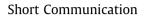
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# Effect of thermal–alkaline pretreatment on the anaerobic digestion of streptomycin bacterial residues for methane production



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#### HIGHLIGHTS

• Anaerobic digestion was a solution with streptomycin bacterial residue treatments.

- Thermal-alkaline pretreatment can enhanced methane production significantly.
- Thermal-alkaline pretreatment can enhance reactor OLR by more than double.

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#### ABSTRACT

The anaerobic digestion of streptomycin bacterial residues, solutions with hazardous waste treatments and bioenergy recovery, was tested in laboratory-scale digesters at 35 °C at various organic loading rates (OLRs). The methane production and biomass digestion were efficient at OLRs below 2.33 gVS  $L^{-1} d^{-1}$  but were deteriorated as OLR increased because of the increased total ammonia nitrogen (TAN) concentration from cell protein decay. The thermal–alkaline pretreatment with 0.10 NaOH/TS at 70 °C for 2 h significantly improved the digestion performance. With the thermal–alkaline pretreatment, the volumetric reactor productivity and specific methane yield of the pretreated streptomycin bacterial residue increased by 22.08–27.08% compared with those of the unpretreated streptomycin bacterial residue at an OLR of 2.33 gVS  $L^{-1} d^{-1}$ . The volatile solid removal was 64.09%, with less accumulation of TAN and total volatile fatty acid.

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#### 1. Introduction

The biopharmaceutical industry has important contributions to the national economy of China. In 2009, China produced 14.7 million tons of antibiotics, accounting for >70% of the total global market and ranking first in the world. Bulk (90%) of the antibiotics globally produced by China is streptomycin. A mass of waste bacterial residues is generated during the production of streptomycin. Bacterial residues were previously used as food additives and fertilizers. However, doubts have been raised on the suitability of streptomycin bacterial residues as feedstock and fertilizers because of their small amount of antibiotics and considerable amount of degradation products. Streptomycin bacterial residues are considered hazardous wastes in China. Therefore, bacterial residues should be managed strictly in accordance with the hazardous waste regulations (Guo et al., 2012; Li et al., 2012a,b). Incineration and landfill are currently the main treatment and disposal technologies for bacterial residues. However, these methods have significant disadvantages, such as high cost and serious secondary environmental pollution (Cassone et al., 2012). Therefore, the development of safe and effective treatments for streptomycin bacterial residues is important.

Anaerobic digestion, coupled with energy production in the form of methane and waste treatment, has been employed to treat several types of organic wastes. Bacterial residues contain rich high-quality protein, fat, cellulose, enzymes, and other components, which can be used for methane by anaerobic digestion (Guo et al., 2012). The feasibility of the anaerobic digestion of various bacterial residues has been investigated by many researchers (Li et al., 1992; Li and He, 1988). However, microbial cell walls contain glycan strands crosslinked by peptide chains, causing resistance to biodegradation. Thermal-alkaline pretreatment promotes the dissolution and decomposition of microbial cells and organic material to improve anaerobic digestibility (Bougrier et al., 2008; Skiadas et al., 2005). Our previous study (Li et al., 2012a) showed that the optimal conditions of the thermal-alkaline pretreatment of streptomycin bacterial residues are 0.10 NaOH/TS, 70 °C, and 2 h.



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In this study, we investigated the effect of thermal-alkaline pretreatment on the anaerobic digestion of streptomycin bacterial residues at various organic loading rates (OLRs) in laboratory-scale digesters with continuous feed mode. The results demonstrated that thermal-alkaline pretreatment can significantly enhance methane yield. The loading rate limitation of the anaerobic digestion of streptomycin bacterial residues was due to ammonia accumulation. The optimal hydraulic retention time (HRT) and OLR of the anaerobic digestion of streptomycin bacterial residues were identified for further application and reactor configuration modification.

#### 2. Methods

#### 2.1. Feedstock

Streptomycin bacterial residues were obtained from the North China Pharmaceutical Company, Hebei, China and were stored in a refrigerator at 4 °C prior to use. According to our previous study (Li et al., 2012a), we performed the thermal–alkaline pretreatment under the optimal conditions of 0.10 NaOH/TS, 70 °C, and 2 h. The characteristics of the streptomycin bacterial residues are listed in Table 1.

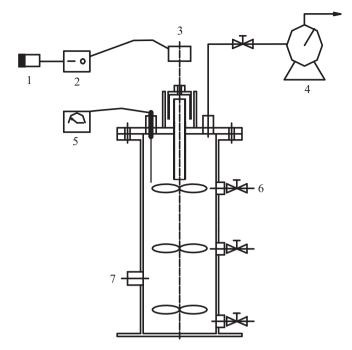
#### 2.2. Reactor and operational procedures

Two identically sized completely stirred tank reactor (CSTR) digesters with a working volume of 5 L (14 cm ID  $\times$  45 cm height) were fabricated with a double-wall Perspex cylinder (15 mm thickness) (Fig. 1). The temperature was maintained at 35 °C by recirculating the temperature-controlled water through the water jacket of the reactor. The reactors were sealed with a Perspex cover, which had three ports on the top. The first port was for substrate feeding. The second port was connected to a wet gas flow meter (LML-2, Jinzhiye Watch Industry Co., Beijing, China). The gas sample was collected via a silicone tube using a 10 µL pressure-tight gas syringe (104, Techcomp, China). The third port was inserted with a high-power magnetic stirrer (JB-2, Jintan Ronghua Instrument Factory, China) for intermittent mixing at 50 rpm for 24 min every 2 h. The daily biogas production was recorded using the gas flow meter (Fig. 1). The inoculum of the anaerobic reactor was anaerobic granular sludge from the streptomycin wastewater treatment plants of North China Pharmaceutical Company, Hebei, China. The sludge contained  $50.76 \text{ g L}^{-1}$  total solid (TS) and  $35.95 \text{ g L}^{-1}$  volatile solid (VS). The experimental results (average values of the two reactors) were collected at the steady state, i.e., a constant CH<sub>4</sub> production (<10% of deviation) for 5 d. The samples removed from the reactors were stored at -4 °C prior to analysis. The desired OLRs were 1.11, 2.33, and 3.68 gVS L<sup>-1</sup> d<sup>-1</sup>, and the related HRTs were 50, 25, and 12.5 d, respectively. Experiments were conducted for 75, 125, and 42 d for each test condition.

#### Table 1

Characteristics of the streptomycin bacterial residue.

Constituent	Streptomycin bacterial residue
TS (g/L)	55.62 (2.64)
VS (%-TS)	84.90 (7.34)
рН	2.90 (0.28)
TNK (mg/L)	5373 (379)
Protein (%-TS)	60.37 (5.76)
Total chemical oxygen demand (mg/L)	53,854 (6204)
Soluble chemical oxygen demand (mg/L)	5593 (745)
Moisture content (%)	93.87 (1.20)
TAN (mg/L)	168 (23)



**Fig. 1.** Laboratory scale CSTR digesters with 5.0 L working volume. (1. Time controller; 2. Speed governor; 3. Stirrer; 4. Wet gas flow meter; 5. Temperature controller; 6. Outlet).

#### 2.3. Analytical methods

Biogas volume was monitored daily using the wet gas flow meter. The measured volume was converted to that at standard temperature (0 °C) and pressure (1 atm) conditions.  $CH_4$  and  $CO_2$ were analyzed using a gas chromatograph (GC7900, Techcomp, China) equipped with a packed column (Shimadzu,  $6' \times 1.8''$  OD, 80/100, Japan) and a thermal conductivity detector. CH<sub>4</sub> production was determined by multiplying the CH<sub>4</sub> content with the volume of the biogas produced. Gas samples were injected directly using the 10 µL pressure-tight gas syringe after sampling. The pH value was directly measured with a Mettler-Toledo pH/conductivity meter using a combined pH electrode LE409 (FE20, Mettler-Toledo, Switzerland). TS, VS, total nitrogen Kjeldahl (TNK), and total ammonia nitrogen (TAN) were measured following previously described methods (Li et al., 2012a). The total chemical oxygen demand (COD) and soluble COD were measured with COD analysis systems (Lianhua Technology Company, China) composed of a spectrophotometer (5B-3), a COD reactor (5B-1), and a COD digestion reagent following the manufacturer's instructions. Analysis of the liquid composition of digester content, including total volatile fatty acids (TVFAs) and ammonia, was conducted in triplicate using the supernatant of mixed slurry after centrifugation (SIGMA, 10,000 rpm for 30 min at 2 °C). Volatile fatty acid (VFA) analysis using gas chromatography was performed as previously described (Zhong et al., 2012). The concentration of TVFA was calculated by summarizing all VFAs. TAN was measured in triplicate according to the APHA standard methods (APHA, 1998). Each analytical result was the mean value of at least three measurements.

#### 3. Results and discussion

#### 3.1. Characterization of biomass

The characteristics of the streptomycin bacterial residues are summarized in Table 1. The streptomycin bacterial residue biomass generally contains 85% VS in TS and is an attractive substrate for anaerobic digestion. The TNK (5373 mg  $L^{-1}$ ) and protein

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