

Original Research

Potential of Rapid Anaerobic Fermentation on Animal Slurry for Biogas Production and Storage of Biogas Slurry

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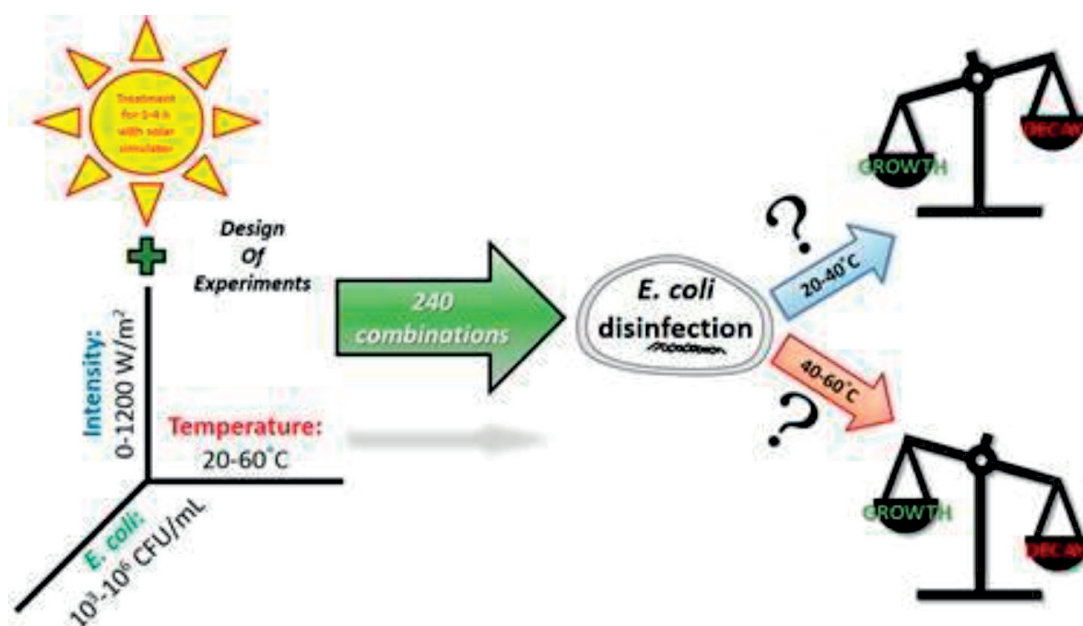
Abstract

A study was designed aiming at the degradation of organic matter in liquid phase of pig manure, the inactivation of pathogenic microorganisms and biogas production at different storage temperatures. A low cost rapid anaerobic fermentation, and biogas sewage storage system was constructed. After the four-day anaerobic fermentation, three treatments were set up: the average temperature of spring and autumn (16.1°C), summer (25°C), and winter (0.6°C) in Yantai City was used as storage temperature for 90 days, respectively. The results showed that the biogas potential of anaerobic fermentation and biogas sewage storage at 16.1°C and 25°C was higher than that stored at 0.6°C. During the experiment, total solids (TS) and organic matter contents in fecal sewage were decreased with time, and the value was determined at 25°C. Total phosphorus and potassium contents in biogas sewage did not change significantly over time, but the total nitrogen content decreased. The content of the 5 day biological oxygen demand (BOD₅), the chemical oxygen demand (COD_{Cr}) and suspended solid concentration (SS) in fecal sewage showed a downward trend over time. The egg mortality of *Ascaris lumbricoides* increased in different degrees under the three storage conditions. The number of *E. coli* in biogas sewage stored at 0.6°C and 16.1°C showed a downward trend, and the number of *E. coli* in biogas sewage stored at 25°C was significantly higher than at 0.6°C and 16.1°C. The comprehensive analysis showed that at temperature 25°C, the an-aerobically produced fecal sewage after fermentation had best potential for biogas production.

Keywords: anaerobic fermentation, substrate concentration, liquid phase of pig manure, temperature, post storage

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Graphical Abstract



Novelty Statement

The degradation of organic matter in liquid phase of pig manure, the inactivation of pathogenic microorganisms and the development of biogas at different storage temperatures has not yet been reported in the literature. Hence the process of biogas production and biogas sewage storage, the total output of biogas and physico-chemical characteristics of the manure were studied through developing a set of low cost fast and anaerobic fermentation biogas sewage storage system.

Introduction

In 2011, China raised 471 million pigs. It accounts for about 50% of the world's pig production [1]. The generation and inappropriate management of animal manure cause serious environmental problems. Animal manure is usually spread on land near confined feeding operations, which leads to a series of problems, such as the contamination of surface water and groundwater with pathogens, odor emission, loss of a potential green energy source, accumulation of excess phosphate (PO_4^{3-}) in soil, and deterioration of biological ecosystems [2, 3]. Anaerobic digestion technology is regarded as one of the most sustainable technologies due to its low energy consumption, high efficiency and new energy production in the process of treating livestock and poultry wastes [4]. Since China's traditional biogas project anaerobic fermentation gas production and aerobic fermentation fertilizer production are independent of each other, and the cycle of gas production fertilizer production is long, and the quality of organic fertilizer is poor, which affects the efficient operation of the project [5],

with small farming enterprises and holdings it is quite difficult to popularize the feces treatment of small and medium-sized aquaculture as an enterprise. Therefore, anaerobic fermentation for biogas production is a reliable technology at present. It is of great significance to increase the proportion of this technology in the treatment of livestock and poultry manure in China. The use of biogas technology is very important for nutrient recycling in agriculture [6-7]. The liquid produced along with methane as a result of anaerobic fermentation is rich in organic matter, nitrogen, phosphorus, potassium and some other elements. Moreover, the fermented residues could be used as semi-finished fertilizer. The approaches used for treating the wastewater from farms usually are based on anaerobic digestion. However, further process improvements are still needed to solve the issues associated with biogas residues, which may be more expensive. In general, environmentally sustainable and cost-effective approaches to treat livestock manure would be beneficial for addressing the urgent concerns of surrounding livestock waste [8-9]. Although anaerobic digestion of manure has been achieved in rural Asia, farm-scale factory capital cannot always be offset by biogas production [10-11]. Therefore, developing a low cost rapid anaerobic treatment plants and biogas production is the key to solve the problem of manure treatment and its application to the field as a source of nutrients.

Soft biogas project consists of anaerobic reactor in which impermeable membrane are used to seal the bottom and top of the tank. It has the characteristics of low operation cost, low project cost, high sewage treatment efficiency, and high gas production. In recent years, it has been preferred by livestock and poultry breeding enterprises, especially small and medium-sized aquaculture enterprises. Most

of the soft biogas engineering systems adopt room temperature fermentation, which often requires a large pool capacity, while most of the conventional biogas engineering systems adopt medium and moderately high temperature fermentation with smaller volume. For safe return of biogas sewage into the field, the retention time of material water conservancy is generally 7 days for fermentation under high temperature and 21 days for fermentation under medium temperature. In both cases, the material hygiene is ensured and fecal pollution is well controlled. On the other hand, when fermentation is done at room temperature, the retention time of the material is prolonged up to 60-90 days, increasing the interval period for fecal pollution. If the soft biogas project is equipped with heating equipment, it means that the construction and operation costs will be greatly increased. The results of preliminary pre-experiment showed that under the condition of medium temperature fermentation, the biogas production of the material with a total solid concentration of 5% exceeded 50% of the biogas potential in 4 days, and then the biogas sewage was stored at room temperature for secondary fermentation, hence reducing the investment cost. During the whole process of biogas production and biogas sewage storage, the total output of biogas and physico-chemical characteristics of the manure were studied. Since the literature has not yet been reported, therefore, liquid phase of pig manure was taken as the research object in this study, aiming at the degradation of organic matter in liquid phase of pig manure, the inactivation of pathogenic microorganisms and the development of biogas at different storage temperatures. This study was conducted on a laboratory scale and the objectives of this study were (1) to construct a low cost rapid anaerobic fermentation and biogas sewage storage system, (2) to provide a scientific basis for fecal sewage treatment and resource utilization in small and medium-sized aquaculture enterprises.

Materials and Methods

Test Materials

This experiment started in March 2019 and was conducted at the Biomass Energy Science and Technology Research Center, Yantai Research Institute of China Agricultural University. The feces water (fecal sewage), used in the experiment, was taken from a pig farm in Muping District, Yantai City. After the initial preparations, fecal sewage (raw material) was analyzed for the total solid (TS) content (5%), organic matter (2.56%), pH (7.39), suspended solid concentration (SS) (1447.2 mg/L), the content of the 5 day biological oxygen demand (BOD_5) (4734.0 mg/L), the chemical oxygen demand (COD_{Cr}) (11237.0 mg/L), *Escherichia coli* population (17000 flu/mL), and mortality of ascaris egg (37.5%).

Experimental Design

A self-designed rapid anaerobic fermentation and biogas sewage post-storage device (Fig. 1) was used in the experiment. The effective volumes of the anaerobic reactor and biogas sewage storage tank were 20 L and 520 L, respectively. The film-coated materials were manufactured with high density polyethylene (HDPE) models. In the anaerobic reactor, fermentation was done at medium temperature (30°C) with retention time of 4 days for water conservancy. In order to accurately simulate the changes in physical and chemical properties of biogas sewage during storage in the Yantai City, Shandong Province, the average temperature of spring and autumn (16.1°C), summer (25°C) and winter (0.6°C) was used as storage temperature for 90 days, respectively.

Methods for Sample Collection and Parameter Determination

After 4 days of anaerobic fermentation of liquid phase of pig manure, 50 ml biogas sewage sample was collected after every 10 days before the biogas sewage storage, and then after storage, the samples were collected at 60-day interval for the determination of physical and chemical properties of biogas sewage. These properties included TS content, organic matter, pH, total nitrogen, total phosphorus, total potassium, suspended solid concentration (SS), the 5 day biological oxygen demand (BOD_5), the chemical oxygen demand (COD_{Cr}), *Escherichia coli* (*E.coli*) flora number and *Ascaris lumbricoides* egg mortality. Duplicate samples for analysis of TS were analyzed following the oven drying method [12]. Total organic carbon (TOC) were determined by the Wet Oxidation Method with 133 mmol/L $K_2Cr_2O_7$ at 170-180°C [13] and pH was determined with the pH meter and pH probe. Total phosphorus was determined by using Model 727 spectrophotometer. Total nitrogen concentrations were determined by the Kjeldahl method (4500-Norg) [14]. For BOD_5 determination, a 300 ml airtight bottle, half-filled with saturated oxygen solution (aerated water), was used. One ml mixed sample was transferred into the bottle, and then the bottle volume was made by the aerated water. The BOD_5 was computed from the difference between the initial and final dissolved oxygen concentrations after 5-day incubation under 20±1°C. To confirm the *E. coli* in samples, a single colony per sample was randomly selected from a countable plate of each medium type (black colony on XLD agar, blue colony with associated gas on the Petrifilm *E. coli*/Coliform Count plate) and streaked onto Eosin methylene blue AGAR (EMB). The Eosin methylene blue AGAR plates were incubated for 24 h at 37°C. Isolates were confirmed based on Gram stain reaction, cell morphology, and biochemical characterization. Isolation of eggs of intestinal parasites was preceded by loosening its structure by adding

Table 1. Effect of anaerobic fermentation and storage of biogas sewage on the TS in animal sewage.

Treatment		TS content (%)		
Raw animal sewage		5		
Anaerobic fermentation for 4 days		4.93		
Biogas sewage storage	Time (d)	0.6 °C	16.1 °C	25 °C
	10	4.93a	4.93a	4.92a
	20	4.93a	4.92a	4.92a
	30	4.93a	4.91b	4.90b
	45	4.92a	4.91b	4.88c
	60	4.92a	4.89c	4.84d
	75	4.91b	4.89c	4.82e
	90	4.91b	4.88c	4.82e

1600 ml of a detergent (0.0025% Tween-20 solution) and long-lasting (4 h) mechanical mixing of a sample of 50 g of sludge, thereby releasing eggs from the so-called floccules. Subsequently, flotation was used in a high volume of flotation solution NaNO_3 with a specific gravity 1.36 g/mL (the ratio between the sample mass and water volume was 1:16) followed by centrifugation (2500 g for 10 min). Eggs of the *Ascaris* spp settled on filters and were confirmed based on microscopic observations (zoom 200X), and followed by periodic observation under a microscope at 3-day intervals for a period of 2 weeks. Eggs with a clear deformation (granulated cytoplasm, damaged sheath, empty sheath) were considered to be dead (non-invasive) [15].

The volume of the biogas was recorded during the process of anaerobic fermentation of the manure and storage of the biogas sewage. Gas production and methane content were determined by Gas Flowmeter MF5700 and PTM-600 portable compound gas analyzer, respectively. The theoretical methane production test of liquid phase of pig manure is based on the fully automatic methane potential test system provided by Hubei Rockek Instrument Co., Ltd.

Statistical Analysis of Data

Data were statistically analyzed by using Excel 2010 and SPSS 19.0. The least significant difference (LSD) test was used to determine the significant difference among treatment means.

Results

Table 1 shows that TS content in the liquid phase of pig manure was 0.07% lower than that in the raw manure after 4 days of anaerobic fermentation at medium temperature (30°C). During the biogas sewage storage at 0.6°C, the TS content did not change significantly up till 60-day storage. After 90 days of storage, TS content decreased by 0.02%. While at 16.1°C storage, the TS content of biogas sewage did not change in the first 20 day-storage. The TS content decreased significantly by 0.02% after 30 days and 0.05% after 90 days. At 25°C, the decrease rate for TS content was more rapid. The TS contents decreased significantly almost after every 20-day interval. After 75 and 90 days, there was

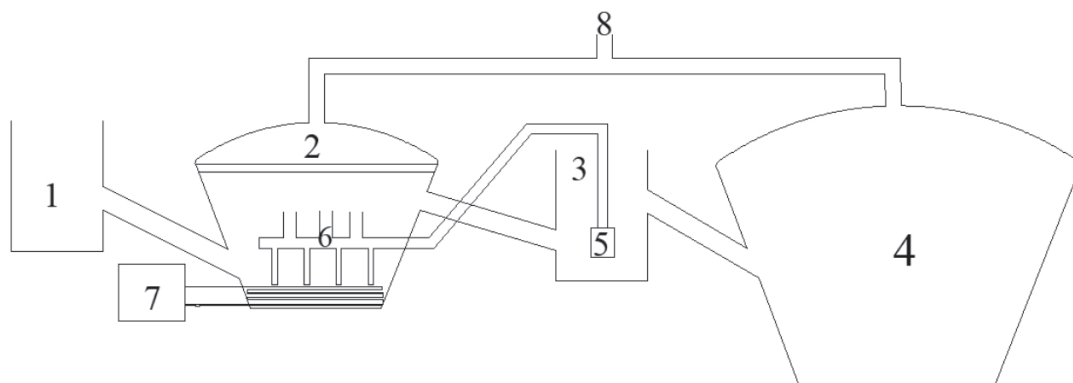


Fig. 1. Schematic diagram of various parts of anaerobic fermentation reactor and biogas sewage storage system. Feed Pool (1), Anaerobic reactor (2), Outfall pool (3), Biogas sewage storage pool (4), High pressure pump (5), Sand removal and shell breaking pipeline (6), Heating equipment (7), and Biogas utilization (8).

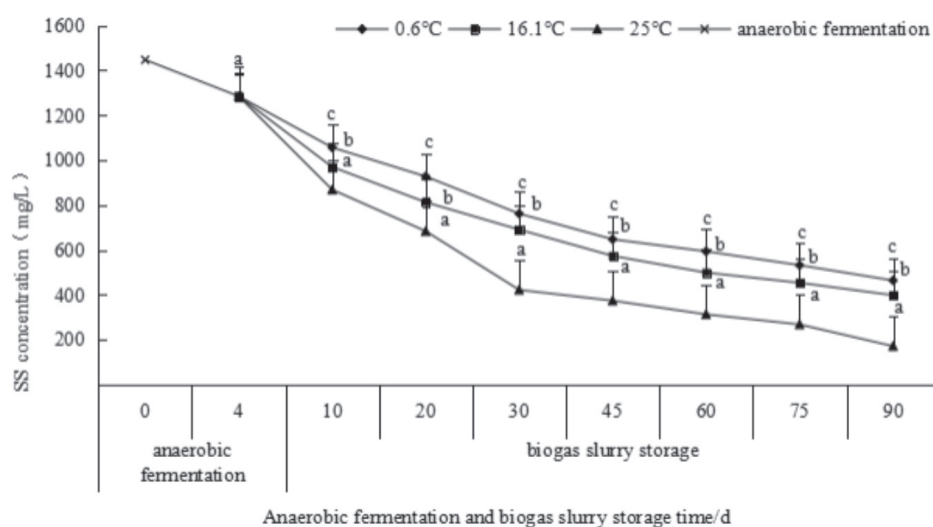


Fig. 2. Effect of anaerobic fermentation and biogas sewage storage on the SS content of biogas sewage.

0.18% decrease in the TS content. Data shows that TS content changes during the storage of biogas sewage are inversely related to storage time and temperature.

The SS content in fecal sewage and biogas sewage decreased whether it was fermented or stored after biogas sewage (Fig. 2). The SS content of biogas sewage decreased by 63.9% and 69.0% after storage at 0.6°C and 16.1°C for 90 days, respectively, while the SS content decreased by 86.7% after storage at 25°C for 90 days, which was 1.36 times and 1.26 times higher than at 0.6°C and 16.1°C, respectively. In case of SS content also, inverse relationship between the duration and temperature for the biogas sewage was observed.

After 4-day anaerobic fermentation (at 30°C), methane production reached 86.9% of biogas production capacity (Table 2). When the biogas sewage was stored at 0.6°C for 90 days, no biogas was produced, and when the storage temperature raised to 16.1°C and 25°C, the methane production was 7.8% and 12.1% of the biogas production capacity, respectively. For the total methane production, the biogas potential of anaerobic fermentation and biogas sewage storage at 0.6°C is only 86.9%, while the biogas potential of anaerobic fermentation and biogas sewage storage at 16.1°C and 25°C is 8.98% and 13.92%, respectively, higher than when stored at 0.6°C.

The initial pH concentration of raw fecal sewage was 7.39, which reduced to 6.63 after 4-day fermentation. In the post-storage stage, the variations among pH were negligible and trends were similar at 0.6°C, 16.1°C and 25°C. The lowest value for the pH was of 6.29 on the 10th day of storage, while the lowest pH values at 16.1°C and 0.6°C were observed on 20th day of storage which were 6.34 and 6.47, respectively. After storage for 20 to 90 days, the pH concentrations of biogas sewage at 0.6°C and 16.1°C remained basically unchanged, while the pH concentration of biogas sewage at 25°C increased gradually at first and reached 6.8 on the 30th day, and then gradually decreased to 6.68 on the 90th day.

The COD_{Cr} and BOD₅ content of fecal sewage decreased by 2.3% and 11.2%, respectively (Table 3). The COD_{Cr} and BOD₅ content of biogas sewage stored at 25°C decreased by 99.1% and 99.0% after 90 days, and at 16.1°C decreased by 83.0% and 87.2%, respectively, and at 0.6°C decreased by 82.0% and 80.8%, respectively. The data showed a positive correlation between storage time and storage temperature for the COD_{Cr} and BOD₅ content in the biogas sewage.

Total phosphorus and potassium contents in fecal sewage did not change significantly during the whole experimental period (Table 4). After the anaerobic fermentation (25°C), the total nitrogen content of fecal sewage decreased by 0.02%. During the post-storage

Table 2. Effect of anaerobic fermentation and storage of biogas sewage on the biogas potential of animal sewage.

Treatment	Actual methane production (mL)	Theoretical methane production (mL)	Biogas potential (%)
Anaerobic fermentation for 4 days	6395.2a	7359.3	86.9a
Biogas sewage storage at 0.6°C	0d	7359.3	0d
Biogas sewage storage at 16.1°C	574.0c	7359.3	7.8c
Biogas sewage storage at 25°C	890.5b	7359.3	12.1b

Table 3. Effect of anaerobic fermentation and storage of biogas sewage on COD_{Cr} and BOD₅ of biogas sewage.

Treatment		COD _{Cr} (mg/L)			BOD ₅ (mg/L)		
Raw animal sewage		11250.0			4734.0		
Anaerobic fermentation for 4 days		10994.0			4205.5		
Biogas sewage storage	Time (d)	0.6°C	16.1°C	25°C	0.6°C	16.1°C	25°C
	10	9037.0a	8593a	7987.0a	3802.8c	3615.9a	3360.9a
	20	7837.3b	6352.4b	5947.0b	3597.9a	2873.1b	2502.5b
	30	6417.7c	4237.6c	3643.1c	3090.6bc	2083.2c	1533.0c
	45	5131.4cd	3678.4c	2317.4d	2093.6c	1500.8c	945.5d
	60	4217.3d	2812.5cd	1928.3d	1720.7c	1157.5d	786.7d
	75	3124.1d	1897.6d	1123.4e	1274.6c	774.2d	458.3e
	90	1978.4e	1317.5d	100.5f	807.2d	537.5e	41.0f

period, the total nitrogen content of biogas sewage stored at 0.6°C decreased by 0.01%, at 16.1°C decreased by 0.03% in 90 days, and at 25°C decreased by 0.07% in 90 days.

The organic matter content of fecal sewage decreased by 0.35% compared with that of raw fecal sewage (Fig. 4). The organic matter content of biogas sewage stored at 0.6°C, 16.1°C and 25°C decreased by 0.09%, 0.14% and 0.21%, respectively, after 90 days of storage. The data showed that the change in organic matter content of biogas sewage during post-storage was positively correlated with storage temperature.

As evident from Table 5, the number of *E. coli* groups increased slightly to 19000 flu/mL after the anaerobic fermentation (at 30°C). In the post-storage stage, number of *E. coli* were decreased significantly at 0.6°C, 16.1°C and 25°C after 90-day storage. The number of *E. coli* increased slightly on the 10th to 30th day after storage at 25°C, decreased on the 45th to 90th day, and finally decreased to 900 flu/mL. On the 10th day

of storage, the egg mortality of *Ascaris lumbricoides* stored at 25°C reached 95.1%, and on the 30th day after storage, the mortality rate of *Ascaris lumbricoides* eggs was 95.1%. The egg mortality of *Ascaris lumbricoides* reached up to 95.0% and 96.1% at 0.6°C and 16.1°C, respectively.

Discussion

The pH concentration of different treatments reached the lowest value on the 10th and 20th day after the start of storage stage (Fig. 3). It might be due to organic acids production during anaerobic digestion, which affected the pH, such as acetate, and that the reaction rate can be obtained by adjusting the pH [16-19]. Then pH raised slowly under each treatment condition. This was due to a period of adaptation and cultivation of methanogens occurs later, the acidic products are used for methane formation, and the pH increases accordingly [20].

Table 4. Effect of anaerobic fermentation and storage of biogas sewage on total nitrogen, total phosphorus and total potassium content of biogas sewage.

Treatment		Total nitrogen (%)			Total phosphorus (%)			Total potassium (%)		
Raw animal sewage		0.061			0.051			0.052		
Anaerobic fermentation for 4 days		0.059			0.050			0.052		
Biogas sewage storage	Time (d)	0.6°C	16.1°C	25°C	0.6°C	16.1°C	25°C	0.6°C	16.1°C	25°C
	10	0.059a	0.059a	0.059a	0.050a	0.050a	0.050a	0.052a	0.051a	0.050a
	20	0.059a	0.058a	0.057b	0.050a	0.049a	0.050a	0.052a	0.050a	0.050a
	30	0.058a	0.058a	0.056b	0.050a	0.049a	0.050a	0.051a	0.050a	0.050a
	45	0.058a	0.058a	0.056b	0.050a	0.049a	0.048b	0.051a	0.050a	0.050a
	60	0.058a	0.057b	0.055c	0.050a	0.049a	0.048b	0.051a	0.050a	0.049b
	75	0.058a	0.056b	0.054c	0.050a	0.048b	0.048b	0.050b	0.050a	0.049b
	90	0.058a	0.056b	0.052d	0.049a	0.048b	0.048b	0.050b	0.049	0.049b

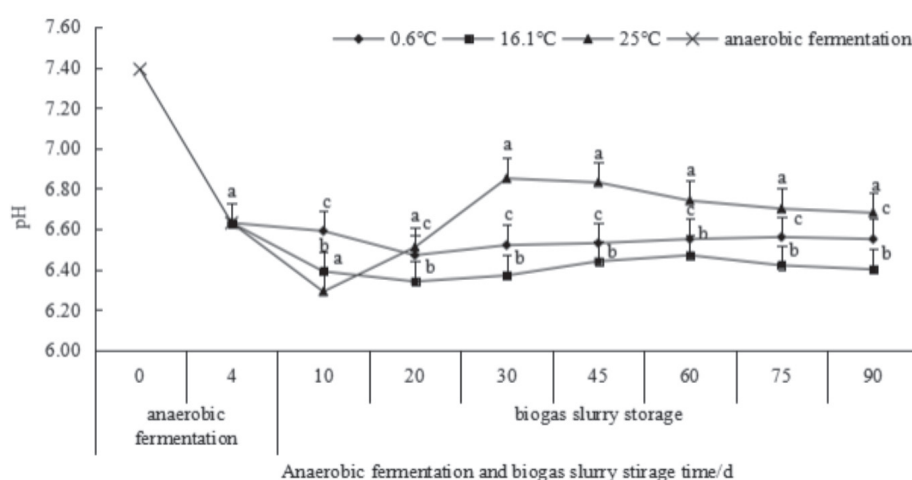


Fig. 3. Effect of anaerobic fermentation and biogas sewage storage on the pH of biogas sewage.

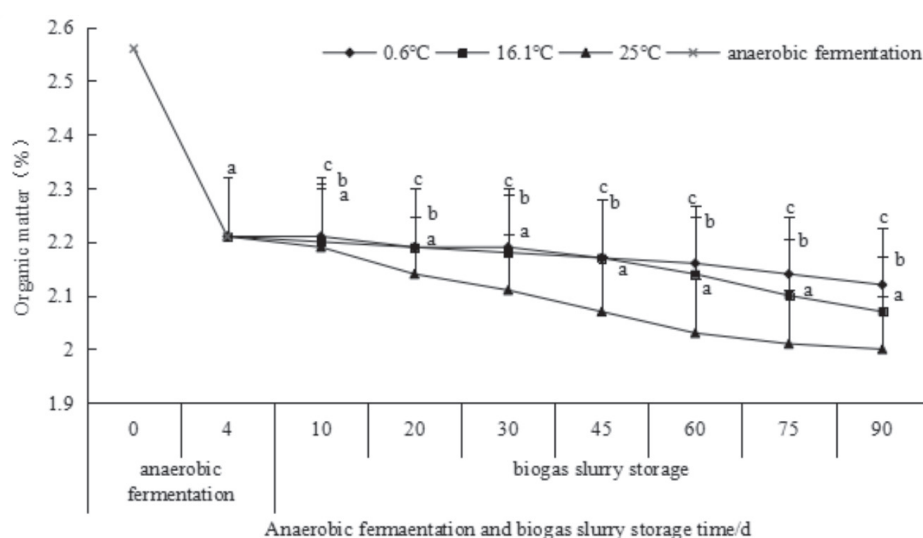


Fig. 4. Effect of anaerobic fermentation and biogas sewage storage on the organic matter of animal sewage.

The downward trend of COD_{Cr} and BOD_5 concentrations in all treatments (Table 3) were due to nutrients usage by various microbial groups in the fermentation system during anaerobic fermentation and post-storage, and the conversion of fermented microorganisms into some gases by using various nutritional elements in the fermented materials. The COD_{Cr} and BOD_5 contents in fecal sewage decreased with the fastest rate at 25°C. The COD_{Cr} and BOD_5 removal rate may increase with raising temperature within a certain temperature range. The removal rate began to increase with continual increase of temperature, because there were obvious differences in the categories and action of bacteria crowd in the fermentation system under different fermentation temperatures [21]. The decrease of temperature decreased the removal rate of COD_{Cr} and BOD_5 [17].

Table 4 shows that during the whole experimental period, the content of total nitrogen in biogas sewage

showed a downward trend. This might be due to the conversion of some nitrogen into ammonium nitrogen in biogas sewage [22]. However, there was no significant difference in the contents of total phosphorus and potassium in biogas sewage during storage time. This may be due to very low consumption of phosphorus and potassium by methanogens and other microorganisms during the experiment.

The data in Table 1 and Fig. 4 show that the concentrations of organic matter and TS in fecal sewage decreased rapidly during anaerobic fermentation, but there was no significant change in organic matter content in the later-storage stage, which indicated that the organic matter in fecal sewage could produce more methane rapidly in the stage of anaerobic fermentation.

Methane production by anaerobic digestion is generally divided into four stages: hydrolysis, acid production, acid dehydrogenation and production of methane. Methane (CH_4) is the main component in

Table 5. Effect of anaerobic fermentation and storage of biogas sewage on the number of *Escherichia coli* and the mortality of *Ascaris lumbricoides* eggs.

Treatment		The number of <i>Escherichia coli</i> (flu/mL)			The mortality of <i>Ascaris lumbricoides</i> eggs (%)		
Raw animal sewage		17000			37.5		
Anaerobic fermentation for 4 days		19000			76.8		
Biogas sewage storage	Time (d)	0.6°C	16.1°C	25°C	0.6°C	16.1°C	25°C
	10	10000a	9000a	20000a	81.3c	83.9c	90.3c
	20	10000a	7000a	21000a	88.4c	94.9b	95.1c
	30	9000a	6000b	22000a	95.0b	96.1a	97.6b
	45	7000b	6000b	15000b	96.4a	96.4a	97.9b
	60	5000c	4000c	9000c	96.8a	97.1a	98.4a
	75	3000c	2900c	3100d	97.2a	97.2a	98.5a
	90	1000d	1700d	900d	97.4a	97.2a	98.9a

gas production, so the concentration of CH_4 gradually increases [1]. The factors affecting biogas production by anaerobic fermentation include temperature and pH [23-26]. The results showed that the biogas potential of anaerobic fermentation and biogas sewage storage at 16.1°C and 25°C was 8.98% and 13.92% higher than that at 0.6°C, respectively. Fermentation temperature is an important factor affecting anaerobic fermentation. Generally speaking, most methanogens can survive at 10-30°C. Therefore, under the storage condition of 0.6°C, methanogens activity was inhibited and it was difficult to produce methane. Biogas sewage stored at 16.1 °C and 25 °C. There was significant difference in methane content in the gas produced (Table 2), which was due to the good storage conditions available at 25°C for rapid decomposition of organic matter, more and more carbon source release, methanogen bacteria had a nice development and reproduction period, the activity of methanogens in biogas sewage is higher, and CO_2 produced by microbial respiration in biogas sewage is more converted into methane [27-29].

Fig. 2 shows that under all temperature conditions, the content of SS in fecal sewage showed a downward trend, and the rate of decrease of biogas sewage stored at 25°C was higher and decreased by 86.7% in the later-storage stage. This might be due to the natural deposition of suspended matter in fecal sewage during the test period. In a certain temperature range, high temperature enhances the settling rate of solid particles.

The number of *E. coli* groups were lower in biogas sewage stored at 0.6°C and 16.1°C than that stored at 25°C (Table 5). The number of *E. coli* in biogas sewage stored at 25°C were also higher than that in raw fecal sewage and medium temperature anaerobically fermented biogas sewage. This may be due to spring and autumn (16.1°C), and winter (0.6°C) conditions, that were clearly more detrimental to *E. coli* than the summer (25°C) conditions. Anaerobic fermentation has an effective killing effect on *E.coli* which is consistent

with the research results of Ye [30]. In addition, the inactivation rate of *Escherichia coli* at high temperature reached 94.3%, higher than that at the medium temperature, which was similar to the findings of Song [31]. Frequent fluctuation of ambient temperature, ca. 0.6°C, in winter conditions may have caused more rapid bacterial death. During anaerobic fermentation and post-storage, the egg mortality of *Ascaris lumbricoides* in biogas sewage increased to varying degrees under all the three treatments. The mortality rate of larvae eggs in all samples can reach above 95%, meeting the standard of innocuous excrement of livestock and poultry. This may be due to the acid produced during the experiment, which inhibits *Ascaris* eggs.

Conclusion

The results showed that low cost rapid anaerobic fermentation could be achieved by controlling the total solid concentration of fecal sewage at about 5% and fermentation at 30°C. Better gas production effect and harmless treatment effect can be obtained by storing the fermented biogas sewage at 25°C. Therefore, considering the safety and cost of biogas sewage, this kind of software biogas project can be carried out to solve the current problems arising out of livestock and poultry breeding waste treatment and returning the extracted nutrients back to the field.

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Conflict of Interest

The authors declare no conflict of interest.

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