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Processing of mycotoxin contaminated waste streams through anaerobic digestion

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ABSTRACT

Food and feed stocks heavily contaminated with mycotoxins are rendered unfit for consumption and therefore discarded as waste. Due to the lack of guidelines and in accordance with the prudent avoidance principle, these waste streams are often incinerated. For better valorization, these streams could be used as input for anaerobic digestion. However, the degradation of multiple mycotoxins during anaerobic digestion and their effect on the methane production is currently unknown. In batch tests spiked with mycotoxins, aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone and T-2 toxin were degraded for more than 90%. For mesophile and thermophile digestion respectively, fumonisin B1 was degraded for 70% and 85%, and most ergot alkaloids for 64% and 98%. Neither biogas production, nor methane production were influenced by the presence of the mycotoxins. Subsequently, semi-continuous reactors fed with contaminated maize resulted in more than 99% degradation for all mycotoxins after 1.8 hydraulic retention time with stable biogas production and process parameters. This study shows that mycotoxin contaminated organic waste can be safely valorized to methane while the digestate is void of mycotoxin residues.

1. Introduction

When food and feed stocks get infected by filamentous fungi, this may lead to a contamination with multiple mycotoxins, thereby rendering these stocks unfit for human or animal consumption. Mycotoxins are fungal secondary metabolites with diverse chemical structures which contaminate many of the most frequently consumed food and feed commodities worldwide (Binder et al., 2007; Miller and Greenhalgh, 1988). They occur all around the world as natural contaminants in numerous commodities of plant origin, especially in cereals grains, but also in nuts, oilseeds, fruits, dried fruits, vegetables, cocoa and coffee beans, wine, beer, herbs and spices (Marin et al., 2013; Smith et al., 2016; Stoev, 2013). The fungal and mycotoxin contamination pattern is complex and differs worldwide, as recently reviewed by Aiko and Mehta (2015). Concentrations in for example feed and feedstuffs range worldwide range from 1–90 ppb aflatoxin B1 (AFB1), 1–271 ppb zearalenon (ZEN), 51–1947 ppb deoxynivalenol (DON), 109–3121 ppb fumonisin B1 (FB1) and 1–15 ppb ochratoxin (OTA) (Pinotti et al., 2016). The UN Food and Agriculture Organization estimated that each year, 25% of the world's crops are affected by mycotoxins resulting in annual losses of around 1 billion metric tons of food and food products (FAO, 2010).

Although several decontamination strategies have been explored or developed (Zhu et al., 2016), heavily contaminated food and feed stocks are irreversibly lost for consumption. So in addition to a lost income from the crop, the farmer faces an additional financial cost of waste disposal through for example incineration. A more attractive alternative would be the valorization of contaminated food and feed stocks towards energy and fertilizer by anaerobic digestion. At this point, neither maximum limits nor guidance levels have been established in the EU for contaminated lots intended for industrial purposes (e.g., bioethanol or biopolymer production) (Pinotti et al., 2016), such that for example in Belgium, these lots are being incinerated in accordance with the prudent avoidance principle. In order to safely process mycotoxin contaminated materials through anaerobic digestion, two important aspects need to be considered.

Firstly, the microbial community of the anaerobic digestion process may be inhibited by the presence of the mycotoxins,

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potentially hampering methane production. The effects of mycotoxins on eukaryotic organisms, specifically humans and animals, has been studied extensively (Bryden, 2012; da Rocha et al., 2014), but regarding prokaryotic organisms less information concerning possible growth and metabolism inhibition is available. No significant growth inhibition of *E. coli* K12 exposed to 2 ppm DON was observed (Park et al., 2014), nor were *Ruminococcus albus* and *Methanobrevibacter ruminantium* negatively affected by 100 μg/mL DON. However, in a screening of ten bacterial strains exposed to five mycotoxins, several inhibitory growth effects were detected (Ali-Vehmas et al., 1998). Also, considering a similar microbial ecosystem to anaerobic waste digestion, the acetate and propionate production were significantly reduced in *in vitro* rumen fermentation exposed to 40 mg/kg dry matter DON (Jeong et al., 2010).

Secondly, is important to know whether the mycotoxins are degraded such that the digestate may be safely applied as a fertilizer, and which process parameters influence this degradation. It is well established that several mycotoxins are prone to biological degradation (Vanhoutte et al., 2016), also in the rumen similarly harboring anaerobic fermentative conditions (Upadhaya et al., 2010). However, to our knowledge, only three studies investigated the processing of mycotoxin contaminated organic waste through anaerobic digestion technology. AFB1 was degraded for 69–87% after 60 days in batch tests at 37 °C while no significant effect on the biogas quantity and quality was observed (Salati et al., 2014). In a follow-up experiment, a daily dose of 7.2 μg/kg AFB1 was also shown to degrade for 47% after 120 days in a continuously stirred tank reactor operated at an hydraulic retention time of 40 days. Biogas and methane production were similar between the two reactors and all other process parameters were within normal range. Frauz et al. (2007) reported no inhibitory effects on the anaerobic digestion process of cereals with 20.000 ppb DON compared to the control cereals. In another study (Goux et al., 2010), seven DON contaminated wheat flour samples were subjected to anaerobic digestion in triplicate lab-scale batch reactors, in which no significant effect was observed on the biogas quantity and quality. Reductions of DON after 30 days varied from 65% to 100% depending on the start concentration. In both studies, only one mycotoxin was investigated, AFB1 and DON respectively. However, in practice, mycotoxin contaminated batches will always harbor a mixture of often structurally not related mycotoxins. The simultaneous degradation of multiple mycotoxins, and their cumulative effect on the anaerobic digestion process itself, is currently unknown.

In this study, the processing of mycotoxin contaminated waste streams through anaerobic digestion was investigated, with respect to the residual presence of the mycotoxins in the digestate and the effect of on the methane production. First, separate mycotoxins representing different chemical mycotoxin categories were assessed in spiked lab-scale batch tests, in order to detect possibly problematic effects on the anaerobic digestion of each mycotoxin. Next, a naturally contaminated sample harboring a mixture of mycotoxins was used in a pilot scale semi-continuous test.

2. M&M

2.1. Mycotoxins and mycotoxin contaminated material

The individual mycotoxin calibration standards of ergometrine (Et, 500 μg), ergosine (Es, 500 μg), ergotamine (Em, 500 μg), ergocornine (Eco, 500 μg), α-ergokryptine (Ekr, 500 μg), ergocristine (Ecr, 500 μg), aflatoxin B1 (AFB1, 20 μg/mL), ochratoxin A (OTA, 10 μg/mL), deoxynivalenol (DON, 100 μg/mL), zearalenone (ZEN, 100 μg/mL), fumonisin B1 (FB1, 50 μg/mL), T-2 toxin (T-2, 100 μg/mL) and deepoxy-deoxynivalenol (DOM, internal standard, 50 μg/mL) were obtained from Romer Labs - Food Risk Management (The Netherlands). The other internal standards methylergometrine (MeEm, 10 mg), dihydroergotamine (DHEt, 50 mg) and zearalanone (ZAN, 1 mg) were purchased from Sigma Aldrich (Belgium). The stock solution of the reference components of the ergot alkaloids was a mixture containing 3 μg of each of the 6 ergot alkaloids. The dried standards were stable for at least 14 months at −18 °C (Lauber et al., 2005), however, the expiry date should not exceed the date mentioned on the certificate of analysis. All other mycotoxin standards were dissolved in methanol (1 mg/mL), and were storable for a minimum of 1 year at −18 °C (Spanjer et al., 2008). Water was obtained from a Milli-Q® SP Reagent water system from Millipore Corp. (Belgium). Methanol (LC-MS grade), acetonitrile (LC-MS grade) and acetic acid (UPLC-MS grade) were purchased from BioSolve (The Netherlands), while acetonitrile (Analar normapur), methanol (HiperSolv chromanorm), *n*-hexane (Hipersolv chromanorm), ammonium acetate, ammonia (25%) and ammonium sulphate were obtained from VWR International (Belgium). Acetic acid (glacial, 100%) was supplied by Merck (Germany). Disinfectol® (denaturated ethanol + 5% ether) was supplied by Chem-Lab (Belgium). Ammonium bicarbonate was purchased from Sigma Aldrich (Belgium), while ethyl acetate was obtained from Across Organics (Belgium).

The contaminated maize used for the semi-continuous test was obtained through Synagra, the Belgian Federation for Grain Trading and Other Agricultural Commodities. The maize was milled and fully homogenized after which it was stored at 4 °C and a sample was taken for analysis.

2.2. Mycotoxin analysis

2.2.1. Analysis of digestate on the presence of mycotoxins: method 1

The digestates were homogenized with a spatula, and 5 g (±0005 g) of the representative sample was weighed on an analytical balance in an extraction tube of 50 mL. Six blank digestate samples (5 g ± 0005 g) were also weighed to set-up a matrix-matched calibration curve. The calibrators were spiked in the following ranges: AFB1 (10–40 μg/L), OTA (25–100 μg/L), DON (200–800 μg/L), ZEN (50–200 μg/L), T-2 (50–200 μg/L) and FB1 (200–800 μg/L). Unknown and blank samples were spiked with 100 μL ZAN (200 μg/L on sample) and 25 μL DOM (250 μg/L on sample). Because of the diversity in physico-chemical properties of the mycotoxins, an extensive clean-up needed to be performed. Sample clean-up and LC-MS/MS analysis were performed according to Monbaliu et al. (2010). LC-MS/MS analysis was performed on a Waters Acquity HPLC coupled to a Quattro Premier XE mass spectrometer (Waters, MA, Milford, USA). Two SRM-transitions were followed during the LC-MS/MS analysis as presented in Table S1. Data were analyzed and interpreted according to Masslynx™ and Quanlynx™ software (Waters, Ma, Milford, USA). Limit of detection and quantification, decision limit, detection capability, recovery rate and measurement uncertainty are shown in Table S2.

2.2.2. Analysis of digestate on the presence of ergot alkaloids: method 2

The digestates were vigorously homogenized with a spatula to obtain a representative sample. On an analytical balance 5 g (±0.005 g) were weighed in an extraction tube of 50 mL. Next to the unknown samples, five blank samples were foreseen (5 g ± 0.005 g) to establish a matrix-matched calibration curve. To all unknown and blank samples, 10 μL of MeEm (40 μg/l on sample) and 50 μL of DHEt (200 μg/l on sample) was added. The calibration curve was set up by spiking the 5 blank samples with the 6 ergot alkaloids (5 μg/mL) in a range from 10 μg/L to 100 μg/L (10 μg/L, 25 μg/L, 50 μg/L, 75 μg/L and 100 μg/L). The clean-up and

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