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Elimination patterns of worldwide used sulfonamides and tetracyclines during anaerobic fermentation

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HIGHLIGHTS

- Veterinary antibiotics can be detected in biogas input and output samples.
- Sulfonamides and tetracyclines are partly eliminated during anaerobic fermentation.
- Semi-continuous lab-scale fermenters were used for simulation of biogas plants.
- Elimination rates depend on structure of target compound and matrix utilized.
- Biogas plants might serve as sink for veterinary antibiotics.

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ABSTRACT

Antibiotics such as sulfonamides and tetracyclines are frequently used in veterinary medicine. Due to incomplete absorption in the animal gut and/or unmetabolized excretion, the substances can enter the environment by using manure as soil fertilizer. The anaerobic fermentation process of biogas plants is discussed as potential sink for antibiotic compounds. However, negative impacts of antibiotics on the fermentation process are suspected. The elimination of sulfadiazine, sulfamethazine, tetracycline and chlortetracycline in semi-continuous lab-scale fermenters was investigated. Both biogas production and methane yield were not negatively affected by concentrations up to 38 mg per kg for sulfonamides and 7 mg per kg for tetracyclines. All substances were partly eliminated with elimination rates between 14% and 89%. Both matrix and structure of the target molecule influenced the elimination rate. Chlortetracycline was mainly transformed into iso-chlortetracycline. In all other cases, the elimination pathways remained undiscovered; however, sorption processes seem to have a negligible impact.

1. Introduction

Anaerobic fermentation of biomass delivers gas mainly consisting of methane and carbon dioxide. This conversion occurs in four stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. Each stage is supported by microorganisms that differ both in pH optima and tolerance towards oxygen (Angelidaki et al., 1993; Weiland, 2010). The conversion of biomass to gas is exploited in biogas plants. According to the European Biogas Association (EBA), over 13,800 biogas plants contribute to the so-called sustainable green energy in Europe (status 2012), almost 8000 plants are installed in Germany (German Biogas Association). The main substrates utilized are maize silage due to its high biogas delivering potential and liquid manure (Weiland, 2010).

Liquid manure and the fermentation residue of biogas plants are used as soil fertilizer. This way, the substrates contribute to a reduced usage of mineral fertilizers. Insam et al. discussed several advantages for the fermentation residues used as soil fertilizer like a possibly enhanced microbial activity in the soil or facilitated application due to homogenization of the material during fermentation (Insam et al., 2015). However, beside nutrients liquid manure can contain loads of antibiotic active compounds. Antibiotics are frequently used in veterinary medicine. Since 2012, the Federal Office of Consumer Protection and Food Safety (BVL) publishes the amount of antibiotics delivered to veterinarians in Germany. In 2013, 1452 tons were supplied with tetracyclines and penicillines being the most distributed active compounds (454 tons respective 473 tons), followed by sulfonamides (152 tons). Most pharmaceuticals are not completely absorbed

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and/or metabolized by the animal, leading to excretion rates of 30–90% of the mother compound (Sarmah et al., 2006 and references therein). These substances can enter the environment by using liquid manure as soil fertilizer as well as via the anaerobic fermentation process in biogas plants when manure is used as substrate. Antibiotics influence the metabolism, growth and viability of various microorganisms. Therefore, several studies investigated the impact of diverse antibiotics on the anaerobic fermentation process. Previous studies delivered varying results due to differences in the setup (batch reactor vs. continuous process), the substrate utilized (e.g. manure, activated sludge, biogas plant fermenter content), the initial compound concentration, or the duration of the experiment. In some cases no influence was found, whereas other studies revealed a decrease of the biogas and/or methane yield (Álvarez et al., 2010; Bauer et al., 2014; Cetecioglu et al., 2013; Lallai et al., 2002; Massé et al., 2000; Mitchell et al., 2013; Mohring et al., 2009; Sara et al., 2013).

Another aspect under investigation is the fate of the antibiotic compounds themselves. Studies have shown the elimination of selected veterinary antibiotics during the anaerobic fermentation (Álvarez et al., 2010; Cetecioglu et al., 2013; Mitchell et al., 2013; Mohring et al., 2009; Riemenschneider et al., 2014). Thus, the process in biogas plants is discussed as potential sink for veterinary antibiotics. However, currently only little information is available about possible metabolites and their antibiotic potential. The elimination pathways (abiotic due to i.e. temperature, pH, sorption, anaerobic conditions, biotic due to potential transformation or degradation via microorganisms) often remain unclear as well.

A sampling of biogas plants was conducted to estimate the elimination potential of the anaerobic fermentation process for antibiotic compounds. For this, the antibiotic content of fermenter input and output samples was analyzed over the course of one year. For a detailed study, a simulation of biogas plants in laboratory scale was performed using semi-continuous fermenters. Via this setup, the elimination pattern of the most important tetracyclines (chlortetracycline, tetracycline) and sulfonamides (sulfadiazine, sulfamethazine/sulfadimidine) in veterinary medicine was observed. As sulfonamides are often applied with trimethoprim for synergistic action, this compound was included in the study as well. Furthermore, an estimation of the impact of abiotic and biotic factors on the observed elimination was conducted.

2. Methods

2.1. Reagents and materials

Standard substances (VETRANAL™ grade, if available), citric acid, formic acid, and EDTA were obtained from Sigma–Aldrich (Taufkirchen, Germany). Analytical solvents were of UHLC–MS grade and purchased from Actu-All Chemicals (Oss, Netherlands).

Biogas plant samples for the analysis of the antibiotic content during the course of one year were taken at one biogas plant in Hesse (plant A) and Bavaria (plant B), respectively. For the input samples, liquid manure and manure were taken from the storage containers at the plant site and mixed in the ratio utilized in the respective plant (e.g. three parts liquid manure and one part manure). Therefore, a volume of approx. 30 L was mixed and an aliquot was filled in a 250 mL PE bottle for analysis. Output samples were taken from the secondary fermenter as 1 h mixed sample on the same day like the input samples. For this purpose, 5 L were taken from the fermenter in intervals of 10 min to get a final volume of approx. 30 L. This volume was stirred and an aliquot was filled in a 250 mL PE bottle for analysis. Samples were transported in a cold box and stored at −20 °C before extraction.

2.2. Sample preparation and analysis

Sample preparation and LC–MS/MS analysis were performed as previously published (Spielmeyer et al., 2014). Briefly, 1 g sample was extracted using citric acid buffer (1 M, pH 4.7, containing 0.05 M EDTA) and dichloromethane, ethanol, methanol (1:3:1, v/v/v). The analysis was performed in the multiple reactions monitoring (MRM) modus. Samples were analyzed within 24 h after preparation. Antibiotic free cattle feces were used for matrix calibration. Analyses and quantification of tetracyclines included the respective 4-epi-isomers.

2.3. Semi-continuous fermenter studies

The experiments were conducted using 36 L fermenters (replica according to plans from the Bavarian State Research Centre for Agriculture, made from plastics and stainless steel) with an operating volume of 28 L. For incubation, fermenter contents of two field biogas plants were utilized to receive an established microbiota for the anaerobic fermentation process. Liquid manure was obtained from the same plants to simulate the respective plant in laboratory scale, maize silage came from one of the biogas plants. The materials of the biogas plants are referred to as matrix I and matrix II, respectively.

During a 3 weeks run-in phase, 120 g maize silage were added daily and 430 g liquid manure once per week at the top of the fermenter. Once per week, 400 g fermenter content was removed at the fermenter's bottom to keep a constant operating volume. Within the last five days of the start-up phase, the procedure was changed to a daily addition of 360 g liquid manure and 120 g maize silage and a daily removal of 300 g fermenter content. The removal was performed prior to the addition of new organic matter. This procedure was kept for the rest of the experiment and corresponds to a volumetric loading of 2 kg organic dry matter (oDM) per m^3 and day. Fermentation was performed employing mesophilic conditions with an average temperature of 41.7 °C. The fermenter content was continuously stirred for 10 min followed by a pause of 5 min. Samples for analyses were withdrawn from the fermenter's bottom.

For each matrix, three fermenters were utilized. For matrix I, fermenter 1 was fed with a non-fortified liquid manure/maize silage mixture and served as control. In case of fermenter 2, the added organic matter was fortified with respective 15 mg per kg sulfadiazine and sulfamethazine within the first 4 days. From day 5 onwards, the fortification was increased to 50 mg kg^{-1} and, furthermore, trimethoprim was added with 5 mg kg^{-1} as well. Fermenter 3 was treated with tetracycline and chlortetracycline, each with 15 mg kg^{-1}. For matrix II, fermenter 4 served as control as described for fermenter 1. Fermenter 5 and 6 were treated accordingly to fermenter 2 and 3. However, in case of fermenter 5, a fortification of 50 mg kg^{-1} for sulfadiazine and sulfamethazine, respectively, and 5 mg kg^{-1} trimethoprim was used from the first day on (Table 1). The addition of the fortified liquid manure/maize silage mixture was performed for 33 days. Thereafter, a 30 days wash-out phase started using a non-fortified liquid manure/maize silage mixture for the daily substrate exchange. The fortification was performed daily by adding the respective amount of an aqueous stock solution to the substrates right before fermenter feeding.

Gas production and methane yield were automatically determined daily by drum type gas meters (Ritter Apparatebau, Bochum, Germany) and an infrared 2-beam sensor (Awite BioEnergie, Langenbach, Germany), respectively. Samples for the analyses of pH and (organic) dry matter were taken once a week, samples for antibiotic analyses were taken two times a week. Dry matter (DM) and oDM determination was performed in triplicates, the other parameters were determined via single analyses.