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Effects of anaerobic digestion on chlortetracycline and oxytetracycline degradation efficiency for swine manure

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ABSTRACT

Manure containing antibiotics is considered a hazardous substance that poses a serious health risk to the environment and to human health. Anaerobic digestion (AD) could not only treatment animal waste but also generate valuable biogas. However, the interaction between antibiotics in manure and the AD process has not been clearly understood. In this study, experiments on biochemical methane potential (BMP) were conducted to determine the inhibition of the AD process from antibiotics and the threshold of complete antibiotic removal. The thresholds of the complete antibiotic removal were 60 and 40 mg/kg·TS for CTC and OTC, respectively. CTC and OTC with concentrations below thresholds could increase the BMP of manure. When the CTC and OTC concentrations exceeded the thresholds, they inhibited manure fermentation, and the CTC removal rate declined exponentially with concentration (60–500 mg/kg·TS). The relationship between OTC antibiotic concentration and its removal rate in AD treatment was described with exponential (40–100 mg/kg·TS) and linear equations (100–500 mg/kg·TS).
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1. Introduction

Chlortetracycline (CTC) and oxytetracycline (OTC) are tetracyclines that are widely used in the livestock industry (Zhang et al., 2013) for prophylactic and therapeutic treatment, as well as for growth promotion, because of their broad spectrum of activity, low cost, and low side effects (Ince et al., 2013). In China, approximately 150,000–200,000 tons of antibiotics are used annually, and half of such amount is used for animal feed (Larson, 2015). In animal husbandry, a wide range (60–90%) of antibiotics are excreted in urine and feces, thereby posing potential human and ecological health risks, particularly when waste is applied to the environment (Kumar et al., 2005). For example, CTC and OTC concentrations in animal manure samples have been reported to be in the range of 0–524.4 and 0–121.8 mg/kg·TS, respectively (Wang and Wei, 2013). A high proportion of antibiotics can enter aquatic ecosystems directly or indirectly through the discharge of wastewater via the leaching and runoff of agricultural soils amended with manure from livestock. Hence, the potential impacts of antibiotics on the environment require further attention.

Anaerobic digestion (AD), a well-established technology, is the most frequently used in the treatment of animal manure via anaerobic fermentation with biogas production. If the concentrations of active antibiotics are above critical levels, the AD process and its application in the treatment of manure and production of biogas could be inhibited because of methanogenic consortia inhibition (Ince et al., 2013). Hence, the effects of antibiotics on AD process performance, as well as the threshold of complete antibiotic removal in batch mesophilic fermentation, should be clearly understood (Aydin et al., 2015).

The effect of antibiotics on biogas production has been reported by some researchers. Stone et al. (2009) and Ince et al. (2013) reported that the CTC and OTC levels of manure slurry decrease from 27.0 mg/L to 11.6 mg/L and from 20 mg/L to 0 mg/L and exhibit a 27.8% and 50% inhibition in methane generation during AD, respectively. Ke et al. (2014) found that when OTC concentrations are 20, 50, and 80 mg/L during the batch mesophilic AD of cow manure, biogas decreases by 43.83%, 65.1%, and 77.79%, respectively. Álvarez et al. (2010) explained that methane production declines by 56%, 60%, and 62% at CTC and OTC concentrations of 10, 50, 100 mg/L, respectively. Thus far, only a small number of molecule classes have been tested for AD inhibition. Moreover, little information is available with regard to the threshold concentrations of different classes of antibiotics (CTC and OTC) in manure that can be removed during the AD process and the interaction between AD and antibiotics. The present research was designed to fill the information gaps related to the threshold concentrations

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Nomenclature

Acronyms

AD	anaerobic digestion
CTC	chlortetracyclin
OTC	oxytetracycline
BMP	biochemical methane potential
TS	total solids
VS	volatile solids
HPLC	high performance liquid chromatography

of different classes of antibiotics(OTC and CTC) in the sludge that are able to completely degrade during AD. Therefore, the objectives of this study are to determine the inhibition of AD from antibiotics, the antibiotic removal efficiency and threshold of complete antibiotic removal during the AD process.

2. Materials and methods

2.1. Sludge sampling and characterization

The two antibiotics analyzed in this study, namely, CTC (99.0% purity, CAS No. 64-72-2) and OTC (98.5% purity, CAS No. 2058-46-0), were purchased from the China Institute of Veterinary Drugs Control. The swine manure used as substrate held a green food label and was collected from a pig farm in Beijing, China. The manure was stored in a refrigerator under 4 °C. To reduce the influence of temperature, when it was fed into the digester, the manure allowed to slowly accommodate to higher ambient temperatures. The inoculum sludge was collected from an operational digester that was used to produce biogas from fecal sludge collected in the University of Science and Technology of Beijing, China. The characteristics of the materials are presented in Table 1.

2.2. Experimental setup

The biochemical methane potential (BMP) test setup (Fig. 1) consisted of 1 L jars as digesters and 1 L graduated cylinders as biogas collectors. The digesters were kept in a thermostat water bath at a constant temperature of 37 ± 1 °C to maintain a constant reaction temperature. A gas-tight rubber pipe was used to transport the biogas generated in the digesters to the graduated cylinders. In this action, the water was pressed out of the graduated cylinders into the water tank. The volume of the headspace of the graduated cylinders equated to the volume of the biogas generated in the digesters.

In this experiment, the AD of manure with antibiotics was studied under 10 different antibiotic concentrations of 0, 20, 40, 60, 80, 100, 200, 300, 400, and 500 mg/kg·TS (labeled as R1 to R10). A blank test labeled as R11 was added to the experiment using only a substrate of the inoculum sludge (Table 2). The biogas generated was collected through a gas-tight tube inserted into the digester headspace, which allowed the generated biogas to flow into the graduated cylinder via water displacement. For each test, the volume of the gas produced and the concentration of methane determined by a biogas analyzer (Geotech-Biogas check) were measured daily.

Table 1
Initial characterization of raw sludge.

Analytical parameters	Feed manure	Inoculum sludge
Total solid (TS) (%)	8.0	0.9
Volatile solids (VS) (%)	81.4%	75.8%
Total carbon (C) (g/g·TS)	38.81%	22.1%
Total nitrogen (N) (g/g·TS)	3.4%	2.7%
Total oxygen (O) (g/g·TS)	37.68%	15.56%
Total hydrogen (H) (g/g·TS)	5.23%	3.27%
pH	7.08	7.63
Chlortetracycline (CTC) (mg/kg·TS)	0	0
Oxytetracycline (OTC) (mg/kg·TS)	0	0

2.3. Analytical methods

TS and VS were determined according to the APHA standard methods (APHA, 1998). The pH level was measured using a pH meter (HI 9125N). C, N, O, and H were analyzed with an elemental analyzer (CCAS, 1985). CTC and OTC concentrations were determined via high performance liquid chromatography (HPLC) (Huang et al., 2014; Wu et al., 2011).

The HPLC system (Waters, USA) consisted of a quaternary pump, a degasser, an auto sampler, and a column oven set to 35 °C. Chromatographic separation was performed on a C18 SecurityGuard column (100 mm × 2.1 mm, 1.7 μm) at a flow rate of 0.3 mL/min under a gradient elution that comprised mobile phases of 0.65% formic acid in water and was identified with an ultraviolet detector at 275 nm. The injection volume was 10 μL for all the tests. Each sample at 2 mL tested sludge was extracted with 20 mL McIlvaine-Na_2EDTA buffer prepared by mixing 0.1 mol/L citric acid monohydrate solution with 0.2 mol/L disodium hydrogen phosphate dodecahydrate solution and then adding 0.1 mol/L ethylenediamineteraacetic acid disodiumsalt (Na_2EDTA). Thereafter, ultrasonic treatment was performed for 20 min, and centrifugation was carried out at 15,000 rpm/min at 4 °C for 10 min. The same extraction procedure was repeated twice, and all the supernatants were combined and filtrated with 0.22 μm membrane before being subjected to ultra performance liquid chromatography.

For the experiment results, all the values represent the average of three measurements.

2.4. Data analysis

The first order kinetic was selected to simulate the cumulative biogas results for the manure with different concentrations antibiotic. The biogas production rate (k) was used to evaluate the effect of antibiotics on the rate of biogas production under different concentration.

The expression of First order kinetics is $$y_t = y_e(1 - e^{-kt}) \quad (1)$$

where y_t is cumulative biogas yield at day t (T/gVS_{added}), y_e is ultimate biogas yield, k is biogas production rate constant (day^{-1}), t is the digestion time (days), e is exp(1) = 2.718282.

To determine the effect of antibiotics on the performance of fermentation and BMP of manure at different antibiotic concentrations, the cumulative biogas production volume of the inoculum sludge was first measured (R11). This value was subtracted from the total volume of biogas for each group (R1 to R10) and then divided by the corresponding weight of sludge. The result is the volume of the cumulative biogas production of sludge (V_{cbp}, mL/g), as shown in Eq. (2). Finally, the volume of biogas production, i.e., mL/g·TS_{added} and mL/g·$TS_{removed}$, for each group (V_{cbptsa}, V_{cbptsr})

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