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Removal of antibiotics during the anaerobic digestion of pig manure

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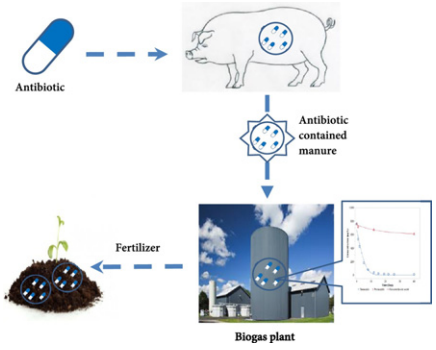
HIGHLIGHTS

- Digestion is able to remove some antibiotics while others are persistent.
- Sulfadiazine and sulfamethizole are persistent during manure digestion.
- Sulfamethoxazole, erythromycin and trimethoprim are degraded rapidly.
- Some transformation products are characterized.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotics are frequently used in animals to treat sickness and prevent infection especially in industrial meat production. Some of the antibiotics cannot be completely metabolized and, as an unavoidable result, are excreted and thus end up in manure which is then spread in the environment. Currently increasing amounts of manure is used in biogas production before spreading the residuals on agricultural fields. In this study, the removal patterns of sulfonamides (sulfadiazine, sulfamethizole, sulfamethoxazole) and macrolides (clarithromycin, erythromycin), as well as trimethoprim, were investigated during the anaerobic digestion of pig manure. Batch kinetic tests were conducted both at thermophilic and psychrophilic condition for 40 days. Some of the antibiotics (clarithromycin, sulfadiazine, sulfamethizole) were persistent in all experiments. Thus, no biodegradation was found for sulfadiazine and sulfamethizole in this study. From the studied compounds, only erythromycin was clearly removed and probably degraded during anaerobic digestion with 99% and 20% removal under thermophilic and psychrophilic condition. The removal of erythromycin was fitted to a single first-order kinetic reaction function, giving reaction rate constant of 0.29 day^{-1} and 0.005 day^{-1}, respectively.

1. Introduction

Antibiotics are frequently used in humans and animals to treat and prevent infections (Kümmerer, 2008). The conventional livestock husbandry has been changed into intensive livestock production due to population growth and economic progress (Hjorth et al., 2010; Udo et al., 2011). The raise of intensive livestock production is inevitably linked with higher demands on animal health thus more antibiotics are used to treat and prevent infections (Kemper, 2008; Widyasari-Mehta et al., 2016). Additionally, another major use of antibiotics is to enhance growth and feed efficiency in animals (Levy, 2013). However this practice is legally outphased in the EU in 2005

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(European Parliament and Council, 2003)), while in the United States, the Food and Drug Administration (FDA) has initiated a voluntary withdrawal of antibiotics for growth promotion by the pharmaceutical companies (Van Boeckel et al., 2015), which have been argued by U.S. food animal and pharmaceutical industries that such restriction have been detrimental to their food animal production. Canada, China, Australia, Brazil and Ukraine do not have any formal national restrictions on antimicrobial use for the purposes of growth promotion (Maron et al., 2013).

Depending on the compound used, 50% to 90% of the antibiotics will be absorbed quickly but are excreted via urine and feces after several days (Spielmeyer et al., 2017) resulting in high concentrations in manure (Hamscher et al., 2005) and thus high exposure to antibiotics via dust (Hamscher et al., 2003). Additionally, high concentrations of antibiotics in other compartments of the environment, such as soil and water have been described (Kemper, 2008) as well as their fate in soil (Thiele-Bruhn, 2003) or in treatment systems, such as a biogas plants (Widyasari-Mehta et al., 2016; Wolters et al., 2016). In Denmark, for instance, there are approximately 80 agricultural biogas plants digesting 2.5 million tons of manure (6–7% of all manure produced) (Luostarinen, 2013). Insam et al. (2015) reported advantages of utilizing digestates from fermenters as organic fertilizer. However, the application of fertilizer containing antibiotics has a risk of altering soil microbial constitution and function, and increased occurrence and abundance of antibiotic resistant genes in various soil bacteria (Knapp et al., 2009). Sengeløv et al. (2003) observed higher occurrence of tetracycline resistance after spreading of pig manure containing 48–698 μg/L of tetracycline. Zhu et al. (2013) also found that the genes potentially conferring resistance to sulfonamide, florfenicol and quaternary ammonium compounds were also found to be enriched broadly in farm samples. The occurrence of antibiotic resistant genes are not only diverse but also offers a higher statistical probability of transfer to the environment (Zhu et al., 2013). Therefore, it is essential to determine reaction rates and residual concentrations of antibiotics in anaerobic digestion to assess the risk connected to the use of this material as fertilizer.

In the present study, batch kinetic tests were conducted to determine the bio-degradability of antibiotics at anaerobic digestion of pig manure as a model scenario. Both, thermophilic and psychrophilic (as manure storage) anaerobic digestion were investigated. As a mechanistic and not a merely monitoring study was intended, we focusing on several groups of antibiotics with little isomers to gain clear data: Six commonly used antibiotics (clarithromycin, erythromycin, sulfadiazine, sulfamethizole, sulfamethoxazole, and trimethoprim) were spiked into pig manure which was then digested while sampling during the incubation for quantification.

2. Materials and methods

2.1. Substrate and chemicals

40 L of fresh pig manure was collected from a pig farm (Bjørnkærvej 1, Øster Velling, Denmark) on October 2015. The operations at this pig farm mostly produce 7–30 kg pigs. Total solids (TS) and volatile solids (VS) for the manure were 3.14 ± 0.04% and 2.16 ± 0.04%, respectively. It had a pH of 7.05 ± 0.1. After sampling, the coarse particles contained in pig manure were screened out by a 2 mm mesh (Spielmeyer et al., 2015).

Half of the pig manure was pre-incubated at 52 °C for 2 weeks serving as inoculum in both biogas yield test and biodegradation kinetic test. The rest was stored at −18 °C prior to using.

Clarithromycin, erythromycin, sulfamethoxazole and trimethoprim were obtained from Sigma-Aldrich while sulfadiazine and sulfamethizole were obtained from Dr. Ehrenstorfer, (Wesel, Germany). HPLC-grade methanol and formic acid were obtained from Merck (Darmstadt, Germany) and HPLC-MS-grade water was obtained from Sigma-Aldrich (Brøndby, Denmark).

2.2. Instruments

Gas chromatography (Agilent technologies 7890A, Santa Clara, CA 95051, USA) equipped with a thermal conductivity detector (TCD), an Alltech® CTR 1 double column (Grace, MD, USA), and helium as the carrier gas was used to determine the biogas composition. The temperature of oven, injector port, and detector was 120, 150, and 150 °C, respectively.

Biodegradation experiments were conducted by using an incubation unit (Bioreactor simulator, Bioprocess Control, Lund, Sweden) consisting of 2 L reactors with rubber plug, mechanical agitation (100 rpm) and a temperature control system. HPLC-MS/MS was used to quantify the antibiotics in the manure. The HPLC was equipped with dual low-pressure mixing ternary-gradient system Ultimate 3000 (Dionex, Germering, Germany). The system had a pump of the 3000 series (DGP-3600 M), a 3000 TSL autosampler (WPS 3000 TSL) and a column oven and degasser also from the Dionex 3000 series. The HPLC operated with two ten-port Valco valves. The mass spectrometer was an API 4000 (ABSciex, Framingham, MA, USA). The API 4000 was operated with an ESI source in positive mode which was set at 400 °C with a capillary voltage of 5500 V. The method used methanol and water, both containing 0.2% of formic acid, as mobile phases. The column used was a Synergy 4 μm Polar-RP (150 × 2 mm) (Phenomenex, Torrance, CA, USA). Further details of the HPLC-MS/MS conditions are described in (Escolà-Casas et al., 2015). The details of the mass spectrometrical operation including the conditions for the multi reaction monitoring (MRMs) are shown in the supplementary material Table S1. The pH value was measured using portable pH meter (Portamess 911, Knick, Germany).

2.3. Sampling and sample preparation

10 mL of manure were centrifuged for 15 min with 6000 G (6000 rpm) for the quantification of antibiotics during the anaerobic digestion. Subsequently 100 μL of the aqueous phase were taken and placed into an HPLC vial, together with 1000 μL of distilled water. 20 μL of labelled internal standard of erythromycin ^{13}C D_3, sulfadiazine $^{13}C_6$ and trimethoprim D_3 were added to reach a concentration of 1 ng/mL in the samples. 10 μL of each prepared sample were injected into the HPLC-MS/MS in MRM mode. For the identification of transformation products, separate incubations for the individual compounds were conducted: 5–10 mL of manure (containing 1 mg of single antibiotics) were centrifuged for 20 min with 6000 G (6000 rpm). Then, 1 mL of the aqueous phase was transferred to an HPLC vial. First, 10 μL were injected into the HPLC-MS/MS in full-scan mode. After identifying possible transformation products, 5 μL were injected in the HPLC-MS/MS in product ion scan and MRM mode in order to verify and quantify them.

2.4. Incubations

Three batch assays were conducted to 1) determine the influence of antibiotics on biogas production from pig manure; 2) investigate the reaction kinetics; and to 3) identify the transformation product. Table 1 shows the concentration (μg/L) of antibiotics in the original unspiked pig manure used for the experiments. It can easily be seen that the conventional operation from which the manure originated, used not only one but several antibiotics. However, since our spike level was at mg/L and not at μg/L as the background, the quantitation and kinetic results should be uninfluenced by the background values. However, the microbial communities will to some extent be adapted to these antibiotics.

2.4.1. Determining the influence of antibiotics on biogas production

Anaerobic incubations were performed to determine the influence of antibiotic on biogas production (bio-methane yield). The experiments were carried out following the procedures suggested by Moset et al. (2015). Incubation bottles (1 L) containing a mixture of pig manure and pre-incubated inoculum at a mixing ratio of approximately 1:1 based on volume, were dosed with all targeted antibiotics to reach