Process optimization and feature analysis of an anaerobic fermentation microbial community in a cold region

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ABSTRACT

Anaerobic digestion can be inhibited at low temperatures in cold regions. This study investigated the effects of fermentation temperature, total solids (TS) concentration and stirring speed on swine manure biogas production by anaerobic fermentation experiments based on the quadratic regression orthogonal rotation combination method. The results showed that the fermentation temperature and TS had significant effects on swine manure biogas production rates. The theoretical and experimental amounts of gas production at the optimal process parameters were 2857.46 mL and 2820.0 mL, respectively. Methanobacteria and Methanomicrobia were the main methane-producing genera for samples in six different anaerobic digestion phases, with relative abundances of 2.25 to 5.59% and 0.85 to 3.02%, respectively. The acetotrophic bacterium Methanoseta was absolutely dominant, attributable to acetic acid metabolic pathways occurring more easily at low temperatures and Methanoseta was more competitive when the acetic acid concentration was in the range of 38.8 to 252.2 mg L\textsuperscript{-1}.

1. Introduction

Due to the development of the rural economy and changes in agricultural land use practices, the Chinese animal husbandry industry has developed rapidly, establishing China as the world’s top pork-producing country [1,2]. To date, the large amount of manure produced by Chinese pig farms has been directly discharged into the environment without treatment, posing major ecological concerns. Since pig manure represents the main source of agricultural pollution in China, developing processes for effective treatment and recycling of pig manure is a pressing societal problem [3,4].

Anaerobic digestion is one of the main ways to treat pig manure and convert it into a useful and sustainable product, such as biosolid compost. Fermentation temperature, total solid (TS) concentration, and mechanical stirring are important factors affecting anaerobic digestion. Medium and high temperatures can promote anaerobic digestion processes, but to ensure the reaction temperature of the material in engineering applications, the energy consumed will also be increased [5–7]. Thus, it is important to identify a suitable temperature for efficient biogas production under low temperature conditions. Methanogens that are active during anaerobic digestion can be divided into four types based on their optimal colony growth temperatures: psychrophilic methanogens (Topt < 25°C), mesophilic methanogens (Topt \approx 35°C), thermophilic methanogens (Topt \approx 55°C) and extremely thermophilic methanogens (Topt > 80°C) [8,9]. A high total solids concentration in the anaerobic reaction system will increase the utilization efficiency of the reactor volume but will also cause toxic intermediate products to accumulate [10,11], resulting in anaerobic digestion instability or even failure [4]. Mechanical stirring can affect anaerobic digestion, but studies have been inconclusive as to the degree [12,13]. To ensure efficient and stable manure processing and biogas production in cold regions, it is urgent to identify how to optimize these three factors.

Anaerobic digestion is completed through the combined action and cooperation of microorganisms at different stages. Thus, it is important to identify the relevant microbial community structure, succession law, and function during the anaerobic digestion process, since a clear understanding of these factors is crucial for improving the stability of anaerobic digestion and increasing biogas production for subsequent use as biofuels [14]. To date, research on microorganisms involved in anaerobic digestion has primarily focused on: (1) the selection of low-temperature methanogenic bacteria, (2) the addition of external assistance and promotion factors, and (3) the establishment of anaerobic digestion kinetic models [15–19]. Additional insight is needed to better understand the mechanisms at play in these studies. Under low temperature conditions,
methanogenic bacteria cannot easily be maintained. Decreased methanogen activity is the main factor in the decreased gas production rate during anaerobic digestion. Therefore, research on the community structure characteristics of low-temperature anaerobic digestion microorganisms and identification of dominant microflora are of great significance, both in the selection of high-efficiency and low-temperature methanogenic bacteria and solving the issue of low biogas production rates in cold regions.

To address this gap in existing research, our study focuses on swine manure as an anaerobic digestion substrate under low temperature conditions. Using the quadratic regression orthogonal rotation combination method, we examine the anaerobic digestion process and optimize three process parameters: temperature, TS, and mechanical stirring. We also analysed the succession process of microorganisms under optimized conditions to determine the dominant methanogenic microflora. Our findings will provide a scientific basis and standardized approach for the utilization of anaerobic digestion of swine manure in cold regions.

2. Materials and methods

2.1. Materials and preliminary preparation

Fresh swine manure was collected from a farm in Harbin, Heilongjiang Province, and was immediately stored at 4°C upon collection. Physical and chemical analyses and inoculation were completed within 24 hours of collection. The inoculated activated sludge was taken from long-term domesticated sludge in our laboratory. The basic physical and chemical properties of the test materials, which consisted of fresh manure inoculated with long-term domesticated sludge, were analysed (Supplementary Materials Table S1).

2.2. Experimental design

The experiment was modelled after the completely randomized design developed by Lu et al. [20] using the three factors of substrate concentration, temperature, and stirring speed. We utilized the three-factor quadratic regression orthogonal rotation combination design method to study the influence of the above factors on the fermentation of raw swine manure materials. There were three replicates for each experimental treatment.

As previously stated, the quadratic regression orthogonal rotation combination method was applied [21,22]. To conduct a three-factor, five-level test, we selected the following: a temperature between 12 and 20°C, a total solids concentration between 3 and 8%, and a stirring speed between 60 and 210r·min⁻¹, with X1, X2, and X3 representing the coding value of each factor (Supplementary Materials Table S2).

2.3. Experimental methods

The anaerobic digestion device we used is shown in Figure 1. We weighed appropriate amounts of pig manure and inoculum into the fermentation bottle, adjusted the mixture with deionized water to 400.0 mL, and set up a separate bottle containing only the inoculum as the control. The remainder of the bottle was filled with nitrogen, and the bottle was then shaken and thoroughly mixed. We carried out three repeated experiments for each group at different temperatures, with a fermentation period of 45 days.

The anaerobic digestion test was carried out under optimal process conditions. From the first day of fermentation, samples were taken every five days, with 15 mL sampled and placed in a centrifuge tube each time. We took a total of six samples, marked as LBY1A-6A, which we stored in a refrigerator at −80°C. We commissioned Shenzhen Huada Gene Technology Co., Ltd., to analyse the characteristics of the sample microbial communities.

2.4. Measurement method

In this study, the total solid content was determined using the drying method [23], while the volatile solid (VS) content was determined using the burning method [24]. Total nitrogen (TN) was determined using a Kjeldahl apparatus [25], while total carbon (TC) was measured using the potassium dichromate oxidation method [26]. Biogas production was measured using the standard drainage method.

2.5. Analysis methods

We used data processing software (DPS) to process the cumulative gas production data. We next established and optimized a gas production mathematical model and then performed a reduction analysis to identify and obtain the best anaerobic digestion conditions. To conduct a microbial community feature analysis, we selected representative sequences from each operational taxonomic unit (OTU) 16S rDNA marker gene sequence we detected. We compared these sequences

Figure 1. Components of experimental device for carrying out anaerobic fermentation of swine manure. (a) Water bath; (b) Gas jar; (c) Recorder jar; (d) Fermentation bottle; (e) Gauge mark; (f) Stirring rod.
3. Results and discussion

3.1. Establishment of a mathematical model for anaerobic digestion of swine manure

Using the coded values X1, X2, and X3 as independent variables and the total gas production Y as the response value (Table 1), we constructed a mathematical model. Applying DPS statistical analysis software, we were able to obtain the regression equation in the coding space, generating Formula (1):

\[
Y = 1227.99 + 529.30X_1 + 237.22X_2 + 73.23X_3 + 27.12X_1^2 - 114.71X_2^2 - 207.05X_2^2 + 83.58X_2X_3 + 31.08X_3 - 171.67X_2X_3
\]

(1)

We used DPS to analyse all results and carried out an analysis of variance (ANOVA). We used the F test method to test the regression coefficients and the significance of the regression equations. Our results are presented in Table 2.

The analysis of the results shows that our quadratic regression model has \( F = 10.36 > F_{0.01} \) and \( P < 0.01 \), indicating that the selected model is significant. Moreover, the adjusted determination coefficient of the model is 0.8159, indicating that the predicted value of the model fits well with the real value.

| Table 1. Plans and results of experiment. |
|------------------------------------------|
| No. | X1 | X2 | X3 | Total gas production (Y/mL) |
|-----|----|----|----|-----------------------------|
| 1   | 1  | 1  | 1  | 1391.67 ± 92.33             |
| 2   | 1  | 1  | –1 | 1850.00 ± 117.53            |
| 3   | 1  | –1 | 1  | 1583.33 ± 106.53            |
| 4   | 1  | –1 | –1 | 493.33 ± 78.57              |
| 5   | –1 | 1  | 1  | 697.00 ± 38.61              |
| 6   | –1 | 1  | –1 | 423.33 ± 39.94              |
| 7   | –1 | –1 | 1  | 366.33 ± 25.71              |
| 8   | –1 | –1 | –1 | 262.33 ± 36.75              |
| 9   | –1.6818| 0  | 0  | 288.33 ± 23.16              |
| 10  | 1.6818| 0  | 0  | 2461.00 ± 95.42             |
| 11  | 0  | –1.6818| 0  | 501.33 ± 26.72              |
| 12  | 0  | 1.6818| 0  | 1445.33 ± 54.76             |
| 13  | 0  | 0  | –1.6818| 713.33 ± 28.44             |
| 14  | 0  | 0  | 1.6818| 711.00 ± 18.77             |
| 15  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 16  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 17  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 18  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 19  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 20  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 21  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 22  | 0  | 0  | 0  | 1224.67 ± 38.04             |
| 23  | 0  | 0  | 0  | 1224.67 ± 38.04             |

3.2. The influence of different factors on the biogas fermentation processes

3.2.1. Analysis of a single factor in biogas production during anaerobic fermentation

To analyse the influence of a single factor on biogas production during anaerobic fermentation, dimensionality reduction analysis can be used, and multiple problems can be transformed to monadic problems. That is, other factors in the model are controlled at the same level, and the regression model, including the single factor and biogas production from fermentation, can be obtained [27,28]. Figure 2 is a one-dimensional model curve of another variable factor when other factors are set at zero, showing that within the test temperature range, as the temperature rises, the gas production from anaerobic digestion trends upwards, indicating that as the temperature increases, the growth and reproduction speed of microorganisms in the system accelerate, and the biogas yield also increases. The influence of substrate concentration on biogas yield shows a slow upward trend, indicating that with an increase in substrate concentration, an increase in the number of microorganisms in the anaerobic digestion system occurs, thereby increasing both gas production and competition among microorganisms, affecting overall gas production efficiency [21]. Within the test range, gas production varies with increasing rotation speed. After reaching the maximum value, the value slowly decreases with increasing rotation speed, indicating that a rotation speed that is too high will destroy the balance among the microflora, thereby affecting gas production.

3.2.2. Influence of factor interactions on biogas production during anaerobic fermentation

We used the DPS data processing system to analyse the interaction among the three factors in our experiment and to obtain the response surface diagram of the interaction. The steeper the response surface is, the greater the effect of the interaction among the factors. The flatter the response surface is, the smaller the influence of the interaction among the factors. Figure 3(a) shows that the larger slope of the response surface representing anaerobic digestion indicates that
the interaction between fermentation temperature and substrate concentration is large. When the substrate concentration is not optimal, total gas production increases rapidly with a rise in temperature.

**Figure 2.** Relationship among individual factors and total gas production.

**Figure 3.** Response surface of biogas fermentation temperature and substrate concentration. (a) Temperature-substrate concentration. (b) Temperature-rotation speed. (c) Substrate concentration-rotation speed.
Biogas production from anaerobic digestion is highest when the temperature is between 1 and 1.68, and the substrate concentration is between 0 and 1. Figure 3(b) shows that the interaction between temperature and rotation speed was relatively large. When the speed is not optimal, the total gas production increases faster with increasing temperature. When the temperature is at the level of 1 to 1.682, the rotation speed is at the level of –1 to 0, and the anaerobic digestion biogas production is highest. Figure 3(c) shows that the slope of the response surface is relatively gradual. When the substrate concentration is at the level of 1 to 1.68 and the rotation speed is at the level of –1 to 0, the anaerobic digestion biogas production is highest.

Among the three influencing factors of biogas fermentation in this study, the most to least influential is as follows: fermentation temperature>TS>stirring speed. Through pairwise interactive analysis, we found that when the experimental stirring speed was constant, cumulative fermentation system biogas production increased with increasing fermentation temperature and TS within a certain range, indicating that a suitable fermentation temperature and TS of the biogas fermentation system played a key role in maintaining and ensuring the most efficient operation of the biogas fermentation system. However, this study only entailed a small-volume laboratory batch experiment. Further research using verification tests and continuous biogas projects will be necessary to ascertain whether the trends we observed in our study hold true in large-scale biogas projects. Given the promising insight of our results, it is worth exploring whether the influence of various factors on cumulative gas production can be optimized by this method in the operation of large-scale biogas projects.

3.3. Model optimization

We applied DPS data analysis software to perform our optimization analysis. Based on the conversion between the coded value and the original value, we found that biogas production is highest when the temperature is 20°C, the substrate concentration is 7.0%, and the rotation speed is 180 r·min⁻¹. The predicted gas production under these conditions was 2857.46 mL, while the test value of gas production proved to be 2820.0 mL. The test value was close to the theoretical value, with a relative deviation between the two of 1.31%. Compared with the values listed in Table 1, the predicted gas production value is higher than what was actually produced. The reason may be that the different levels of various factors have a great influence on gas production. Under the best combination of factor levels – that is, the best process conditions – better gas production can be obtained. Guo et al. [21] studied the mixed anaerobic digestion of kitchen waste and cow manure to produce biogas at a yield of 14.51 L, where the relative deviation between the predicted value and the experimental value was 0.48%, indicating that the analysis method can better predict the actual biogas. Our yield rate research result appears to be consistent with the findings of Guo et al. Although the biogas yield rate in our study was significantly lower than the biogas yield rate of the experimental result in Guo et al. [21], which may be attributable to the different substrate and substrate concentrations. Follow-up experimental research is needed to verify whether such patterns may be a consistent outcome.

3.4. Microbial community analysis

3.4.1. Analysis of alpha diversity of bacterial community

The four parameters identified by Shannon, Simpson, Ace, and Chao can reflect the richness and diversity of the microbial community in a sample [29,30]. As shown in Figures 4 and 5, as the number of sample sequences increases, the Shannon and Chao index curves both rise rapidly and then become flat, indicating that our study fully reflects the richness and diversity of the microbial community structure in the samples we collected. The sample diversity analysis results are shown in Table 3. The coverage of LBY1A through LB6A samples was above 0.99, which indicates that the sequencing results of these six samples objectively reflect the diversity of the sample microbial communities. The Ace and Chao parameters for sample LBY3A are higher than those parameters in other periods of anaerobic digestion, indicating that as anaerobic digestion progresses, the abundance of the microbial community gradually increases and then slowly decreases [31].

3.4.2. Sample Venn diagram analysis

As shown in the Figure 6 Venn diagram presenting the OTU level, there were 1265 bacterial OTUs in the anaerobic digestion system, of which 602 were common, accounting for 47.59% of the total OTUs, showing that these common bacterial groups play a role in the process of anaerobic digestion. In our study, the six samples LBY1A through LB6A had 29, 12, 12, 10, 11, and 10 unique OTUs, indicating that the unique functional flora in each stage is closely related to the fermentation process, with an obvious succession phenomenon at play.

3.4.3. Analysis of bacterial phylogeny during stable operations at different times

We carried out a phylogenetic analysis by phylum of microorganisms that demonstrated stability in different phases of anaerobic digestion, as shown in Figure 7. Among these phyla, Firmicutes, Bacteroidetes,
Figure 4. Rarefaction curve of sample Shanno index during the stable operation period at different time intervals.

Figure 5. Rarefaction curve of sample Chao index during the stable operation period at different time intervals.

Table 3. Richness and diversity of microbial communities during digestion.

| Samples | Sequence No. | OTU No. | Chao index | Shannon index | Simpson index | ACE index | Coverage |
|---------|--------------|---------|------------|---------------|---------------|-----------|----------|
| LBY1A   | 48097.00     | 1066.00 | 1191.11    | 4.94          | 0.022         | 1188.12   | 0.9961   |
| LBY2A   | 50293.00     | 1068.00 | 1230.79    | 4.69          | 0.035         | 1220.70   | 0.9957   |
| LBY3A   | 49169.00     | 1104.00 | 1262.06    | 4.87          | 0.023         | 1287.98   | 0.9953   |
| LBY4A   | 49800.00     | 1075.00 | 1221.58    | 4.89          | 0.019         | 1255.57   | 0.9954   |
| LBY5A   | 49937.00     | 1045.00 | 1203.49    | 4.84          | 0.023         | 1222.42   | 0.9956   |
| LBY6A   | 50136.00     | 1015.00 | 1189.77    | 4.72          | 0.024         | 1196.15   | 0.9954   |
Proteobacteria, Chloroflexi and Actinobacteria were the main dominant bacterial phyla, with relative abundances in LBY1A through LBY6A of 14.5 to 30.2%, 16.1 to 26.3%, 8.8 to 27.1%, 13.8 to 29.1%, and 1.2 to 1.8%, respectively, accounting for more than 60% of the total bacterial sequence. Firmicutes bacteria are isolated mainly from human and animal faeces. Bacteroides bacteria are isolated mainly in an anaerobic environment, such as anaerobic digestion of sludge and intestinal tract contents. Proteobacteria bacteria are derived mainly from anaerobic digestion of sludge and manure [32, 33], consistent with the result of pig manure as a substrate in this study. Studies have found that Firmicutes and Bacteroidetes are the main microflora in the hydrolysis process during anaerobic digestion, Proteobacteria, Chloroflexi, Firmicutes and Bacteroidetes are the main flora in the acidification process, and these microorganisms play important roles in the anaerobic digestion of organic waste [34]. Therefore, the research results of the dominant microflora obtained in this study are consistent with the characteristics of the four-stage microflora of anaerobic fermentation. Meanwhile, this study found that the abundance of Proteobacteria gradually decreased with the progress of anaerobic digestion, while the abundance of Firmicutes gradually increased with the progress of anaerobic digestion. In the process of anaerobic digestion, the bacterial community may gradually change from Proteobacteria to Firmicutes, which is similar to the results of Lim et al. [35].

In addition, each fermentation broth also contained Acidobacteria, Spirochaetes, Planctomycetes and Verrucomicrobia. Acidobacteria can degrade cellulose under anaerobic conditions and produce acetic acid [36], some bacteria in Actinobacteria produce propionic acid [37], and Spirochaetes can convert carbohydrates or amino acids into acetic acid, hydrogen, and carbon dioxide [38]. Although the abovementioned microorganisms are relatively low in abundance, their metabolites provide abundant raw materials for the metabolism of acetic acid and hydrogen methanogens, promote the progress of anaerobic digestion, and play an irreplaceable role in the anaerobic digestion system.

As shown in Figure 8, the abundance of each bacterial group at the taxonomic level of class under conditions of different anaerobic digestion phases exhibits certain differences. The dominant microflora in all
samples were Anaerolineae, Bacteroidia and Clostridium. Methanobacteria and Methanomicrobia demonstrate stability in different phases and are the primary dominant populations, with relative abundance ratios ranging from 0.62 to 0.94% and 3.42 to 7.62%, respectively.

Figure 8 shows that the growth rate and stability of hydrolysing bacteria far exceed the growth rate and stability of methanogens, which may be because methane production is very sensitive to temperature. Because methanogenic bacteria are domesticated at a certain temperature, when the temperature fluctuation exceeds 0.6°C, the temperature fluctuation will affect the effect of anaerobic digestion [39]. Therefore, methanogens, compared with hydrolytic bacteria, grow faster and more stably and are more adaptable to environmental factors such as pH and temperature, consistent with the results of Pan et al. [11].

Figure 9 presents the microbial community structure of the sample at the genus level. The dominant bacterial microflora were Clostridium and Flavobacterium, with relative abundance ranges of 3.15 to 7.34% and 0.15 to 1.20%, respectively. Methanobacterium, Methanoseta and Methanosarcina were the main dominant genera for methanogenesis in this experiment, and they demonstrated stability in different phases, with relative abundance ranges of 0.35 to 0.56%, 2.25 to 5.59%, and 0.85–3.02%, respectively. The relative abundance of Methanospermum was the highest, followed by the relative abundance of Methanosarcina and Methanobacterium, indicating that the methanogenic bacteria of this anaerobic digestion system are mainly acetotrophic methanogens. According to the metabolic kinetic equation of anaerobic digestion methanogenic bacteria (2–5), the acetic acid metabolism methanogenesis pathway (Equation (2)) and the H$_2$/CO$_2$ methanogenesis pathway (Equation (3)) are both exothermic reactions. However, the energy released by the H$_2$/CO$_2$ methanogenesis pathway is significantly higher than the energy released by the acetic acid metabolism methane pathway. Equations (4) and (5) are the processes of propionic acid and butyric acid metabolism to produce hydrogen, respectively, both of which are endothermic reactions. Chin et al. [40] found that low temperatures can lead to the accumulation of a large quantity of fatty acids in anaerobic digestion, creating conditions that can only produce acetic acid. Therefore, the importance of acetic acid methane production under low-temperature environments is much greater than H$_2$/CO$_2$ methane production. Wu et al. [41] also concluded that under low temperatures, the methane-producing pathway of acetic acid metabolism is more likely to occur. These findings are consistent with the results of our study.

Acetic acid methane production

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 3\text{H}_2\text{O} \rightarrow \text{CH}_3\text{CHOH} + \text{CO}_2 \quad \Delta \text{G}^0 = 88.2 \text{kJmol}^{-1}
\] (2)

H$_2$/CO$_2$ methane production

\[
4\text{H}_2 + \text{H}_2\text{O} + 3\text{CO}_2 \rightarrow 2\text{CH}_4 + 6\text{H}_2\text{O} \quad \Delta \text{G}^0 = -135.6 \text{kJmol}^{-1}
\] (3)

\[
\text{CH}_3\text{CH}_2\text{COOH} + 3\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{CH}_3\text{CHOH} + 3\text{H}_2 \quad \Delta \text{G}^0 = 76.1 \text{kJmol}^{-1}
\] (4)

Figure 8. Histogram of microbial relative abundance at the class level.
CH$_3$CH$_2$CH$_2$COOH + 3H$_2$O2CH$_3$COOH
+2H$_2$ΔG$^\circ$ = 88.2kJmol$^{-1}$  

However, in most anaerobic digestion systems, *Methanosarcina* is the dominant microflora [42], which is not what we found in our study, possibly because *Methanosaeta* is an obligate acetotrophic methanogen that uses the adenosine phosphate (AMP)-acetyl-coenzyme A synthetase pathway to generate acetyl-coenzyme A (CH$_3$CO-S-CoA) [43,44]. *Methanosarcina* can not only use acetic acid but also use H$_2$/CO$_2$, as well as methanol and C1 methyl compounds, to produce methane. The sensitivity of *Methanosaeta* to acetic acid is much greater than the sensitivity of *Methanosarcina* [45]. When the acetic acid concentration was within the range of 93.7 to 140.6 mg·L$^{-1}$, *Methanosaeta* was the dominant genus, while *Methanosarcina* was the dominant genus when the acetic acid concentration was within the higher range of 234.3 to 468.6 mg·L$^{-1}$ [45,46]. The concentration of acetic acid in our study was in the range of 38.8 to 252.2 mg·L$^{-1}$, and within this concentration range, *Methanosaeta* exhibited a stronger competitiveness than *Methanosarcina* [47]. Grosskopf et al. [48] found that under low temperature conditions, there are many types of methanogens in the anaerobic digestion system, but they are mainly acetotrophic methanogens such as *Methanoseta*. Zhang et al. [49] found a significant difference in the degree of inhibition of the activity of different nutrient types of methanogens by temperature reduction. Acetic acid-trophic methanogens showed better tolerance at 20–30°C. These results are also consistent with the results of this study.

4. Conclusions

We applied the quadratic regression orthogonal rotation combination method to a biogas production experiment using swine manure as a substrate. We used a built model to optimize the process parameters of anaerobic digestion. We found that fermentation temperature and TS had extremely significant effects on the biogas production efficiency in this experiment. Under optimal process conditions, *Methanosaeta* and *Methanosarcina* were the primary dominant genera of methanogens. Acetotrophic *Methanosaeta* has an absolute advantage, indicating that under low temperature conditions, the acetic acid metabolic methane production pathway is more likely to occur than the H$_2$/CO$_2$ methane production pathway. The concentration of acetic acid in the anaerobic digestion system may also be in the range of 38.8 to 252.2 mg·L$^{-1}$, and *Methanosaeta* is more competitive within this concentration range.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
Authors’ contributions

Binyu Lu and Je Liu conceived and designed the article. Meiyu Jia reviewed and edited the manuscript, Zhanjiang Pei, Fengmei Shi and Yabing Gao performed the experiments and data analysis, and all authors read and approved the manuscript.

Consent for publication

All authors agree with publication on environmental pollutants and bioavailability.

Disclosure statement

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Ethics approval and consent to participate

The article complies with ethics approval and all authors consent to participate.

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References

[1] Heilongjiang Provincial Bureau of Statistics. Heilongjiang statistical yearbook. Beijing: China Statistics Press; 2016.
[2] Rui JP, Li JJ, Li JB, et al. Prokaryotic community structures in biogas plants with swine manure. CIESC J. 2014;65(5):1784–1791.
[3] Zhu FL, Ma YH, Zhou J, et al. Analysis on present situation of pollution and utilization of animal excrement in China. Anhui Agric Sci Bull. 2008;14(13):48–50. (in Chinese with English abstract).
[4] Wang C, Meng J, Li JL, et al. Pollutant removal efficiency in upflow microaerobic biofilm reactor treating manure-free piggery wastewater with low COD/TN ratio and high - 4 NH+ N. CIESC J. 2018;69 (9):4001–4011.
[5] Kettunen RH, Rintala JA. The effect of low temperature (5-29°C) and adaptation on the methanogenic activity of biomass. Appl Microbiol Biotechnol. 1997;48 (4):570–576.
[6] Ahring BK, Ibrahim AA, Mladenovska Z. Effect of temperature increase from 55 to 65°C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. Water Res. 2001;35 (10):2446–2452.
[7] Khanal SK, Wong JWC, Sánchez A, et al. Recent advances in anaerobic digestion. Bioreasur Technol. 2020;11(316):123–127.
[8] Pei ZJ, Li J, Zheng WJ. Biomethanation under psychrophilic conditions and the psychrophilic methanogens. Environ Sci Surv. 2013;32(6):11–13.
[9] Kotsyurbenko OR. Trophic interactions in the methanogenic microbial community of low-temperature terrestrial ecosystems. FEMS Microbiol Ecol. 2005;53:3–13.
[10] Chang H, Li HH, Yan ZY. The effect of total solid concentration on swine manure continuous anaerobic fermentation under medium temperature. J Shannxi Univ Ser Technol. 2017;35(4):27–31.
[11] Pan YF, Li WZ. Discussion on controlling techniques of fuel evaporative pollutant of gasoline automobile. J Agric Mechanization Res. 2008;30(1):193–196.
[12] Hoffmann RA, Garcia ML, Veskivar M, et al. Effect of shear on performance and microbial ecology of continuously stirred anaerobic digesters treating animal manure. Biotechnol Bioeng. 2008;100(1):38–48.
[13] Halalsheh M, Kassab G, Yazajeen H, et al. Effect of increasing the surface area of primary sludge on anaerobic digestion at low temperature. Bioresour Technol. 2011;102(2):748–752.
[14] Qiao JT, Guo RB, Yuan XZ, et al. Phylogenetic analysis of methanogenic corn stalk degrading microbial communities. Environ Sci. 2013;34(4):1531–1539.
[15] Allen MA, Lauro FM, Williams TJ, et al. The genome sequence of the psychrophilic archaeon, methanococcoides burtonii: the role of genome evolution in cold adaptation. Isme J. 2009;3(9):1021–1035.
[16] Zhang G, Jiang N, Liu X, et al. Methanogenesis from methanol at low temperatures by a novel psychrophilic methanogen, “Methanolobus psychrophilus” sp nov. prev. Appl Environ Microbiol. 2008;74(19):6114–6120.
[17] Conrad R, Klose M. Anaerobic conversion of carbon dioxide to methane, acetate and propionate on washed rice roots. FEMS Microbiol Ecol. 1999;30(2):147–155.
[18] Wang DQ, Meng J, Zhang Q, et al. Microorganism bacteria colony to enhance biogas output. Environ Sci Technol. 2009;32(1):31–34.
[19] Zhang X. Study on exogenous promoter of manure biogas fermentation at cold region. Shenyang: Shenyang Jianzhu University; 2014. (in Chinese with English abstract).
[20] Lu BY, Pei ZJ, Shi FM, et al. Optimization on pig manure anaerobic digestion based on response surface methodology. China Biogas. 2018;36(4):7–12. (in Chinese with English abstract).
[21] Li Y, Li L, Dalie Z, et al. Process optimization of anaerobic fermentation with mixed material of food garbage and cow dung. Trans Chinese Soc Agric Mach. 2012;43(Z1):180–185.
[22] Wu WF, Chen JY, Cheng RM, et al. Optimization of microwave-assisted extraction parameter for unsaturated fatty acids in raw potato flours and its application. Trans Chin Soc Agric Eng (Transactions of the CSAE). 2018;34(6):278–284. (in Chinese with English abstract).
[23] Díaz-de-cerio E, Arráez-Román D, Segura-Carretero A, et al. Establishment of pressurized-liquid extraction by response surface methodology approach coupled to HPLC-DAD-TOF-MS for the determination of phenolic compounds of myrtle leaves. Anal Bioanal Chem. 2018;410(15):1–11.
[24] Duan YF. Analysis and optimization of the influence of medium temperature mixed anaerobic fermentation of biogas production. Harbin: Harbin Institute of Technology; 2015. (in Chinese with English abstract).
[25] Chengdu Institute of Biology, Chinese Academy of Sciences. Conventional analysis of biogas fermentation. Beijing: Beijing Science and Technology Press; 1984. p. 48–51. (In Chinese with English abstract).
[26] State Environmental Protection Administration. 2002 water and wastewater monitoring and analysis method. 4th. Beijing: China Environmental Science Press; 2002. p. 211–281. (In Chinese with English abstract).
[27] Wang XH, Xu X, Shan Q, et al. Optimization of pretreatment process for corn straw anaerobic digest. Trans Chin Soc Agric Eng (Transactions of the CSAE). 2018;34 (23):246–253. (In Chinese with English abstract).
[28] Rostamifasi Z, Pasalari H, Mohammadi F, et al. Heterogeneous catalytic degradation of methylpara-ben using persulfate activated by natural magnetite; optimization and modeling by response surface methodology. J Chem Technol Biot. 2019;94(6):81–86.
[29] Huan HL, Gu HR, Zhang X, et al. Change research of carbon, nitrogen and microbial community structure in different periods of fermentation bed for pig. Trans Chin Soc Agric Eng (Transactions of the CSAE). 2018;34 (Supp.):27–34. (In Chinese with English abstract).
[30] Tian H, Fotidis IA, Mancini E, et al. Acclimation to extremely high ammonia levels in continuous biomethanation process and the associated microbial community dynamics. Bioresour Technol. 2017;247 (1):616–623.
[31] Ma W. Methane-producing composite strains anaerobic digestion and microbial community structure analysis. Harbin: Harbin Institute of Technology. 2015. (In Chinese with English abstract).
[32] Bomberg M, Mäkinen J, Salo M, et al. High diversity in iron cycling microbial communities in acidic, iron-rich water of the Pyhäsalmi Mine, Finland. Geofluids. 2019;2019(3):1–17.
[33] Tian XL, Wang CB, Bao XG, et al. Crop diversity facilitates soil aggregation in relation to soil microbial community composition driven by intercropping. Plant Soil. 2019;19(2):809–821.
[34] Wang P, Wang HT, Qiu YQ, et al. Microbial characteristics in anaerobic digestion process of food waste for methane production—a review. Bioresour Technol. 2017;248:29–36.
[35] Lim JW, Ge T, Tong YW. Monitoring of microbial communities in anaerobic digestion sludge for biogas optimization. Waste Manage. 2018;71:334–341.
[36] Wang GH, Liu Jl, Yu ZH, et al. Research progress of acidobacteria ecology in soils. Biotechnol Bulletin. 2016;32(2):14–20.
[37] Nelson MC, Morrison M, Yu Z. A meta-analysis of the microbial diversity observed in anaerobic digesters. Bioreasur Technol. 2011;102(4):3730–3739.
[38] Fernandez A, Huang S, Seston S, et al. How stable is stable? Function versus community composition. Appl Environ Microbiol. 1999;65(8):3697–3704.
[39] Wei HJ. Operation characteristics analysis of anaerobic digestion of sludge in Bailinggang wastewater treatment plant. Water Wastewater Eng. 2018;44(10):53–55.
[40] Chin K-J CR. Intermediary metabolism in methano-genic paddy soil and the influence of temperature. FEMS Microbiol Ecol. 1995;18(2):85–102.
[41] Wu MR, Zhang R, Zhou J, et al. Effect of temperature on methanogens metabolic pathway and structures of predominant bacteria. CIESC J. 2014;65(5):1602–1607.
[42] Kong DW, Zhang KQ, Fang F, et al. Study of microbial community and biogas production in anaerobic digestion of pig manure with digested slurry recirculation. J Agro-Environ Sci. 2018;37(3):559–566.
[43] Smith KS, Ingram-Smith C. Methanosaeta, the forgotten methanogen? Trends Microbiol. 2007;15(4):150–155.
[44] Jetten MSM, Stams AJM, Zehnder AJB. Methanogenesis from acetate: a comparison of the acetate metabolism in Methanotherbo seohrigenii and Methanosarcina spp. FEMS Microbiol Lett. 1992;88(3–4):181–198.
[45] Liu YC, Whitman WB. Metabolic, phylogenetic and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci. 2010;1125(1):171–189.
[46] De Vrieze J, Hennebel T, Boon N, et al. Methanosaeta: Therediscovered methanen for heavy duty biomethanation. Bioresour Technol. 2012;112(5):1–9.
[47] Yu D, Kurola JM, Lahde K, et al. Biogas production and methanogenic archaeanac community in mesophilic and thermophilic anaerobic co-digestion processes. J Environ Manage. 2014;143(10):54–60.
[48] Grosskopf R, Janssen PH, Liesack W. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16 S rRNA gene sequence retrieval. Appl Environ Microbiol. 1998;64(3):960–969.
[49] Zhang LG, Ban QY, Li JZ. Response of methanogens on temperature stress in an UASB reactor. China Environ Sci. 2016;36(4):1082–1086. (In Chinese with English abstract).