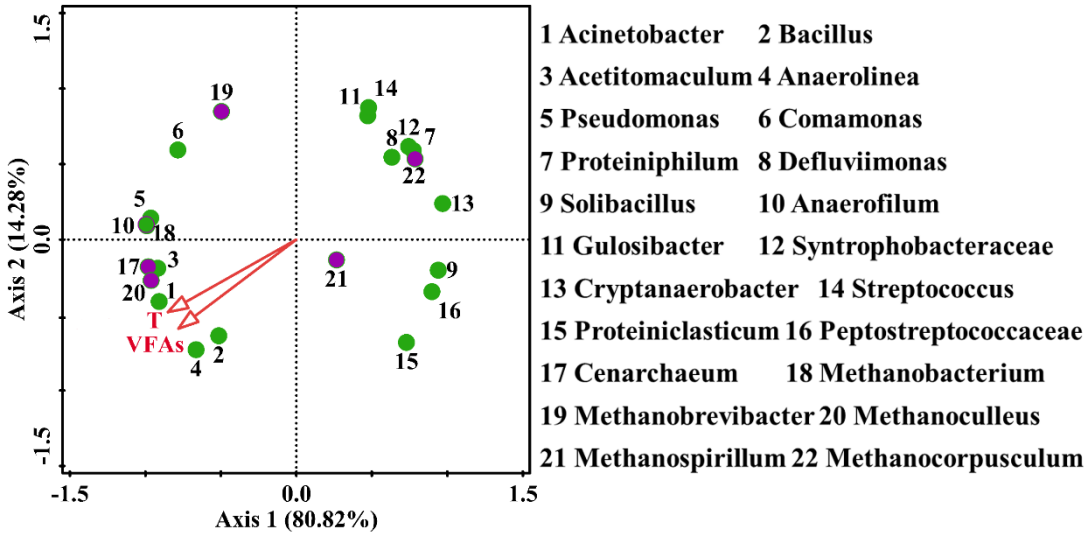

Figure 6. RDA on the correlation of system microbial composition with different environmental variables (temperature and VFAs).

4. Conclusions

In this study, the performance of a two-phase anaerobic digestion process for co-digestion cow manure and corn straw was systematically investigated at different temperatures. The acidogenic phase demonstrated the most efficient VFA production ability, and the methanogenic phase achieved the optimal biogas production efficiency at the temperature range of 35–25 °C. The analysis of microbial community structure indicated that the dominant functional bacteria were *Acinetobacter*, *Acetitomaculum*, and *Bacillus* in the acidogenic phase and *Cenarchaeum* in the methanogenic phase. The low temperature (20 °C) had a negative effect on the performances of the acidogenic phase and the methanogenic phase. The content of SCOD and VFA in the acidogenic phase and biogas production in the methanogenic phase decreased at 20 °C. The richness and variety of the microbial community in the acidogenic phase and the methanogenic phase decreased to lower levels at 20 °C. The microbial community structure performed significant differences compared with that worked at the temperatures of 35–25 °C. Therefore, the two-phase anaerobic digestion process for co-digestion cow manure and corn straw could maintain high biogas production efficiency at a moderated temperature above 25 °C.

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