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**Original Research Paper** 

# Start-up Performance of Chicken Manure Anaerobic Digesters Amended with Biochar and Operated at Different Temperatures

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## ABSTRACT

Batch anaerobic digestion was conducted using chicken manure (CM) and biochar over the course of 30 days in separate 15, 25, 35, 45, 55, and 65°C reactors. Daily volumetric methane production, hydrogen sulfide concentration, pH, oxidation-reduction potential (ORP), alkalinity (Alk), and soluble chemical oxygen demand (SCOD) were measured. Results showed that the anaerobic reactors started up successfully under all temperature conditions. It needed one week for 35°C and 45°C reactors to peak the methane production rate, 18 days for 25°C and 65°C reactors, and over one month for 55°C reactor, but it was not the peak as the methane production rate for 15°C reactor. The hydrogen sulfide concentrations at various temperature conditions were not more than 538 ppm, and the difference was small, except 65°C reactors with 1148 ppm concentration. Based on the process parameters of the 15°C reactor, the hydrolysis processing was smooth, but the poor activity and slow growth of methanogens were the key problems to make the efficiency poor. Comparison of the added biochar reactor with a control reactor without biochar operated at 35°C showed that the addition of biochar reduced the lag phase by 41%, enhanced the maximum methane production rate by 18%, and reduced the hydrogen sulfide by more than 95%, although no difference was observed in the cumulative methane production.

## INTRODUCTION

With the development of poultry breeding industry, the treatment of chicken manure (CM) has attracted attention recently. Land application of CM as fertilizer is generally convenient and economical, but it brings about environmental pollution (Salminen & Rintala 2002). Anaerobic digestion could overcome the above problems and is thus a good choice of treatment for CM.

Anaerobic digestion is divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each phase is controlled by special microorganisms and affected by external environmental conditions. Temperature is one of the most important factors in anaerobic digestion (Labatut et al. 2014), as it affects the material's hydrolysis rate (Mahmoud et al. 2004), microbial population (Li et al. 2014), ammonia inhibition (Rajagopal et al. 2013), and the yield and quality of biogas (Sanchez et al. 2001).

Mesophilic and thermophilic anaerobic digestion are more popular than psycrophilic digestion due to the higher efficiency. One of the most important issues in anaerobic digestion is the "start-up" process, when a new biogas plant is just built or restarted again (Normak et al. 2015) since the adapted microbial community has yet to be formed (Pandey et al. 2011). Due to the general lack of successful thermophilic anaerobic digestion plants, there are few relative inocula currently available for new engineering projects (Ahring 1994). To start up a thermophilic anaerobic digestion reactor, the inocula are typically borrowed from a mesophilic anaerobic digestion plant (Bouskova et al. 2005, Lepisto & Rintala 1997, Yilmaz et al. 2008). As of now, two start-up strategies have been established for transforming mesophilic sludge to thermophilic sludge: one-step or step-wise temperature increase (Bouskova et al. 2005, Iranpour et al. 2002). Either method has its benefits and drawbacks, although the one-step strategy generally performs better than the stepwise temperature increase strategy because it requires a shorter start-up time (Bouskova et al. 2005). However, the sharp changes in temperature may accumulate intermediate products and even lead to failure, and the process parameters will provide evidence for understanding the digestion process.

Biochar, the waste produced by biomass carbonization, may prove economically beneficial if its value chain is expanded. Biochar has begun to be applied more commonly to anaerobic digestion in recent years as it promotes direct electron transfer between microorganisms (Chen et al. 2014), mitigates ammonia inhibition (Mumme et al. 2014), increases methane content (Torri & Fabbri 2014), and can be directly applied to soil after anaerobic digestion without endangering the environment (Luo et al. 2015). The use of biochar for CM digestion can thus be beneficial, but previous studies have not yet to look at the start-up performance. The study explored the start-up performance of chicken manure anaerobic digesters that are amended with biochar and psycrophilic inoculum and are operated at different temperatures  $(15, 25, 35, 45, 55, 65^{\circ}C)$ . At each temperature, we measured methane yields and methane concentration, pH, alkalinity (Alk), oxidation-reduction potential (ORP), and soluble chemical oxygen demand (SCOD) to evaluate the start-up performance.

## MATERIALS AND METHODS

**Experimental materials:** The raw CM was taken from a chicken farm in Yangling (Shaanxi, China), with the feathers removed from the material. The CM was stored in a refrigerator at -4°C. The inocula used was the digestate from laboratory-based anaerobic digestion of melon stems and leaves. The residue was stored under ambient environment (approximately 15°C) and oxygen-free conditions for six months before use. Biochar was produced by Yixin Bioenergy Technology (Shaanxi, China) from the pyrolyzation of wood at 550°C in anaerobic conditions. The biochar was crushed to a particle size of 0.3~0.45 mm. The total solids (TS), volatile solids (VS), and carbon-nitrogen ratio (C/N) of the raw materials are presented in Table 1.

**Experimental design:** Anaerobic digestion experiment was conducted using seven 10 L reactors at six temperatures: 15, 25, 35, 45, 55 and 65°C. Each reactor contained 25% inocula and CM (1:3 w/w) with TS (excluding the biochar) of 8%, tap water, and 5% biochar TS. A control reactor using the same materials with the exception of biochar was operated at 35°C. The anaerobic reactor running volume was 8 L and the temperature of each reactor was controlled externally within  $\pm 1^{\circ}$ C by circulating water. The reactor was sealed with water using a U-shaped inlet and overflow

Table 1: Characteristics of raw materials.

| Materials      | %TS   | %VS   | C/N  |
|----------------|-------|-------|------|
| Chicken manure | 29.36 | 21.15 | 8.35 |
| Inoculum       | 6.16  | 3.78  | -    |
| Biochar        | 97.01 | 83.93 | -    |

outlet, and stirred at 50 rpm with a three-layer blade motor agitator operated continuously for 10 min twice daily (at 8:00 am and 8:00 pm.)

Analysis methods: The TS and VS values were measured according to Standard Methods for the Examination of Water and Wastewater (Lenore S. Clescerl 1999). The daily yield of biogas was monitored when it flowed past a wet gas flowmeter (LMF-01, Changchun, China), recorded at 9:00 am every day, after which biogas was collected in a 2 L aluminium foil bag. Methane concentration was analysed by a gas chromatograph (GC2014C, Shimadzu, Japan) with a thermal conductivity detector. The furnace and detector temperatures were 90 and 100°C, respectively. High-purity argon was used as a carrier gas at a flow rate of 30 mL/min. The temperature of the liquid sample was adjusted to about 20°C, after which the pH and ORP were measured using a water quality analyser (JDZ-706, Lei-ci, China). The Alk was measured by automatic potentiometric titrator (ZDJ-3D, bjxqwf, China) with a pH end point of pH 3.8 using 0.1 M HCl. The Alk was calculated using the equivalent CaCO<sub>3</sub> concentration (according to a blank sample of water). Samples were centrifuged for 10 min (rotary speed 8000 rpm), then the supernatant liquid was diluted to 1/20 times its original concentration with deionized water. The potassium dichromate method was used to analyse the SCOD, with the mixture digested for 2 h at 120°C (Orion COD165, Thermo Fisher Scientific, USA). Once the mixture had cooled, it was analysed with a water quality analyser (Orion AQ3700, Thermo Fisher Scientific, USA) in 0-1500 mg measurement range. H<sub>2</sub>S was measured by a Biogas Analyser (Gasboard 3200L, Wuhan Cubic Optoelectronics, China).

#### **RESULTS AND DISCUSSION**

**Methane produced at different temperatures:** The daily volumetric methane production under different temperature conditions is shown in Fig. 1. With the exception of the 55°C reactor, more than 95% of the final biogas and methane yields were obtained after thirty days of anaerobic digestion (Fig. 1A). These results are similar to those obtained in a previous study (Li et al. 2013).

The volumetric methane production peaks appeared for 35 and 45°C reactors on the 7<sup>th</sup> day, and the peak values were 0.92  $L\cdot d^{-1}\cdot L^{-1}$  and 0.86  $L\cdot d^{-1}\cdot L^{-1}$ , respectively, which was likely due to the anaerobic flora adapting to temperature changes and growing rapidly. The volumetric methane production peaks appeared for 25 and 65°C reactors on the 15<sup>th</sup> day, and the peak values were 0.44  $L\cdot d^{-1}\cdot L^{-1}$  and 0.68  $L\cdot d^{-1}\cdot L^{-1}$ , respectively. In fact, these two reactors had different curves of methane production. The daily volumetric methane production of the 65°C reactor stayed at approxi-





Fig. 1: Methane production, methane and hydrogen sulphide concentration during digestion of chicken manure with biochar at various temperatures.

mate 0.06  $L \cdot d^{-1} \cdot L^{-1}$  in the first nine days. There were no obvious peaks of methane production in the 15 or 55°C reactors throughout the experiment, and the reason for 55°C reactor was similar to that of 65°C reactor, but its daily volumetric methane production stayed at approximate 0.05  $L \cdot d^{-1} \cdot L^{-1}$  for a longer duration.

Previous studies have found that the Methanosarcinales Order (genera *Methanosarcina* and *Methanosaeta*) dominated mesophilic conditions, but Methanomicrobiales and Methanobacteriales were more common under thermophilic conditions (Jimenez et al. 2014), and that there are no visible differences in diversity or abundance of archaeal populations between ambient and mesophilic conditions (Ju & Zhang 2014). So the psycrophilic inoculum could quickly adapt the mesophilic reactor. Other researchers have reported similar phenomena; when the temperature of a reactor increased, the heat potentially kills microbial communities necessary for the digester to function efficiently (Leitao et al. 2006). Therefore, there were more days to enrich the thermophilic methanogenic bacteria in thermophilic reactors.

In addition, the optimum growth temperature range of *Methanothermobacter* is 65-70°C (Zeikus & Wolfe 1972), the recovery time of the 55°C reactor was much longer than that for the 65°C reactor (Fig. 1A).

The curve of methane production accumulation at six different temperatures is shown in Fig. 1B. The curves of 35 and 45°C reactors were fairly similar (p<0.05), and the curve of accumulation methane production in 25°C appeared almost in the middle of those reactors that ran at 15 and 35°C. Changes in methane concentration in different temperatures are shown in Fig. 1C, where, in general, the methane con-



Fig. 2: Process parameters for the anaerobic digestion of chicken manure with biochar under different temperatures.

centration of each reactor reached 60% (the 15°C reactor was the only exception). If the start-up time is defined as the time for methane concentration to reach 60%, then the start-up time increased in the following order: 45°C, 35°C, 65°C, 25°C, and 55°C. Start-up times were vastly different-the 45 and 35°C reactors needed approximately seven days, the 25 and 65°C reactors needed approximately 18 days, and the 55°C reactor needed 29 days.

In addition, the hydrogen sulfide concentration in  $65^{\circ}$ C reactor reached a peak 1200 ppm on the 13<sup>th</sup> day, and it was significantly higher than other reactors (Fig. 1D). The hydrogen sulfide concentration of  $45^{\circ}$ C reactor peaked at 550 ppm on the 10th day. The peak values of the concentration of hydrogen sulfide in other reactors were below 400 ppm. Overall, with the exception of the  $65^{\circ}$ C reactor, the temperature did not affect the hydrogen sulfide concentration.

**Influence of temperature on start-up process parameters:** Many process parameters have been suggested to determine process stability, including pH, biogas output flow rate, methane flow rate, Alk, and ORP value (Rodriguez et al. 2006, Steyer et al. 1999, Waewsak et al. 2010, Zanetti et al. 2012). In fact, due to significant changes in the environment, process parameters change dramatically at the startup stage and as such, it is necessary to monitor the parameters of the start-up process carefully.

The characteristic curves of liquid samples differed considerably among the various temperature conditions (Fig. 2A-D). The initial pH of each reactor was about 7.10 (Fig. 2A), but as methane production stabilized, the pH of the 25°C, 35°C, and 45°C reactors increased to 7.69, 8.22, and 8.14, respectively. For the 15°C reactor, pH decreased slightly and stayed low for the entire experiment at approximately 6.50. Just as they began to produce methane, the pH of 55 and 65°C reactors sharply declined but eventually rose to over 8.0. Specifically, for the 55°C reactor, pH had dropped to 6.19 on the tenth day, stayed there, then spiked



Fig. 3: Methane production and hydrogen sulphide concentration in the 35°C reactor with biochar (B35) and the 35°C reactor without biochar (C35).

above 8.0 from the  $23^{rd}$  day until the end of the experiment. Conversely, the pH of the  $65^{\circ}$ C reactor increased to 7.48 in the first eight days and then dropped to 6.30 in the following four days before shooting back upto 8.25 by the  $20^{th}$ day and stayed there until the end of the experiment. For the 55 and  $65^{\circ}$ C reactor, the pH drop to approximate 6.2, but there was no further acidification, it was suitable for the late recovery of methane production.

ORP is an indicator of the capacity of molecules to release or gain electrons in wastewater. Generally, the best ORP value range for anaerobes to degrade substrates efficiently is between -200 mV and -350 mV (Morris 1975), though it may be as low as 400 mV for methanogens (Archer & Harris 1986). The ORP values we observed at six different temperatures are shown in Fig. 2B. The ORP values in the 15, 25, 35, and 45°C reactors were similar (between -300 mV and -450 mV) for the entire experiment. Overall, the ORP values were suitable for methanogenic growth under psycrophilic and mesophilic conditions, but the thermophilic reactors encountered some problems as ORP values increased. In the first ten days, the ORP values for the 55 and 65°C reactors increased rapidly to -128 mV and -240 mV, respectively. The ORP value of the 55°C reactor fluctuated quite a bit at approximately -180 mV before decreasing on the 24th day and remaining at that value until the end of the experiment. For the 65°C reactor, the ORP value decreased to -400 mV at the end of 16th day and remained at that value until the end of the 30-day period.

Generally, the Alk contains carbonate Alk  $(CO_3^{2^\circ}, HCO_3,$ and  $CO_2$ ), creating a buffer capacity for the digester (Hou et al. 2014), and it also helps maintain a pH close to neutral inside cells (Agdag & Sponza 2005, Speece 1996), which is beneficial to methanogenic activity. All our reactors showed similar total Alk, which increased steadily throughout the 30-day experiment (Fig. 2C), with the Alk of 45 and 65°C reactors increased from 5.3 g/L and 5.2 g/L to 10.4 g/L and 9.9 g/L, respectively, they were approximate 1-3 g/L higher than the other reactors at the end. Because temperature affected hydrolytic ability and the dissociation equilibrium constant, the Alk was higher with higher temperature, but was unexpected in the 55°C reactor because of the accumulated volatile fatty acids (VFAs) neutralized the Alk partially.

SCOD is readily utilized by anaerobic bacteria, and as such, is responsible for the majority of the methane production (Oh et al. 2015). The SCOD data divided into two groups in the experiment (Fig. 2D). In the first group, the SCOD increased dramatically for the first nine days in the 15, 25, 55, and 65°C reactors and then fluctuated until the end of the experiment. In the other group, the SCOD in the 35 and 45°C reactors reached a peak of approximately 17 g/L on the 6th day, after which the values decreased gradually to 5.9 g/L and 9.7 g/L, respectively. Due to the SCOD being consumed by methanogens faster than they were produced in the second group, the corresponding methane yields were higher than that of the first group. In fact, when the COD removal rate is relatively constant, the reactors ran well and increased the methane yield (Michaud et al. 2002, Zwain et al. 2013).

**Biochar effect on start-up process at 35°C:** The modified Gompertz model is used to quantitatively analyse the methane production, as demonstrated in the Eq. 1 (Luo et al.

| Table 2: Calculated | 1 parameters in | the 35°C reactor | with biochar | (B35) and | the 35°C | reactor v | without | biochar ( | C35) | using the | modified | Gompertz |
|---------------------|-----------------|------------------|--------------|-----------|----------|-----------|---------|-----------|------|-----------|----------|----------|
| equation.           |                 |                  |              |           |          |           |         |           |      |           |          |          |

| Treatment | λ (d) | $CH_4$ production rate, $R_{max}$ (L) | Ultimate CH <sub>4</sub> yield, P(L) | R <sup>2</sup> |
|-----------|-------|---------------------------------------|--------------------------------------|----------------|
| B35       | 2.5   | 0.520                                 | 11.459                               | 0.992          |
| C35       | 4.3   | 0.440                                 | 12.188                               | 0.992          |

2015):

$$Y(t) = P \times \exp\{-\exp\{-\exp[\frac{R_{maax} \times e}{P}(\lambda - t) + 1]\} \qquad \dots (1)$$

Where *t* is the time (d); *Y*(*t*) is the cumulative methane production (L CH<sub>4</sub>/L-volume) at time *t*; *P* is the theoretical value of ultimate methane yield (L CH<sub>4</sub>/L-volume);  $R_{max}$  is the maximum methane production rate (L·d<sup>-1</sup>·L<sup>-1</sup>-volume);  $\lambda$  is the lag phase (d); and *e* is 2.7183.

The cumulative methane productions and the fitting curves for the biochar and without biochar reactors are shown in Fig. 3A, with the fitting parameters listed in Table 2. The squared correlation coefficient R<sup>2</sup> of fitting was 0.992. It was observed that there was a lag phase in C35 throughout the experiment. Compared with C35, the  $\lambda$  and ultimate methane yield (*P*) in B35 were reduced by 42% and 6%, respectively, while the maximum methane production rate  $R_{max}$  was enhanced by 18%.

The hydrogen sulfide production between the two reactors were also different distinctly (Fig. 3B). There were more fluctuations in C35 within the first 14 days and the concentration increased dramatically to a peak value of 4083 ppm in the first week before decreasing below 1000 ppm at the end of the second week and finally to approximately 200 ppm in the final 9 days. In contrast, the hydrogen sulfide concentration in B35 was far lower than C35; it was below 200 ppm throughout the experiment. Comparison of the process parameters showed that there were no noticeable differences in pH, but the SCOD and Alk were slightly higher in the C35 reactor, while the ORP was lower in the C35 reactor (data not shown).

Biochar provides high surface area for enriching functional microbes tightly attached to and therefore, the biochar effectively reduce the lag phase for anaerobic digestion and advanced the methane production peak (Luo et al. 2015). Due to the labile carbon fraction of less than 1% (Mumme et al. 2014), it is expected that biochar do not offer any extra carbon sources for anaerobic digestion, so it does not contribute to methane concentration or biogas yield (Mumme et al. 2014). The effects of additive biochar were similar to these researches. In addition, the addition of biochar also effectively reduced hydrogen sulfide concentration in the methane produced during the start-up phase of the biochar reactor by more than 95%. This is likely due to the adsorption of hydrogen sulfide to biochar (Shen et al. 2015).

## CONCLUSIONS

This study demonstrated the anaerobic digestion of chicken manure with biochar at six different temperatures. The results suggest that start-up was successful almost regardless of temperature, but the clear lag phases were observed in the thermophilic reactors. Furthermore, it was observed that the speed of start up for 65°C reactor was faster than 55°C reactor. According to the low methane production and the stable processing parameters in 15°C reactor, it could be assumed that its hydrolysis process was smooth, but the poor activity and slow growth of methanogens were the key problems to make the efficiency poor. A CK experiment at 35°C showed that adding biochar can shorten the start-up time for anaerobic digestion and decrease the H<sub>2</sub>S concentration, but did not affect methane production and methane concentration.

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