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Research article

# Behaviour of doxycycline, oxytetracycline, tetracycline and flumequine during manure up-cycling for fertilizer production

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ABSTRACT

The fate of four widely used veterinary antibiotics (doxycycline, flumequine, oxytetracycline and tetracycline) during manure upcycling was investigated at laboratory and pilot scale. The pilot was operated continuously, while the laboratory scale in batch mode. Both set-ups consisted of anaerobic digestion, ammonia stripping and a solid liquid separation step. A partial nitritation anammox process was used to treat the laboratory scale effluent. In the pilot installation, pig manure as feed, natural occurring antibiotics levels were reduced by 92% for doxycycline, 88% for flumequine, 95% for oxytetracycline and 100% for tetracycline. In the laboratory scale set-up, antibiotic free sludge was used and the four substances were spiked. The input antibiotics concentration was reduced by 85% for doxycycline, 46% for flumequine, 97% for oxytetracycline and 100% for tetracycline. In both set-ups the centrifuge cake was identified as the major emission pathway for residual antibiotics. Manure upcycling, while producing fertilizers, can be considered effective in reducing the residual antibiotic load.

## 1. Introduction

Antibiotics are widely used in animal husbandry, especially at pig farms to prevent and treat disease outbreaks (Kemper et al., 2008). Until their ban in 2006, antibiotics were also used as growth promotors in the European Union (Castanon, 2007). The release of antibiotics into the environment through manure spreading is of considerable concern because persistent antibiotic residues may lead to development of antibiotic-resistant bacteria (Álvarez et al., 2010; Landers et al., 2012; Youngquist et al., 2016). Once ingested by an animal, these compounds can be metabolised following different pathways. They are eventually excreted, maintaining the same chemical structure or as metabolites that have been transformed into isomers (Kemper et al., 2008). Roughly between 20% and 75% of antibiotics fed to animals are excreted in manure either unaltered or as metabolites (Jjemba, 2002).

Manure treatment has become more important during the last decades due to intensified animal husbandry and more stringent regulations. Anaerobic digestion has been considered as one of the most common manure treatment systems (Chen et al., 2008; Zhang et al., 2012). Through anaerobic digestion, manure is stabilized (dry matter and COD reduction) and energy in the form of biogas is generated. The digested manure is commonly applied on fields as fertilizer. However, due to low nutrient content and varying composition (both nutrient and possible pollutants), especially in regions with high livestock density and nitrate vulnerable zones, direct field application is being reconsidered (Bonmatí and Flotats, 2003; Wäger et al., 2009). Therefore, manure upcycling and production of high grade fertilizers are deemed to be of high importance. While the production of fertilizers from manure is in line with the idea of a circular economy, the contamination and migration of micro pollutants should be considered to mitigate risks for agricultural application.

In order to demonstrate the feasibility of manure up-cycling, a process cascade has been developed by combining several well-established core technologies into a novel process. This cascade was implemented as pilot plant at the premises of a large biogas plant in The Netherlands and was operated for 12 months, treating pig-manure and co-substrates (Pintucci et al., 2016). For the first treatment step, thermophilic anaerobic digestion coupled with an ammonia stripping was used. High rate anaerobic digestion is reached by the thermophilic temperature level (Liu and Sung, 2002) allowing high volumetric loading rates. Ammonia, toxic for the anaerobic process at higher temperatures, was removed by the stripping unit and converted to ammonia sulphate. Since phosphorous recovery through struvite precipitation has become an interesting manure up-cycling option (Kataki et al., 2016), this process was applied after a solid-liquid separation of the digestate. As final step to reach irrigation water quality, nitrogen

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removal via a partial nitritation and anammox was implemented.

During monitoring of the pilot plant and the subsequent laboratory scale experiments a wide range of antibiotic substances were detected. However, it was decided to focus only on four substances, which are widely used in piggery and were present at the highest concentrations at the pilot. Three of them (doxycycline, oxytetracycline and tetracycline) belong to the important group of tetracyclines. Tetracycline concentrations in manure between 0.001 and 150 mg $kg^{-1}$ were reported by various authors (Álvarez et al., 2010; Massé et al., 2014; Van Epps and Blaney, 2016). Flumequine out of the group of fluoroquinolones was chosen as the fourth compound to monitor. Total fluoroquinolones concentrations in manure were reported between 0.005 and 5 mg $kg^{-1}$ (Massé et al., 2014; Van Doorslaer et al., 2014). The huge concentration range reported for both substance groups can be attributed to different animal treatment regimes, manure types, treatment and storage processes. Reported antibiotic removal rates during anaerobic manure digestion are strongly dependent on the manure type and the digester set up. For the group of tetracyclines removal rates between 53 and 82% at mesophilic temperatures were reported by Youngquist et al. (2016). According to Van Doorslaer et al. (2014) the data situation for the removal of flumequine and the whole group of fluoroquinolones during anaerobic digestion is not sufficient to estimate removal rates.

During the operation of the pilot plant in The Netherlands, a dedicated sampling campaign for antibiotics was conducted to study contamination, migration and removal at pilot scale. To verify the pilot plant results, additional experimental trials were conducted at laboratory scale.

## 2. Materials and methods

### 2.1. Pilot scale

The pilot plant was installed and operated at a full-scale biogas plant in Hulst, The Netherlands (Fig. 1 a). Due to on-going process optimisation at the pilot, not all the designed treatment steps were operational at the time of the sampling campaign. Therefore, an up-cycling cascade consisting of anaerobic digestion, ammonia side stream stripping and two-step solid-liquid separation was investigated for antibiotics monitoring at the pilot. The sampling covered raw input materials (manure and co-substrates) and the inputs and outputs of the process steps to identify emission pathways to products and the environment.

Pig manure from The Netherlands' Zeeland-Flanders region was fermented together with Ecofrit as co-substrate to increase biogas production. Ecofrit is a mix of vegetable wastes from supermarkets. The composition in terms of organics, nutrients and dry matter of manure, the co-substrate and the feed mixture is given in Table 1. For feed preparation, the manure was sieved and the co-substrate was shredded and sieved. The substrates were then stored in separate buffer tanks, which were weekly re-filled. Manure and co-substrates were subsequently mixed in a feeding buffer tank according to a specific feeding ratio (Table 1). Samples were taken from the buffer tanks.

**Table 1**
Feed composition of the pilot plant; DM…dry matter, oDM…organic dry matter, COD…chemical oxygen demand, TN…total nitrogen, $NH_4^+$-N…ammonia-nitrogen, TP…total phosphorus, $PO_4^{3+}$-P…phosphate-phosphorus.

| | | Pig manure (n = 16) | Ecofrit (n = 7) | Final feeding |
|---|---|---|---|---|
| Feeding mixture | % (w/w) | 83.7 | 16.3 | 100.0 |
| DM | g $kg^{-1}$ | 86.0 ± 11 | 191.4 ± 14.7 | 107.1 ± 25.9 |
| ODM | g $kg^{-1}$ | 60.0 ± 8.0 | 144.9 ± 14.6 | 77.0 ± 9.3 |
| COD | g $kg^{-1}$ | 83.4 ± 20.9 | 239 ± 14.5 | 114.7 ± 19.6 |
| $COD_s$ | g $kg^{-1}$ | 40.6 ± 16.7 | 111.8 ± 3.8 | 54.8 ± 14.1 |
| TN | g $kg^{-1}$ | 6.9 ± 1.2 | 6.7 ± 0.5 | 6.9 ± 1.1 |
| $NH_4^+$-N | g $kg^{-1}$ | 4.5 ± 0.6 | 1.6 ± 0.2 | 3.9 ± 0.5 |
| TP | g $kg^{-1}$ | 1.9 ± 0.4 | 2.4 ± 0.3 | 2.0 ± 0.4 |
| $PO_4^{3-}$-P | g $kg^{-1}$ | 1.7 ± 0.4 | 2.1 ± 0.2 | 1.7 ± 0.3 |

The substrate was fermented in a continuously stirred reactor (3 $m^3$ working volume) at thermophilic level (51.1 ± 1.0 °C). The digester was inoculated with thermophilic biomass (EcoFuel, Well, The Netherlands) and fed on a daily basis. Biogas production was measured from the reactor and the digestate buffer using mechanical biogas counters. The biogas from the digester was passed into a 1 $m^3$ biofilter composed of organic absorption material. The off-gas from the filter was released to the atmosphere.

Ammonia was removed from the biogas reactor through a batch side stream stripping unit. The stripping column had a volume of 990 L and a working volume of 330 L. The unit was operated at a temperature of 65 °C. A digestate batch was stripped for 6–12 h. The air flow rate was set to 75 L air $L^{-1}$ digestate and h (25% fresh and 75% recycled). Air was injected into the stripping column at two different locations (bottom and middle of the column) by three compressors. After ammonia stripping, the stripped digestate was recirculated back in the digester. The strip gas (ammonia and air) was led into a counter current scrubbing column. The gaseous ammonia was scrubbed out of the strip gas with sulphuric acid (40% w/w) and bound in the form of ammonia sulphate. The cleaned air was partly recirculated. The collected ammonia sulphate solution was pumped into a storage tank after each stripping run.

For solid liquid separation (SL) of the digestate a two-step process was implemented. The first step consisted of a decanter centrifuge with a subsequent ultrafiltration of the centrifugate as second step. A MD-60 pilot scale decanter centrifuge (Lemitec GmbH, Germany) was used for the first step of the SL. The digestate from the biogas reactor was shredded before entering the centrifuge to reduce clogging. The system was operated at a hydraulic retention time (HRT) of 45 s, a G-force of

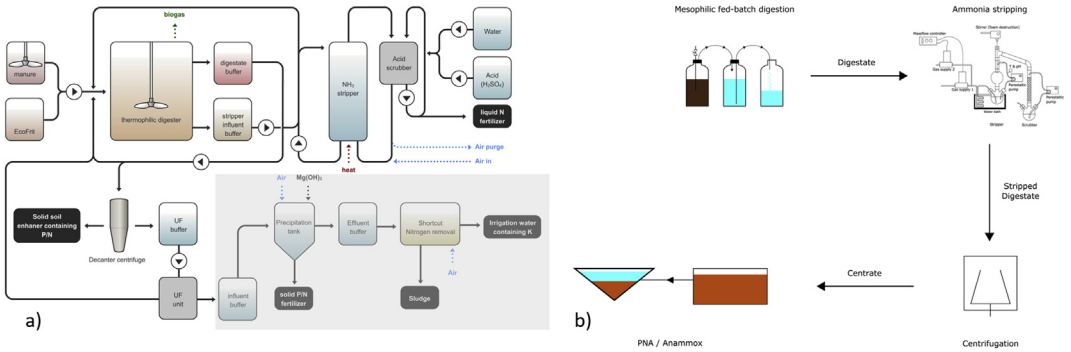


**Fig. 1.** a. Design scheme of the pilot plant. Processes steps shaded in grey were not operational at the time of the monitoring campaign. b. laboratory scale simulation of the MEM process.