Bioresource Technology 149 (2013) 244–252

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

The effects of the antibiotics ampicillin, florfenicol, sulfamethazine, and tylosin on biogas production and their degradation efficiency during anaerobic digestion

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HIGHLIGHTS

- The antibiotic effect on biogas production during anaerobic digestion was studied.
- ⩽280 mg/L sulfamethazine and ⩽91 mg/L tylosin did not inhibit biogas production.
- ⩽350 mg/L ampicillin and ⩽6.4 mg/L florfenicol did not inhibit biogas production.
- Ampicillin, florfenicol, and tylosin were rapidly degraded within 5 d of digestion.
- Sulfamethazine and degradation products from florfenicol and tylosin were persistent.

ARTICLE INFO

Article history:
Received 10 July 2013
Received in revised form 9 September 2013
Accepted 11 September 2013
Available online 20 September 2013

Keywords:
Anaerobic digestion
Antibiotic degradation
Biogas
Methane

ABSTRACT

The impacts of four common animal husbandry antibiotics (ampicillin, florfenicol, sulfamethazine, and tylosin) on anaerobic digestion (AD) treatment efficiency and the potential for antibiotic degradation during digestion were evaluated. Sulfamethazine and ampicillin exhibited no impact on total biogas production up to 280 and 350 mg/L, respectively, although ampicillin inhibited biogas production rates during early stages of AD. Tylosin reduced biogas production by 10–38% between 130 and 913 mg/L. Florfenicol reduced biogas by ~5%, 40% and 75% at 6.4, 36 and 210 mg/L, respectively. These antibiotic concentrations are higher than commonly seen for mixed feedlot manure, so impacts on full scale AD should be minimal. Antibiotic degradation products were found, confirming AD effectively degraded ampicillin, florfenicol, and tylosin, although some products were persistent throughout the process. Contamination of AD solid and liquid effluents with sulfamethazine and antibiotic transformation products from florfenicol and tylosin could present an environmental concern.

Published by Elsevier Ltd.

1. Introduction

Approximately 80% of the 16,000 metric tons of antibiotics sold annually in the U.S. are used in animal husbandry (US FDA, 2012; US FDA, 2009). A wide range of the antibiotics (10–90%) is excreted unchanged in urine and feces, providing potential human and ecological health risks when waste is applied to the environment (Kumar et al., 2005), which is confirmed by widespread detectable antibiotic concentrations in surface waters (Kolpin et al., 2002) and sediments (Massey et al., 2010). Antibiotics originating in manure from livestock operations are a concern because they remain bioactive (Subbiah et al., 2011) and select for antibiotic-resistance (Subbiah et al., 2012).

Prior to manure disposal to the environment, most animal manure is managed through a variety of manure-handling practices including pit storage, pile storage, composting, anaerobic digestion, and aerated lagoons. Anaerobic digestion (AD) is an emerging manure management technology being used by some concentrated animal feeding operations (CAFOs). AD treatment at the commercial-scale has been shown to provide waste stabilization as well as reductions in odors, pathogens, and greenhouse gas emissions (Frear et al., 2011). Beyond these benefits, the AD process is also of interest because the methane (CH_4) rich biogas can be used to generate electricity, heat, or compressed transportation fuel (US EPA, 2006). Furthermore, inclusion of an AD step during manure treatment on CAFOs might result not only in the aforementioned benefits but also lead to further degradation of the active antibiotic compounds through the biological, chemical, and thermal components of the AD process. On the contrary, if concentrations of active

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0960-8524/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.biortech.2013.09.048

antibiotics are above critical levels, the AD process and its treatment of manure and production of biogas/energy could be inhibited.

Most studies of antibiotic inhibition of AD have focused on swine manure as the substrate, and have shown significant AD inhibition primarily at the higher antibiotic concentrations typically found in manure (Alvarez et al., 2010; Chelliapan et al., 2011; Gartiser et al., 2007; Lallai et al., 2002; Shi et al., 2011). For example, Lallai et al. (2002) found that 80 mg/L thiamphenicol in a pig manure slurry decreased biogas production by >60% after 10 d, while 60 mg/L amoxicillin resulted in 25% lower biogas production over the same period. However, macrolide antibiotics, such as tylosin and erythromycin, have been shown to inhibit biogas only at concentrations >100 mg/L (Chelliapan et al., 2011).

Although most research has focused on antibiotic inhibition of biogas production, few studies have been conducted on antibiotic degradation rates and degradation products generated during AD. Antibiotic AD dissipation half-lives have been found to be as short as <1 d for antibiotics such as tetracycline and sulfamethoxydiazine (Shi et al., 2011), while no detectable degradation occurs for other antibiotics such as sulfamethazine and sulfathiazole (Mohring et al., 2009). Moreover, antibiotic degradation product formation was monitored in only a few studies, examining tetracycline, macrolide, and sulfonamide antibiotics (Alvarez et al., 2010; Arikan, 2008; Kolz et al., 2005; Mohring et al., 2009; Teeter and Meyerhoff, 2003). Knowledge of degradation products is important to identify persistent transformation products, which may increase human or ecological risk when AD effluents are applied to the environment.

This study was designed to fill information gaps related to AD inhibition by different antibiotic classes in diluted manures received by anaerobic digesters, particularly cattle manure, and the need to more thoroughly investigate degradation products from the AD process. The general purpose of the research was to determine the effect of concentration of four common cattle husbandry antibiotics (ampicillin, florfenicol, sulfamethazine, and tylosin) on AD biogas production and characterize antibiotic degradation resulting from the AD process. The specific research objectives were to determine: (1) AD biogas inhibition from the four antibiotics in cattle manure at five equal molar concentrations, (2) antibiotic removal rates from the liquid phase, (3) the concentration of antibiotics sorbed onto the solids, (4) the resultant antibiotic transformation products, and (5) the relative persistence of the degradation products during AD.

2. Methods

2.1. Materials

Fresh, screened (0.3 cm slope screen with dewatering augur by US Farm, Tulare, CA, USA) cattle manure was collected from a local source in Pullman, WA, while primary digested sludge, serving as the AD inoculum, was obtained from a wastewater treatment plant (WWTP) anaerobic digester (Pullman, WA, USA). Total solids (TS) and volatile solids (VS) for the manure was 20.1% and 17.8%, while the inoculum had a TS and VS of 1.6% and 1.1%, respectively, which were determined by following EPA Method 1684. The four antibiotics studied were analytical grade and purchased from Sigma–Aldrich (St. Louis, MO, USA). The antibiotics have very different chemical structures and physical properties (Table 1).

2.2. Antibiotic effect on AD

A combined anaerobic toxicity assay (ATA) (ISO, 2003) and biomethane potential (BMP) test (ISO, 1995) was used to measure the potential adverse effect of the antibiotics on the rate of total gas production and to characterize the substrate biodegradability, respectively. The effective concentration (EC) was then calculated from plots of percentage inhibition against the logarithm of the toxin mass concentration (ISO, 2003). The assays were conducted in triplicate using 300 mL glass serum bottles fitted with rubber septum and incubated for 40 d at 37 °C. The bottles contained a mixture of manure, inoculum, antibiotic, and de-ionized water, designed to mimic common dairy AD influent TS concentrations (3–6% TS) and range of antibiotic concentrations (0.001, 0.01, 0.1, 0.5, and 1.0 mM). Additional bottles prepared in parallel included the reference toxin 3,5-dichlorophenol as a comparison for results obtained for the antibiotics. Bottles were prepared by first adding the antibiotics, separately, to 11.3 g of manure. Then, 20 mL of deionized water was added with the mass of antibiotic or reference toxin, and finally, 180 mL of municipal anaerobically digested primary sludge was added to the bottle for a final volume of 200 mL, leaving 100 mL of headspace for gas collection. The headspace was filled with nitrogen gas prior to securing the septum caps. The volume of inoculum utilized allowed for a VS ratio of manure and inoculum equal to one, a ratio previously shown to be effective in ATA and BMP evaluations (Labatut et al., 2011; Ma et al., 2013a). Manure + inoculum and inoculum only controls were also run in triplicate. All bottles were gently mixed by hand each day of sample collection.

The biogas volume produced was measured every day for 7 d and then every few days for 33 d using standard gas syringe methodology (ISO, 1995). A 1.5 mL liquid sample was taken after the biogas measurement using a 15.24-cm needle and 3 mL plastic syringe, and the soluble chemical oxygen demand (SCOD) (EPA Method 410.4) and antibiotic concentrations (EPA Method 1694) were analyzed from the centrifuged (13,000 rpm for 2 min) liquid supernatant. Samples of the controls were taken at time zero and tested for the four antibiotics to ensure that the antibiotics were not detected in the original manure and inoculum matrices. After 40 d, the TS and VS were determined from the mixed bioreactor slurry. The total biogas inhibition after 40 d was modeled using the best fitting logarithmic or polynomial equation obtained through the software program KaleidaGraph 4.0. Methane and carbon dioxide concentrations were measured using a calibrated gas chromatograph (Varian CP-3800 GC) (Frear et al., 2011).

2.3. AD effect on antibiotic degradation

Prior to antibiotic and degradation/transformation product analysis the liquid samples were centrifuged at 13,000 rpm for 2 min. Then, 50 μL of the supernatant was diluted with an aqueous 10 mM formic acid solution to promote efficient compound ionization. In addition, a portion of the solid phase was extracted at the end of the 40 d AD process to determine the mass of antibiotic and degradation/transformation products sorbed to the solids. Methanol was used as the organic solvent. First, 25 mL of the bioreactor slurry was centrifuged for 1 min at 1000 rpm in a polypropylene centrifuge tube. Then, the water was decanted from the tube, and 5 mL of water was added to the solids and gently mixed. The tubes were centrifuged again, decanted, and then 10 mL of methanol was added to the tube, which was then shaken for 1 h on a reciprocal shaker and then by hand for 1 min. The tube was centrifuged again, and a portion of the methanol extract was diluted with 10 mM formic acid in nanopure water prior to analysis.

The antibiotic concentrations and the relative concentration of degradation products were analyzed by LC–ESI-MS with an Agilent 6460 Triple Quad LC/MS following a modified EPA Method 1694 procedure. An Agilent XDB-C18 analytical column with dimensions of 50 mm × 4.6 mm and 1.8 μm was used. Solvents A and B were 10 mM formic acid in nanopure water and 10 mM formic acid in