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Kinetics of inactivation and dilution effects on the mass balance of fungal phytopathogens in anaerobic digesters

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ABSTRACT

Knowledge of fate and behavior of plant pathogens in the biogas production chain is limited and hampers the estimation and evaluation of the potential phytosanitary risk if digestate is spread on arable land as a fertilizer. Therefore, simulation is an appropriate tool to demonstrate the effects which influence the steady state of pathogen infected plant material in both digesters and digestate. Simple approaches of kinetics of inactivation and mass balances of infected material were carried out considering single-step as well as two-step digestion. The simulation revealed a very fast to fast reduction of infected material after a singular feeding, reaching a cutback to less than 1% of input within 4 days even for D_{90}-values of 68 h. Steady state mass balances below input rate could be calculated with D_{90}-values of less than 2 h at a continuous hourly feeding. At higher D_{90}-values steady state mass balances exceed the input rate but are still clearly below the sum of input mass. Dilution further decreases mass balances to values 10^{-5} to 10^{-6} Mg m^{-3} for first-step digestion and 10^{-8} to 10^{-9} for second-step.
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1. Introduction

On-farm anaerobic co-digestion of energy crops and organic wastes to biogas is inextricably linked to environmental benefits like renewable energy production, reduction of greenhouse gas emissions, improvement of livestock waste management and nutrient recovery (Rehl and Müller, 2013; Weiland, 2010). It is common practice to spread the effluent from this biogas production process, the so-called digestate on arable land as a fertilizer and soil conditioner to enhance recycling and conservation of nutrients and organic matter, respectively. In principle, anaerobic digestion technology has been proven to diminish the number of pathogenic microorganisms in manure and hence, reducing the loading rate to farmland and thus contributing to risk reduction (Sahlström, 2003; Saunders et al., 2012; Ziemba and Peccia, 2011). However, two trends, volume increase and feedstock diversification, fuelled a debate on such contemporary agricultural practices having the potential to transmit pathogens from diverse sources to farmland. Most attention is focused on human and livestock diseases (Bøtner and Belsham, 2012; Goberna et al., 2011; Saunders et al., 2012; Venglovsky et al., 2009), but increasingly too, on plant diseases (Bandte et al., 2013; Seigner et al., 2010). Plant pathogenic microorganisms are of particular significance as they are responsible for crop losses and interfere with food security. In the context of energy cropping and feeding infested biomass to biogas plants, recently, plant pathologists tend to emphasize the risk of both introducing new phytopathogens and increasing persistence of phytopathogens in the agricultural environment (Noble et al., 2009; Van Overbeek and Runia, 2011).

Hitherto knowledge of fate and behavior of plant pathogens in the biogas production chain is limited and hampers the estimation and evaluation of the potential phytosanitary risk. Reasons for this are manifold, including:

- the broad spectrum of phytopathogenic microorganisms (bacteria, viruses, fungi) and the occurrence of both infectious propagules and resting structures (fungi)
- limits of sampling regarding (i) the wide range of feedstocks (whole-crop silages of maize, sorghum, rye, triticale, wheat,

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barley, and sugar beet; green wastes and organic livestock wastes like slurry and dung), (ii) the complex and multi-variate process of anaerobic digestion (batch or continuous mode, reactor configuration, temperature, exposure time), and (iii) the different quality of the digestate (liquid, paste, semi-dry) due to digestate processing comprising the use of separation and/or dewatering technologies
- limits of routine microbiological analytical techniques regarding (i) the heterogeneity of matrices and the microbial composition, (ii) identification and quantification of pathogens (traditional detection protocols, based on cultural, morphological and biochemical properties, only provide qualitative results i.e. presence or absence) and (iii) reliability of lab-scale tests (*in vitro* and on rich culture media) for *in vivo* assessments
- higher costs for recent, more sensitive, accurate, specific, and much faster diagnostic techniques (molecular-based techniques, real-time PCR) than conventional approaches
- impossibility of exposure of hazardous organisms to full-scale biogas plants to validate the pathogen removal efficiency.

For making progress in understanding the fate and behavior of plant pathogens in the biogas production chain it is vital to systematize the available information focusing on the sanitizing impact of anaerobic digestion on particular phytopathogen host combinations. Only recently an extended joint project investigated the effect of mesophilic anaerobic digestion in continuous stirred tank reactors (CSTR) on the viability of selected phytopathogens in suitable plant-derived feedstock (Bandte et al., 2012; Liebe et al., 2012; Rodemann et al., 2012). The experiments were conducted both in lab-scale reactors and in full-scale biogas plants. It could be shown, that most pathogens are inactivated within 24–138 h. Some pathogens were already inactivated by ensiling which is common practice to preserve green whole plant crops as feedstock for biomethanation (Herrmann et al., 2011).

It may be an appropriate tool to simulate the effects which influence the steady state of pathogen infected plant material in both digesters and digestate. Therefore, simple approaches of kinetics of inactivation and mass balances of infected material were carried out considering single-step as well as two-step digestion. The simulation distinguishes between the inimitable feeding of the digester with infected material and the continuous feeding during a period of several days. The differences in time for the decrease of infectious propagules in various hosts was expressed as D_{90}-values (90% reductions). These values facilitate the categorization of particular pathogen host combinations.

The aim of this paper is to determine i) the effect of inactivation kinetics and dilution on the mass balance of fungal phytopathogens and ii) its concentration in the effluent of single digesters and optional two-step digesters.

2. Material and methods

2.1. Model parameters of biogas plant

The model biogas plant is set to a methane output of 5455 m^3 per day equivalent to an electric performance of 500 kW and thus representing the median size of German biogas plants. It is considered either a single-step digestion (i.e. one digester and one digestate storage tank) or a two-step digestion comprised of two digesters in row and the storage tank at the end. The digester size is set to 1000 m^3 both for single and two-step digestion thus the average hydraulic retention time of digestion equals either 33 days or 66 days. For the digestate storage the size is not set as it is not considered in simulations. Daily feeding would be composed of 10 Mg of liquid manure and 20 Mg of energy crops, conducted in hourly intervals. The exchange between tanks is by simple overflow thus feeding and discharge is concurrent. The outflow from first to second digester or to digestate storage is set to 24.4 m^3 per day assuming a conversion of 80% of the total biogas production. Applying a conversion efficiency of 80% for the second digester the outflow to the storage tank is 23.3 m^3 per day.

2.2. Parameters of phytopathogenic load

The dwell time necessary to inactivate 90% of phytopathogens, D_{90}-value, is determined on the basis of the results of the experiments described in Bandte et al. (2013) and Rodemann et al. (2012). It is assumed that inactivation follows a logarithmic decay. The test phytopathogens (*Claviceps pupurea*, *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium proliferatum*, *Fusarium verticillioides*, *Rhizoctonia solani*, *Scleotinia sclerotiorum*), host crops and abundance of pathogens after particular time periods in anaerobic digestion are summarized in Table 1. Results were obtained either in lab-scale reactors (L) or in a full-scale biogas plant (F) for a range of plants (maize (*Zea mays*), rye (*Secale cereale*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolour* spp.) potato (*Solanum tuberosum*) and sugar beet (*Beta vulgaris subsp. vulgaris var. altissima*) and plant parts.

2.3. Simulation

Simulation is carried out with simple spread sheet software using Euler algorithm for integration with a time step of 6 min.

The mass of infected material m at time t in the digester is given by:

$$m(t) = m_{\text{in}} - m_{\text{out}} - k \cdot m(t-1) \tag{1}$$

m_{in} is the input of infected material and equals, in the case of two-step digestion, the m_{out} which is the outflow of infected material to the second digester or to the digestate storage. m_{in} and m_{out} are bound to feeding intervals of 60 min k is the rate of inactivation due to simple logarithmic decay.

It is assumed here that the infected material is evenly distributed across the digesters. Thus m_{out} equals the concentration of infected material times the volume of outflow at the time of feeding and discharging, V_{out}, given in Section 2.1:

$$m_{\text{out}} = c(t) \cdot V_{\text{out}} \tag{2}$$

The concentration of infected material is given by the amount of $m(t)$ divided by the digester volume, V_D:

$$c(t) = \frac{m(t)}{V_D} \tag{3}$$

Two scenarios are assumed for the feeding of infected material to the biogas plant:

1. A single feeding of 2 Mg
2. An hourly feeding of 0.0833 Mg i.e. 10% of the energy crop feedstock is infected material over a period of 4 days.

3. Results and discussion

D_{90}-values for inactivation gained from the experiments carried out in lab-scale digesters as well as in a full-scale biogas plants range between almost null (i.e. spontaneous complete inactivation) and 96 h (Table 2). *S. sclerotiorum* in sugar beet, *R. solani* in potato and *F. verticillioides* in ensiled sorghum were almost immediately inactivated in lab-scale reactors. In the case of *S. sclerotiorum* in

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