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Effect of the presence of the antimicrobial tylosin in swine waste on anaerobic treatment

Largus T. Angenent[a], *Margit Mau*[b], *Usha George*[c], *James A. Zahn*[d], *Lutgarde Raskin*[e,*]

[a]*Department of Energy, Environmental and Chemical Engineering, Washington University in St. Louis, St. Louis, MO 63130,USA*
[b]*Department of Environmental Microbiology, TU Bergakademie Freiberg, Freiberg D-09596, Germany*
[c]*Tocklai Experimental Station, Jorhat 785 008, Assam, India*
[d]*Coskata, Inc., Warrenville, IL 60555, USA*
[e]*Department of Civil and Environmental Engineering, University of Michigan, Ann-Arbor, MI 48109, USA*

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ABSTRACT

An anaerobic sequencing batch reactor (ASBR), seeded with a biomass inoculum that previously had not been exposed to the macrolide antimicrobial tylosin (mixture of Tylosin A, B, C, and D), was operated for 3 months with swine waste without Tylosin A and for 9 months with swine waste containing Tylosin A at an average concentration of 1.6 mg/L. When swine waste with tylosin was fed to the ASBR, methane production and volatile solids removal did not appear to be inhibited and a methane yield of 0.47 L methane per gram volatile solids fed to the ASBR was observed. Throughout the operating period, Tylosin A levels in ASBR biomass and effluent were below the detection limit of 0.01 mg/L. However, during the first 3 months of operation, the levels of macrolide–lincosamide–streptogramin B (MLS_B)-resistant bacteria in the ASBR biomass increased substantially as determined by hybridizations with oligonucleotide probes designed to target MLS_B-resistant bacteria. Since no Tylosin A was present in the swine waste during the initial 3 months, the presence of MLSB-resistant bacteria in the swine waste was likely the reason for the increase in resistance. Subsequently, the levels of MLS_B-resistant bacteria in ASBR biomass stabilized with an average of 44.9% for the 9 months of operation with swine waste containing Tylosin A. The level of MLS_B-resistant bacteria in the swine waste fed to the ASBR during this period averaged 18.0%. The results indicate that anaerobic treatment of a waste stream containing tylosin was effective (based on reactor performance) and that the level of resistant bacteria in the ASBR was substantially higher than in the waste stream fed to this system.

1. Introduction

Antimicrobials have been used extensively during the past 70 years to treat and prevent bacterial infections in humans and animals, and to promote growth in confined livestock animals, such as swine and poultry. As a result of the widespread use of antimicrobials, antimicrobial-resistant microbes have become abundant and the rise in their appearance has become a serious public health concern (Mellon et al., 2001). Antimicrobials and antimicrobial-resistant bacteria are introduced into biological waste treatment systems through their presence in hospital, pharmaceutical, and domestic wastewaters (Ferreira da Silva et al., 2006; Guardabassi et al., 1998) and in animal wastes (Aminov et al.,

*Corresponding author. Tel.: +1 734 647 6920; fax: +1 734 763 2275.
E-mail address: raskin@umich.edu (L. Raskin).
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2001; Chee-Sanford et al., 2001). Only a limited number of studies have focused on determining the effects of the presence of antimicrobials and antimicrobial-resistant bacteria on biological waste treatment systems in general and on animal waste treatment systems in particular (Chee-Sanford et al., 2001; Hanzawa et al., 1984; Jindal et al., 2006; Poels et al., 1984; Zilles et al., 2005). In addition, determining the levels of antimicrobials and antimicrobial-resistant bacteria in excess biomass generated by animal waste treatment systems is important since disposal through land application is common, possibly resulting in the contamination of ground and surface waters, soils, and crops with antimicrobials and antimicrobial resistance genes (Chee-Sanford et al., 2001; Jindal et al., 2006; Zilles et al., 2005).

The current study focused on the antimicrobial tylosin (a mixture of Tylosin A, B, C and D), a macrolide consisting of a 16-membered lactone ring. Tylosin belongs to the macrolide–lincosamide–streptogramin B (MLS_B) antimicrobials, some of which are used commonly to treat and prevent infection in the livestock industry (Zilles et al., 2005). Antimicrobials within this group also are used at subtherapeutic levels as feed additives, such as lincomycin (lincosamide) and virginiamycin (streptogramin B) for chicken and turkey, and tylosin for swine and feed cattle (Mellon et al., 2001). Although the antimicrobials within the three classes of MLS_B antimicrobials are chemically distinct, their function and resistance mechanisms are similar. Therefore, they are grouped together as MLS_B antimicrobials and their mechanism of resistance is referred to as MLS_B resistance.

The peptidyltransferase loop of the 23S ribosomal RNA (rRNA), a universally conserved region within the molecule, is the target of action for MLS_B antimicrobials (Weisblum, 1995). Binding of the antimicrobials to this site inhibits protein synthesis. A common mechanism of resistance for most macrolide-resistant strains is target site modification, which consists in a posttranscriptional modification (mono- or dimethylation) of the adenine at position 2058 (A_{2058}, *Escherichia coli* numbering system) in the 23S rRNA by the adenine-N_6-methyltransferase (Andersson and Kurland, 1987; Fluit et al., 2001; Vester and Douthwaite, 1994). This methylation in the 23S rRNA blocks the binding of MLS_B antimicrobials (Leclercq and Courvalin, 1991). Genes encoding enzymes responsible for methylation have been designated *erm* (erythromycin ribosomal methylase). In the present study, we relied on this mechanism of resistance to develop a method that allows for the direct (i.e., without bacterial culturing) determination of the levels of MLS_B-resistant bacteria. While methylation of the 23S rRNA is the major cause of macrolide resistance in animals and humans, it is not the only mechanism of resistance to MLS_B antimicrobials (Douthwaite and Vester, 2000; Nash, 2003; Weisblum, 1995).

The objectives of the current study were to evaluate the effect of the presence of the antimicrobial tylosin and MLS_B-resistant bacteria in swine waste on anaerobic biological treatment efficiency and to quantify the levels of tylosin and MLS_B-resistant bacteria in the biomass from these systems. To carry out these objectives, we fed swine waste without tylosin to a laboratory-scale, high-rate anaerobic sequencing batch reactor (ASBR) for 3 months, then fed swine waste containing tylosin for a 9-month period. The performance of the ASBR, the extent of tylosin degradation, and the level of MLS_B-resistant bacteria were monitored.

2. Materials and methods

2.1. ASBR operating conditions and performance

Diluted swine waste with a concentration of 20 g/L as volatile solids (VS) was treated in a 5-L ASBR (Figure S1, Supplementary information) by sequencing through a feed step (1 min), a react step (23.2 h), a settling step (45 min), and a decant step (2–5 min). Gentle, intermittent mixing was performed by biogas recycling (1 min of biogas recycling every hour at a flow rate of 26 L/h). The temperature of the ASBR was maintained at 25 °C by circulating water through a jacket mounted around the reactor with a heating recirculator (Polyscience, model 210, Niles, IL). At the start of operation, 0.25 L of swine waste was fed per day, which resulted in a volumetric loading rate (VLR) of 1.0 g VS per L reactor volume per day and a hydraulic retention time (HRT) of 20 days. The VLR was increased when the biogas production rate had been stable (fluctuations were less than 5% for weekly averages) for a period corresponding to at least three HRTs. For each stable operating period, at least three samples were obtained from the ASBR before the VLR was increased. The final VLR was 4 g VS/L/d (HRT of 5 days).

The reactor performance was assessed by determining the VS removal efficiency and by monitoring the methane production and total VFA concentration in the effluent. The volumetric methane production rate (VMPR) was obtained by correcting the biogas production (measured with a gas meter, Supplementary information) to standard temperature and pressure (STP) using the ideal gas law and converting this with the wet volume of the reactor and the methane percentage that was present in the biogas. Therefore, the VMPR was expressed as volume of methane per reactor volume per day (L/L/d). The methane yield (at STP) was obtained by plotting pseudo-steady-state daily VMPRs versus VLRs. Least-square linear regression allowed estimation of the slope, which represents the methane yield during the operating period, and is expressed as volume of methane produced per g VS swine waste fed to the digester (L/g VS fed).

The ASBR was inoculated at an initial VS concentration of 20 g/L (VS to total solids [TS] ratio of 0.51) with anaerobic digester sludge from the secondary digester operated by the Urbana-Champaign Sanitary District, Northeast Wastewater Treatment Plant (Urbana, IL) and thus had not been in contact with swine waste.

2.2. Swine waste and other environmental samples

Swine waste was collected once every 1–3 months during and immediately after scraping of finisher swine buildings (University of Illinois at Urbana-Champaign [UIUC] research farms, Urbana, IL). The swine waste was screened through a 1.7-mm screen to remove large debris to prevent clogging of tubing feeding the ASBR, diluted with tap water (City of Urbana, IL) to a concentration of 20 g VS/L (VS to TS ratio of 0.77), and stored at −20 °C in 1-L batches to prevent