Waste Management 78 (2018) 467–473

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Effect of the mycotoxin aflatoxin B1 on a semi-continuous anaerobic digestion process

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ARTICLE INFO

Article history:
Received 16 October 2017
Revised 26 April 2018
Accepted 6 June 2018

Keywords:
Aflatoxin B1
Anaerobic digestion
Biomethane
Phytotoxicity
Waste treatment

ABSTRACT

Cereals are primary crops and are the most important raw material for feed and food production. Increasing aflatoxin B1 (AFB1) contamination of corn is an emerging issue, and disposal procedures for AFB1-contaminated corn are not currently defined. Recovery of contaminated corn through anaerobic digestion may represent a suitable strategy for its valorisation; however, only a few studies concerning the effect of AFB1 on anaerobic processes can be found. Thus, the purpose of the present work was to evaluate the effect of the mycotoxin AFB1 on a semi-continuous anaerobic digestion (AD) process. Semi-continuous trials were carried out, and the biomethane production from ABF1-contaminated feedstocks (25, 50, and 100 μg kg^{-1} AFB1 wet weight) was compared to that from non-contaminated feedstock. Moreover, the feasibility of the agronomic re-use of the digestate, and the fate of AFB1 during AD was assessed. No adverse effect of 25 μg kg^{-1} AFB1 contamination of feedstock on biomethane yield was observed. In contrast, 100 μg kg^{-1} AFB1 in the feedstock resulted in inhibition of the process due to the accumulation of organic acids, and to the decrease of the pH in the digestate (from 8.1 to 5.4). The continuous addition of AFB1-contaminated feedstock led to accumulation of the mycotoxin in the digestates. Consequently, a composting process should always precede the agricultural re-use of digestates in order to remove AFB1 and the residual phytotoxicity.

1. Introduction

Approximately 9 million hectares of corn, and 26 million hectares of wheat are cultivated yearly in Europe. Furthermore, cereals generally constitute approximately 30% of the human diet in industrialized countries, and roughly 50% of the animal feed in Europe (FAOStat, 2013). Therefore, issues related to these crops are of great economic and health concern (Battilani et al., 2016). Increasing aflatoxin B1 (AFB1) contamination of corn is one of the most significant health and environmental issues related to cereal cultivation. The European Food Safety Authority (EFSA) studied the prevalence of AFB1 in several foods and in feed, and found that the 14% of corn samples analysed were contaminated, with a mean AFB1 concentration of 0.26 μg kg^{-1} (EFSA, 2007).

Identified in 1960 as the cause of "Turkey X Disease", the mycotoxin AFB1 is produced by the fungal species *Aspergillus parasiticus*, *A. flavus,* and *A. nomius* (Salati et al., 2014). These widely distributed moulds infect grain and other raw food, and contaminate the food supply (Minto and Townsend, 1997). AFB1 is known to be a mutagenic, carcinogenic, and teratogenic compound, and for these reasons, it is classified by the International Agency for Research in Cancer (IARC) as a class-1 human carcinogen. Indeed, after ingestion, liver cytochrome P450 enzymes convert AFB1 into its carcinogenic form (AFB1-8,9-epoxide), which bonds covalently to DNA and serum albumin, producing AFB1-N7-guanine and AFB1-lysine adducts, respectively (Vlastimil et al., 2014).

The most important factors that contribute to the increased occurrence of aflatoxin in food include weather conditions (temperature and humidity), agronomic practices, and food chain factors such as drying and storage conditions (Guevara-Gonzalez, 2011; Strosnider et al., 2006). The European Regulation 1881/2006 establishes a maximum level of 2 μg kg^{-1} of AFB1 contamination of corn for foodstuffs in Europe. With regard to animal feed, the European Directive 2002/32/CE established a maximum level of 20 μg kg^{-1} for AFB1 contamination of corn. Corn contaminated with AFB1 at concentrations higher than legally allowed is defined as waste, and is disposed of by incineration or landfilling. Biological treatments may represent an alternative, and a more sustainable strategy, for the recovery of AFB1-contaminated corn compared to traditional disposal methods.

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https://doi.org/10.1016/j.wasman.2018.06.014
0956-053X/

Anaerobic digestion (AD) is a microbiological process in which the microorganisms break down biodegradable material in the absence of oxygen (Appels et al., 2011; Kangle et al., 2012). AD is used worldwide as a recycling process for industrial, agricultural, and municipal wastes since it produces bio-energy in the form of biogas. Moreover, the AD process results in a by-product, the digestate, that may have great agronomic value owing to its high content of mineral nitrogen, and its relatively stable organic matter (Cucina et al., 2017; Solé-Bundó et al., 2017; Tambone et al., 2010). Nevertheless, the digestate is often not suitable for direct application to agricultural soils because of undesirable characteristics, such as pungent odour, high water-content, and a high content of volatile fatty acids responsible for phytotoxicity (Alburquerque et al., 2012; Cucina et al., 2017; Di Maria et al., 2014). Moreover, some researchers have noted the potential risks of soil contamination by pathogens, heavy metals, and emerging contaminants that may be present in the digestate (Alburquerque et al., 2012; Cucina et al., 2017).

Considering the potential high biomethane yield from corn (Ward et al., 2008), AD may represent a suitable valorisation strategy for AFB1-contaminated corn. Nevertheless, few studies can be found concerning the influence of AFB1 on the AD process, and on digestate quality (Salati et al., 2014; De Gelder et al., 2018). The purpose of this study was to evaluate the possible recovery of AFB1-contaminated feedstocks through a semi-continuous AD process. In particular, the effect of AFB1 on the anaerobic process, the feasibility of agronomic re-use of the digestate, and the fate of AFB1 during the treatment process was assessed.

2. Material and methods

2.1. Organic materials and AFB1 contamination

The organic materials used in the anaerobic trials were collected from local farms and stored at 4 °C until the start of the experiment. The feedstock was prepared by mixing 10% (w/w) certified AFB1-free chopped corn grain and 90% (w/w) pig slurry, and the mixture blended using a Braun JB 3060 jug blender (Kronberg/Taunus, Germany) to a final particle size <2 mm. The organic materials were collected at two different time periods (December 2016 and March 2017) to provide fresh materials throughout the experiments, and to simulate a full scale plant. Physico-chemical characteristics of the feedstocks used in the anaerobic trials are reported in Table 1. Because of differences in the volatile solids (VS) and total organic carbon (TOC) content, the organic loading rate (OLR, Table 2) varied during the trials and thus, the biomethane yields were normalized to the VS content of the feedstock and expressed as NL kg VS^{-1}.

For the experimental contamination of the feedstocks, an AFB1 stock solution was prepared by dissolving 1 mg of AFB1 reference standard (Sigma Aldrich, Saint Louis, USA) in 1 mL of benzene/acetonitrile (98/2, v/v). The stock solution was diluted in methanol to an appropriate concentration and added to the feedstock to obtain the following final concentrations: 25 (C25), 50 (C50), and 100 (C100) μg kg^{-1} of AFB1 (wet weight). Throughout the experiment, the non-contaminated (NC) feedstock was spiked with the same volume of methanol added to the contaminated feedstocks to evaluate any inhibition of the anaerobic microflora caused by the solvent.

2.2. Semi-continuous anaerobic digestion apparatus and experimental design

The semi-continuous anaerobic trials were conducted in 2-L reactors fitted with displacement gasometers and supplied with an alkaline solution (0.5 N NaOH) to capture the CO_2. Three reactors were used simultaneously throughout the experiment to provide experimental data in triplicate. Biomethane production was normalized to the standard temperature and pressure. Each reactor was filled with 1.2 L of the appropriate feedstock, and operated

Table 1
Physico-chemical characterization of the feedstocks.

Parameter	Unit	NC	C25	C50	C100
Total solids	%	$10.4^{a} \pm 0.1$	$11.5^{a} \pm 0.1$	$6.2^{b} \pm 0.1$	$6.6^{b} \pm 0.2$
Volatile solids	%	$89.0^{a} \pm 0.3$	$89.9^{a} \pm 1.0$	$73.6^{b} \pm 0.5$	$88.7^{a} \pm 1.0$
Total organic C	%	$47.9^{a} \pm 0.8$	$48.0^{a} \pm 1.5$	$40.6^{b} \pm 0.1$	$47.9^{a} \pm 1.9$
Total N	%	$6.1^{ab} \pm 0.5$	$5.0^{b} \pm 0.9$	$6.9^{a} \pm 0.5$	$6.7^{a} \pm 0.1$
Ammonia-N	%	$3.4^{a} \pm 0.4$	$2.7^{b} \pm 0.2$	$5.6^{c} \pm 0.5$	$3.7^{a} \pm 0.3$

NC, non-contaminated.
C25, 25 μg kg^{-1} Aflatoxin B1 (AFB1).
C50, 50 μg kg^{-1} AFB1.
C100, 100 μg kg^{-1} AFB1.
Data expressed as the Mean ± SD, n = 3.
All data are expressed on a dry weight basis.
Means in the same row followed by different letters (a, b, or c) are significantly different at $P < 0.05$.

Table 2
Organic loading rate (OLR), biomethane yield, organic matter removal, FOS/TAC ratio, and pH in the non-contaminated and aflatoxin B1-contaminated feedstock tests.

Parameter	Unit	NC	C25	C50	C100
OLR	g VS L^{-1} d^{-1}	$6.2^{a} \pm 0.3$	$6.9^{a} \pm 0.3$	$3.0^{c} \pm 0.1$	$4.7^{b} \pm 0.2$
Biomethane yield	NL kg VS^{-1}	$561.9^{a} \pm 78.6$	$627.6^{a} \pm 59.7$	$440.7^{b} \pm 21.0$	$121.9^{c} \pm 17.6$
OM removal	%	$36.3^{a} \pm 0.5$	$34.4^{a} \pm 0.9$	$25.3^{b} \pm 0.9$	$9.1^{c} \pm 1.1$
FOS/TAC	–	$0.6^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.2^{b} \pm 0.0$	$6.6^{c} \pm 0.4$
pH	–	$8.1^{a} \pm 0.0$	$8.0^{a} \pm 0.1$	$8.1^{a} \pm 0.1$	$5.4^{b} \pm 0.1$

NC, non-contaminated.
C25, 25 μg kg^{-1} Aflatoxin B1 (AFB1).
C50, 50 μg kg^{-1} AFB1.
C100, 100 μg kg^{-1} AFB1.
Data expressed as the Mean ± SD, n = 3.
All data are expressed on dry weight basis.
Means in the same row followed by different letters (a, b, or c) are significantly different at $P < 0.05$.