



Effect of hydrochar on anaerobic digestion of dead pig carcass after hydrothermal pretreatment



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ABSTRACT

Incineration and burial are the current practices for pig carcasses disposal but are not environmentally friendly. Anaerobic digestion can be a better alternative if the process inhibition by carcass digestion can be ameliorated. This study successfully mitigated the inhibition in anaerobic digestion of carcasses by hydrochar addition and by co-digestion with RS and HRS. Biogas production from SP of the pretreated hydrothermal carcasses was enhanced by 60.7 to 90.8% through hydrochar addition. The highest biogas production of 450 mL/g-VS was obtained at 4 g-hydrochar/L addition. The methane content was also increased from 57.5% to up to 69.8%. Each gram of hydrochar removed 25 mg of ammonium and 50 mg of VFA. Hydrochar addition promoted the conversion of VFA to biogas by strengthening the intensity of functional groups and the immobilization of microbial biomass. Co-digestion of SP with RS or HRS also increased the biogas production, and the optimal production of 428 mL/g VS was obtained at 70% SP and 30% RS. The co-digestion of carcass SP with RS and the addition of hydrochar can be a promising solution for improving biogas production from a pig carcass, and can be potentially developed as a sustainable waste management method.

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1. Introduction

China produced 451 million head of pigs in 2016 and is the largest pork production and consumption country in the world (NBSC, 2016). It's unavoidable that a portion of pigs die from various diseases in animal feeding and operation. The mortality can exceed 5% during breeding (Basumatary et al., 2009), corresponding to 22 million deceased pigs for the year 2016 alone. Concerns about parasitic pathogens and environmental pollution issues arise from the large quantities of carcasses, and proper carcasses disposal technology is necessary for the healthy development of the swine industry (Zhong et al., 2017). Incineration and burial are the mainstream disposal methods in China, but these methods either consume a large amount of energy and generate air pollutants or

occupy a large area of land. Burial has been banned in the European Union and biological treatment such as composting and anaerobic digestion are preferred in the USA and Canada (Anon, 2002; Gwyther et al., 2011). The Chinese government also regulates carcass disposal and encourages bio-safety treatment methods (Cheng et al., 2016).

Anaerobic digestion is a promising technology for the treatment of sterilized animal carcass, because this treatment method is environmentally friendly and produces clean energy (Gwyther et al., 2011). The animal carcass must be sterilized at 133 °C and 300 kPa for 20 min before digestion or any other uses by the European Union legislation (Anon, 2002). Hydrothermal treatment can be an alternative method to carcass sterilization or pretreatment because the process controls temperature between 100 and 375 °C and thoroughly kills pathogens. Hydrothermal treatment can promote the decomposition of organic matter in the solid phase, but wastes contain proteins are difficult to be decomposed during hydrothermal treatment (Pavlovic et al., 2013). Part of proteins in carcass are retained in solid products after HTP, which can cause the risk of ammonium inhibition in anaerobic digestion when proteins are degraded and mineralized. Traditional recovery methods for ammonium inhibition mainly include pH adjustment,

Abbreviations: RS, rice straw; HRS, hydrothermal pretreated rice straw; SP, solid product; VFA, volatile fatty acids; HTP, hydrothermal pretreatment; HTC, hydrothermal carbonization; TS, total solids; VS, volatile solids; COD, chemical oxygen demand; FTIR, Fourier transform infrared; BET, Brunauer-Emmet-Teller; NH₃, FA, free ammonia; NH₄⁺, ionized form of ammonium; NME, Net methane energy yield; TME, total methane energy yield.

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microorganism acclimation and co-digestion with another substrate (Chen et al., 2014). The adsorbent addition has been recently developed to remove ammonium and its inhibition in anaerobic digestion (Shen et al., 2017). Numerous carbonaceous adsorbents converted from the biomass have been widely evaluated (Galgani et al., 2014).

Biochar is a carbonaceous solid adsorbent obtained from the thermochemical conversion of biomass in an oxygen-limited environment, including pyrochar and hydrochar (Kambo and Dutta, 2015). Pyrochar is obtained from biomass pyrolysis, and hydrochar is produced from HTC of biomass. Adding hydrochar or pyrochar in anaerobic digestion decreases process inhibitors, increases buffering capacity, immobilizes microbial cell, retains nutrients and stimulates biogas production (Fagbohunbe et al., 2017). However, the investigation of hydrochar on anaerobic digestion of animal carcass is not well known.

The objectives of this study are: (1) to investigate the effect of hydrochar addition on anaerobic digestion of solid product from animal carcass after hydrothermal pretreatment (HTP); (2) to explore the mechanism for the promoted effect of hydrochar on biogas production; (3) and to assess the effects of hydrochar addition and co-digestion with rice straw on digestion of solid product from animal carcass after HTP.

2. Materials and methods

2.1. Feedstock and inoculum

Because the limitation of safety condition, the fresh and healthy pork was as an experimental alternative (Dai et al., 2015) and it was taken from the Walmart (No. 588 Gudun Road, Hangzhou), consists of a hindquarters, was minced and homogenized using a blender (CPEL-23, Shanghai Guosheng, China), and then stored at $-20\text{ }^{\circ}\text{C}$ for further use. The rice straw was taken from a farm in Haiyan County, Zhejiang Province, chopped and sieved to 100 meshes, and then stored at room temperature (about $15\text{ }^{\circ}\text{C}$) in an airtight container for further use. The inoculum sludge was collected from the bottom settlement of a mesophilic biogas plant in Hangzhou, China.

2.2. Hydrothermal treatment

HTP of the pork and the RS was carried out in a 2-L batch reactor equipped with a Parr 4848 controller (USA). Based on our previous study, $170\text{ }^{\circ}\text{C}$ was determined for HTP of pork, because the protein is mostly inside cells and the breakage of cell wall occurred at temperatures above $160\text{ }^{\circ}\text{C}$ (Zhang et al., 2014); 250 g pork and 250 g water were treated at $170\text{ }^{\circ}\text{C}$ for 30 min. 160 and $260\text{ }^{\circ}\text{C}$ were respectively determined for HTP and HTC of RS (Hesami et al., 2015; Yan et al., 2016). 100 g RS and 1000 mL water were treated at $160\text{ }^{\circ}\text{C}$ for 1 h for HTP. 100 g RS and 1000 mL water were treated at $260\text{ }^{\circ}\text{C}$ for 1 h for hydrothermal carbonization. After the HTC experiments, the solid and liquid products were separated by

vacuum filtration. The hydrochar was dried at $105\text{ }^{\circ}\text{C}$ for 24 h and then stored in a dryer for subsequent use. The pH of hydrochar was measured in deionized water at a mass ratio of 1:10 for hydrochar to water. The characteristics of feedstock, inoculum and hydrochar were shown in Table 1.

2.3. Batch anaerobic digestion system

The anaerobic digestion experiments were carried out in a batch mode. Some 500-mL glass bottles were loaded with feedstock and inoculums at designated levels, and the headspace was flushed with N_2 for 5 min. The digesters were then kept at $37 \pm 1\text{ }^{\circ}\text{C}$ in a water bath and shaken manually for 1 min twice daily. The produced biogas was collected in gas collection bottle containing diluted hydrochloric acid solution ($\text{pH} < 3$), and the volume was obtained through water displacement method. The experiments were run in duplicate and digestions were maintained until there was no obvious biogas production for consecutive five days. The period of the digestions was 25 days.

In all digestion, the working volume of bioreactors was 150 mL, the weight of inoculum was 3.98 g of VS and the inoculums to substrate VS ratio was 1.6. Digestion of SP with hydrochar addition, the weight of SP was 2.49 g per bottle (VS, d.b.), and hydrochar (on a dry base) was added at concentrations of 2, 4, 6, 8 and 10 g/L, respectively, and the correspondent weight of hydrochar were 0.3, 0.6, 0.9, 1.2, 1.5 g per bottle. In co-digestion of SP and RS, the total VS of mixtures (2.49 g) kept constant, the VS content of RS or HRS in mixtures was carried out at 10%, 20%, 30%, 40%, and 50% (based on VS). The C/N ratio of the substrates in co-digestion was shown in Table 4. Control groups were set up for mono-digestion of hydrochar, RS, or HRS.

2.4. Analytical methods

TS, VS, COD, ammonium was measured according to APHA standard method (APHA, 2006). The COD was determined (Model DR/2800 Spectrophotometer, Hach) following dichromate digestion. The pH value was determined by a pH meter (PHS-3D, Shanghai, China). Total carbon and nitrogen content were measured by an elemental analyzer (EA 1112, CarloErba, Italy). Protein content was determined by Kjeldahl nitrogen determination apparatus (UDK152, Italy; protein = $6.25 \times$ total nitrogen), lipid content was determined by Soxhlet extraction apparatus (SXT-06, Shanghai). The biogas composition was measured by a gas chromatograph (GC 2014, Shimadzu, Japan) equipped with a thermal conductivity detector. The temperatures of the column oven, injector port and detector were 100, 120 and $120\text{ }^{\circ}\text{C}$, respectively. The carrier gas was argon at a flow rate of 30 mL/min. The biogas potential was calculated by dividing the cumulative biogas production by the added VS weight of feedstock (2.49 g).

The liquid samples were centrifuged at 8000 rpm for 10 min, filtered through $0.22\text{ }\mu\text{m}$ membrane, and measured for the VFA composition. Determination of VFA in the fermented liquid was

Table 1
Characteristics of feedstock and inoculum.

	Pork	RS	HRS	Hydrochar	Inoculum
TS (% w.b.)	45.6 ± 0.3	90.2 ± 0.1	18.7 ± 0.5	58.7 ± 0.2	8.8 ± 1.0
VS/TS (% d.b.)	97.5 ± 0.1	86.4 ± 0.1	85.6 ± 0.1	71.7 ± 0.1	62.4 ± 0.1
C (%)	37.81	37.59	41.47	53.15	25.25
N (%)	4.52	0.73	0.96	1.41	3.64
C/N	8.4 ± 0.3	50.8 ± 0.2	43.3 ± 0.3	37.7 ± 0.3	6.9 ± 0.2
pH	7.2 ± 0.1	ND	ND	7.3 ± 0.1	6.8 ± 0.1

Note: w.b., wet base; d.b., dry base; ND, not determined. RS, rice straw; HRS, hydrothermal pretreated rice straw. Data are mean values ± standard error of three replicates.

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