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Influence of cefazolin contamination on performance of two-stage and single stage anaerobic batch digesters

Suraju A. Lateef *, Masahiro Iwasaki, Takaki Yamashiro, Kazutaka Umetsu

Environmental Hygiene Unit, Department of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

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ABSTRACT

Anaerobic digestion of animal waste is a well-established technology for on-farm waste management and energy generation. Animal wastes often contain toxic substances such as antibiotic, which have been shown to cause varying degrees of biogas inhibition in single stage (SS) digestion system. Currently, useful information on influence of antibiotic contamination of substrates, particularly cefazolin (CFZ), on two-stage (TS) digestion system is limited. The effect of CFZ antibiotic on performance of two-stage and single batch systems digesting mixture of manure and milk from dairy cow was investigated. Batch experiments were conducted at 55 °C in TS (5 and 15 days for first and second stages, respectively) and SS (20 days) digesters. Cefazolin was added at concentration of 5, 10, 20, 40 and 80 mg/l. Control experiments, with no addition of CFZ were also conducted. In TS system, all concentrations of CFZ exhibited complete or near complete inhibition of hydrogen production in the first stage, with methane reductions of 9.2–56.0% in the second stage as compared with control treatment. Corresponding biogas inhibitions for the system ranged from 7.3–29.1%. The inhibition resulted in reduction of energy recovery by 16.7–60.6%. For SS system, CFZ addition reduced 39.8–68.3% of methane production while total biogas inhibition ranged 24.0–39.0%. The inhibition also resulted in reduction of energy recovery by 39.7–68.3%. Evidently, lower biogas inhibitions and reduction in energy recovery were experienced in TS system as compared with SS system. The results thus suggest that TS system can be an alternative to conventional SS system for on-farm management of CFZ contaminated wastes.

Introduction

With anticipated depletion in fossil fuel reserves and environmental problems associated with burning fossil fuels, development of alternative sources of energy has received considerable attention in recent years. Anaerobic digestion, a proven technology for conversion of various biomasses to biogas, is widely regarded as a source of renewable energy and an important technology for achieving pollution reduction. It is one of the waste management technologies often employed for treatment of animal waste in concentrated animal feeding operations (Mitchell, Ullman, Teel, Watts, & Frear, 2013). It has various benefits among which are degradation of the active antibiotic compounds in the manure and reduction of antibiotic resistant bacteria (Arikan, 2008; Arikan et al., 2006; Beneragama et al., 2013). Profitability of anaerobic digestion process depends on the amount of biogas produced from a given feedstock. For animal waste, relatively low biogas production when compared with biogas yield from other feedstocks is the major drawback of using animal waste as sole substrate (Ward, Hobbs, Holliman, & Jones, 2008). The strategy that is commonly employed to improve biogas production from animal manure is co-digestion with other carbon-rich feedstocks drawn from the vicinity of the farm. In this respect, various reports have appeared in the literature (El-Mashad & Zhang, 2010; Guiliano, Bolzonella, Pavan, Cavinato, & Cecchi, 2013; Lateef, Beneragama, Yamashiro, Iwasaki, & Umetsu, 2014; Wu, Dong, Yao, & Zhu, 2011). However, animal manure and other livestock wastes often contain chemical such as antibiotic that can impact negatively on biogas production during anaerobic digestion process.

The use of antimicrobials in raising food animal has contributed significantly to the success of concentrated animal feeding operation in many countries. Antimicrobial agents are indispensable in prevention, control and treatment of bacterial infections in animals and are also frequently used as growth promoters in intensive animal husbandry (Schwarz, Kehrenberg, & Walsh, 2001). In Japan, >700 tons of antimicrobial drugs and feed additives are used yearly in animal production (Harada & Asai, 2010). In spite of their benefits, concerns are growing over their potentials to contribute to environmental problems such as emergence and spread of antibiotic-resistant bacteria and pathogenic bacteria (Alexander et al., 2008).

* Corresponding author at: Department of Environmental Health Sciences, Faculty of Public Health, University of Ibadan, Ibadan, Nigeria.
E-mail address: surpaul2000@yahoo.com. (S.A. Lateef).

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Cefazolin, a β-lactam antibiotic, is widely used for treatment of bacterial infections in cows. In Obihiro University farm, Hokkaido, Japan, it is commonly used for treatment of mastitis (a commonly occurring infection and inflammation of udders) in milking cows. Milk produced by cows undergoing antibiotic treatment for mastitis, which has been estimated to be about 37 l per cow per day (Hensley, Moreira, & Holden, 2016), cannot be added to food supply until after 5 days of treatment because of the residue of the antibiotic (around 0.1% of the initial antibiotic dose) (Hornish & Kotarski, 2002) usually excreted in milk. In addition, presence of antibiotic in manure, excreted in an unaltered form or metabolites of parent compound of administered antibiotic via urine and feces (Jjemba, 2002), poses an ecological risk when dispose of in the environment. In Obihiro University, single stage anaerobic digestion process is employed for treatment of cow manure and waste milk from treated cow.

Anaerobic digestion reactors can be operated as a single stage or multi-stage (usually two stages) system (Ward et al., 2008). In a single stage system, the multistep process of anaerobic digestion: hydrolysis, acidification, acetogenesis, and methanogenesis, takes place in a single reactor. However, in multi-stage system, environmental conditions for operation of different stages: hydrolysis/acidification (also used for hydrogen gas recovery) and acetogenesis/methanogenesis are optimized through separation of the processes in different reactors (Liu, Liu, Zeng, & Angelidaki, 2006). Reactors upset are reported to result from fluctuations in organic loading rate, heterogeneity of wastes or excessive inhibitors. Multi-stage digestion system is regarded as a solution to digestion instability often encountered in anaerobic digestion system (Ward et al., 2008). However, little is known about the ability of two-stage system to reduce the inhibition of biogas production by cefazolin when compared with single stage system.

Inhibitions of anaerobic digestion of swine manure and cow manure by some antibiotics such as oxytetracyclines, ampicillin, sulfamethazine and amoxicillin at different concentrations have been reported in some studies (Alvarez, Otero, Lema, & Omil, 2010; Lallai, Mura, & Onnis, 2002; Mitchell et al., 2013). Knowledge of the effect of cefazolin on biogas production and energy yield from dairy cow manure is still limited. Studies on inhibitory effect of antibiotics on anaerobic digestion have focused mainly on single stage system in spite of benefits of two-stage system. Only few studies have reported impact of antibiotics during multi-stage digestion. Sanz, Rodriguez, and Amils (1996) using volatile fatty acids as substrate reported about 55% decrease in activity of acetogenic bacteria in the presence of β-lactamic antibiotics. Akyol, Aydin, Ince, and Ince (2016) reported higher biogas yield, methane yield and reduction of antibiotic resistance genes in two-stage anaerobic digestion system than in single stage system during anaerobic digestion of oxytetracycline-medicated cattle manure. Lateef et al. (2014), during co-digestion of cow manure and waste milk from medicated cows (treated with cefazolin) in two-stage system reported significant inhibition of hydrogen production. We assumed that the inhibition was caused by cefazolin residues in the waste milk. However, it was difficult to affirm from the data of our previous study the role cefazolin played in the inhibition and the concentration that caused significant inhibition. It is therefore important to investigate further the effect of different concentrations of cefazolin on biogas production in two-stage system and determine the relative benefit of two-stage system in reducing biogas inhibition by cefazolin.

The main objective of this study was to determine the effect of different concentrations of cefazolin on two-stage (for hydrogen and methane production) and single stage (for methane production) anaerobic digestion systems treating mixture of cow manure and waste milk. The specific objectives of the study were to determine: (i) the effects of different concentrations of cefazolin on hydrogen production from co-digestion of cow manure and waste milk, (ii) the effects of possible inhibition of hydrogen production in the first stage on subsequent methane production in the second stage, (iii) the effects of different concentrations of cefazolin on the performances of single stage and two-stage anaerobic digestion systems, and (iv) the benefit of two-stage system over single stage system in reducing inhibition of biogas production by cefazolin residues.

Materials and methods

Materials

The mixture of cow manure and milk from dairy cow that was directly spiked with cefazolin (CFZ) was used in this study instead of using manure and milk collected from cows administered with CFZ. This helped us to proportionally control the CFZ doses within each digester. Cefazolin was obtained from Zigma chemicals.

Cow manure was collected from cows that had not been treated with antibiotics including CFZ for considerable time. The manure was collected from the floor of free-stall barn at Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan. After collection, it was mixed with tap water at a ratio of 2:1 (wt:wt) and sieved through 2-mm sieve to remove coarse materials. The characteristics of sieved manure were: total solids (TS) 76.2 g/l, volatile solids (VS) 59.2 g/l and pH 7.3.

Fresh milk was collected from dairy cow that had not treated with antibiotics including CFZ for considerable time. The milk was obtained from a private farm in Obihiro, Japan. Its characteristics were: TS 132.2 g/l, VS 116.2 g/l and pH 6.7.

Inoculum (seed bacterial culture), used for single stage methane production and the second stage of two-stage system, was digested slurry taken from farm scale thermophilic (55 °C) biogas plant at Ashoro Town, Hokkaido, Japan. Prior to use, the slurry was pre-incubated at 55 °C for 7 days to remove residual organic matters. Its characteristics after pre-incubation were 38.7 g/l, 26.2 g/l and 8.1 for TS, VS and pH, respectively.

Experimental procedure

The experiments were carried out in 1 l digesters at thermophilic temperature (55 °C). For the two-stage system, manure was heat-shock pretreated as previously described by Lateef et al. (2012). A mixture (600 ml) of treated manure and milk at a ratio of 70:30 VS basis, based on recommendation of earlier study (Lateef et al., 2014), and total VS of 35 g/l was placed in each digester. The initial pH of the mixture was adjusted to 6.2 by addition of 2 M HCl. Cefazolin sodium salt was added at concentrations of 5, 10, 20, 40 and 80 mg/l mixture. Control test without CFZ was also conducted to determine hydrogen production from the substrate without CFZ. After 120 h (5 days), each digester was opened and 200 ml of the substrate was removed. The same amount of inoculum for second stage methane production was added and the pH in each digester was adjusted to 7.5 by addition of 2 M NaOH. The duration of methane production experiments was 360 h (15 days).

For single stage system, a mixture (400 ml) of manure and milk at ratio of 70:30 VS basis and total VS of 35 g/l was added to each digester. 200 ml of inoculum was added and the initial pH was adjusted to 7.5 by addition of 2 M HCl. Cefazolin sodium salt was added at concentrations of 5, 10, 20, 40 and 80 mg/l mixture. The single stage methane production experiments were carried out for 480 h (20 days).

All experiments were carried out batch-wise. In each of the cases above, the digesters were flushed with argon gas prior to sealing. The digestions were done in water baths maintained at 55 °C and the digesters were agitated manually once daily. No supplemental nutrients were added to the substrate. Each experiment was conducted in duplicate. Produced biogas was collected in a gas bag. The amount and composition were determined daily. Substrate samples were taken before and after experimentation to determine TS, VS and pH.