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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.

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:

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

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:

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

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

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

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

Table 1
Phytopathogens, host crops and abundance of pathogens after particular time periods exposed to anaerobic digestion in either lab-scale reactors or a full-scale biogas plant.

Pathogen	Host[a]			Scale[b]	Time periods (h)[c]							Ref.[d]
					0	6	24	48	72	96	138	
C. purpurea	Rye	Ergot	f	F	54	27	0	0	0	0	0	[1]
F. avenaceum	Cereal	Whole plant	f	L	60	10	0	n/d	n/d	n/d	0	[1]
	Maize	Whole plant	f	L	81	4	0	n/d	n/d	n/d	0	[1]
F. culmorum	Maize	Grain	f	F	100	100	n/d	n/d	n/d	n/d	0	[1]
		Grain broken	f	F	100	100	n/d	n/d	n/d	n/d	0	[1]
		Whole plant	f	L	100	100	0	n/d	n/d	n/d	0	[1]
			f	F	80	30	3	0	0	0	0	[1]
	Wheat	Grain broken	f	L	100	7	0	n/d	n/d	n/d	n/d	[1]
		Grain	f	L	100	36	0	n/d	n/d	n/d	n/d	[1]
		Grain broken	f	F	100	99	0	0	0	0	0	[1]
		Grain	f	F	100	76	3	0	0	0	0	[1]
F. proliferatum	Sorghum	Whole plant	f	L	100	100	87	0	0	0	0	[2]
			f	F	100	n/d	30	0	13	5	0	[2]
			e	L	100	0	3	0	0	0	0	[2]
			e	F	100	n/d	12	2	n/d	n/d	n/d	[2]
F. verticillioides	Cereal	Whole plant	f	L	62	15	0	n/d	n/d	n/d	0	[1]
	Sorghum	Whole plant	f	L	100	53	1	0	0	0	0	[2]
			f	F	100	98	4	32	0	n/d	0	[2]
			e	L	60	0	0	0	0	0	0	[2]
			e	F	100	75	n/d	15	0	0	0	[2]
	Maize	Grain	f	F	70	70	n/d	n/d	n/d	n/d	0	[1]
		Whole plant	f	L	86	30	0	n/d	n/d	n/d	0	[1]
			f	F	62	51	4	0	0	0	0	[1]
R. solani	Maize	Whole plant	f	L	73	12	0	n/d	n/d	n/d	0	[1]
			f	F	85	2	2	0	0	0	0	[1]
	Potato	Tuber	f	L	80	0	0	0	0	0	0	[2]
S. sclerotiorum	Sugar beet	Tuber	f	L	100	0	0	0	0	0	0	[2]
	Sugar beet	Tuber	f	F	100	0	n/d	n/d	n/d	n/d	n/d	[2]

[a] Host crop material: *f* = fresh; *e* = ensiled.
[b] L = lab-scale reactor; F = full-scale biogas plant.
[c] n/d = not determined.
[d] Ref. = References: [1] = Rodemann et al. (2012); [2] = Bandte et al. (2013).

Table 2
D_{90}-values and corresponding inactivation rates derived from experiments at lab-scale and full-scale biogas plants.

D_{90}-value[a] (h)	k[b]	Scale[c]	Pathogen	Host		
5.99	0.38	F	*C. purpurea*	Rye	Ergot	Fresh
0.75	3.06	L	*F. avenaceum*	Maize	Whole plant	Fresh
1.97	1.17	L		Cereal	Whole plant	Fresh
1.30	1.77	L	*F. culmorum*	Wheat	Grain broken	Fresh
7.12	0.32	F		Maize	Whole plant	Fresh
8.33	0.28	L		Wheat	Grain	Fresh
22.26	0.10	F		Wheat	Grain	Fresh
29.51	0.08	F		Wheat	Grain broken	Fresh
29.85	0.08	L		Maize	Whole plant	Fresh
95.61	0.02	F		Maize	Grain	Fresh
95.61	0.02	F		Maize	Grain broken	Fresh
0.13	17.33	L	*F. proliferatum*	Sorghum	Whole plant	Ensiled
0.63	3.65	F		Sorghum	Whole plant	Ensiled
2.03	1.14	F		Sorghum	Whole plant	Fresh
68.17	0.03	L		Sorghum	Whole plant	Fresh
n/c	n/c	L	*F. verticillioides*	Sorghum	Whole plant	Ensiled
3.06	0.75	L		Cereal	Whole plant	Fresh
6.80	0.34	L		Maize	Whole plant	Fresh
13.76	0.17	F		Maize	Whole plant	Fresh
13.85	0.17	L		Sorghum	Whole plant	Fresh
23.55	0.10	F		Sorghum	Whole plant	Ensiled
37.90	0.06	F		Sorghum	Whole plant	Fresh
40.86	0.06	F		Maize	Grain	Fresh
n/c	n/c	L	*R. solani*	Potato	Tuber	Fresh
0.47	4.91	F		Maize	Whole plant	Fresh
2.39	0.96	L		Maize	Whole plant	Fresh
n/c	n/c	L	*S. sclerotiorum*	Sugar beet	Tuber	Fresh
n/c	n/c	F		Sugar beet	Tuber	Fresh

[a] n/c = not calculated.
[b] k = inactivation rate.
[c] L = lab-scale reactor; F = full-scale biogas plant.

sugar beet immediate inactivation occurred also in a full-scale digester additionally to lab-scale reactors. Thus for these combinations it is not possible to calculate D_{90}-values.

In general as well as in the particular combinations, phytopathogens showed shorter D_{90}-values of inactivation in ensiled material than in fresh material. But there is no clear range of D_{90}-values for host and pathogen combinations, although rye, and wheat tend to have shorter D_{90}-values than maize or sorghum. Whole plant material also has, in general, shorter D_{90}-values than grains.

Particular combinations do not reveal a trend in D_{90}-values between lab-scale and full-scale experiments. *F. verticillioides* in sorghum as well as in maize showed higher D_{90}-values in full-scale digesters than in lab-scale experiments. *F. proliferatum* in sorghum as well as *F. culmorum* in maize and wheat displayed variable behavior, e.g. *F. proliferatum* in fresh sorghum had higher D_{90}-values in lab-scale experiments than in full-scale experiments while in ensiled sorghum it was the opposite.

Simulations of mass balance of infected feedstock and concentration in contaminated output were performed for pathogen-sorghum combinations. These combinations cover almost the entire range of D_{90}-values determined.

It is a common principle to simulate the reduction of infectious propagules according to logarithmic decay as a result of a first order reaction (Astals et al., 2012; Popat et al., 2010; Salsali et al., 2008). The results obtained by these authors coincide with the results observed in our study. The simulation based on single feeding of infected feedstock demonstrates the fast to very fast reduction of infectious propagules within the digester. Up to D_{90}-values of 13.85 h, the mass balance of infected material is almost zero within the first day (Fig. 1). The concentration of it in the output is, at that time, six magnitudes below the input concentration. If D_{90}-values

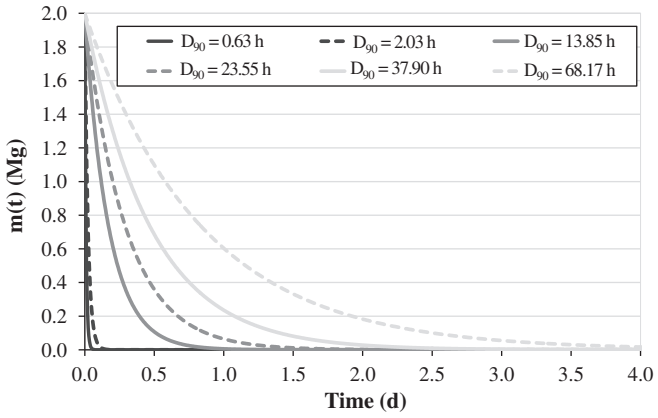

Fig. 1. Decrease of masses of infected material in first-step digester dependent on D_{90}-values after singular feeding of 2 Mg.

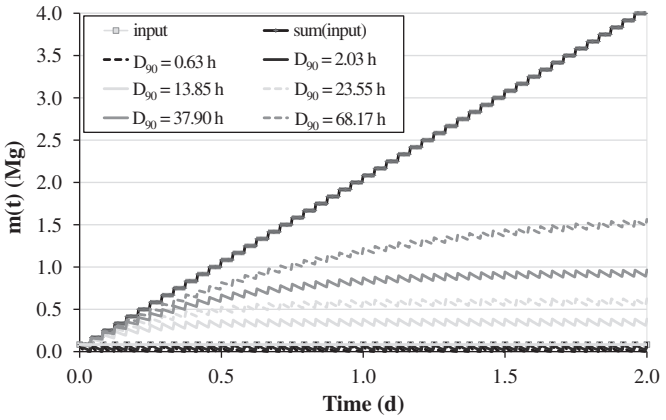

Fig. 3. Steady state mass balances of first-step digesters dependent on D_{90}-values with hourly feeding compared to input rate and sum of input.

are 24 h or higher, the sanitation of this material is still fast and reaches a reduction to values below 1% of the input within 4 days at a D_{90}-value of 68 h Pandey and Soupir (2011) obtained similar results for the kinetics of inactivation of *Escherichia coli* in batch anaerobic digestion at different temperatures. This reveals that the single feeding with infected material can be compared to batch processes as long as the first-step digester is regarded. The inspection of the curves in Fig. 1 reveals also that errors in determining the percentage of inactivated propagules and hence the D_{90}-values have a larger effect in the resulting inactivation with large D_{90}-values while small D_{90}-values reveal a smaller effect. The 3.21-fold increase of the D_{90}-value from 0.19 h to 0.61 h leads to a 2.5-fold increase in the time for a 90% reduction of infected material. The 1.80-fold increase from D_{90}-value 11.41 h to D_{90}-value 20.52 h leads to 1.81-fold increase in the 90% reduction time.

With the introduction of a second-step digester the dilution of propagules play an even more important role on the concentration of the propagules in the output (Fig. 2). In this case the concentration change for the output is mainly dependent on the volume of the second-step digester, i.e. the dilution factor is the inverse of the volume. While the output concentration of the first-step digester falls five magnitudes below the input concentration and the concentration of the second-step output decreases by eight magnitudes compared to the input.

If the feeding with infected plant material continues over a longer time period, a steady state will be built up for the mass balance of the infected material in the digesters (Fig. 3) as well as for its concentration in the output (Fig. 4). The steady states for a particular D_{90}-value oscillates between a maximum value given by the input and a minimum value given by the reduction achievable until the next feeding turn.

D_{90}-values below 2 h lead to steady states in the digester which are below the mass of input. If D_{90}-values are above this two hour limit, the mass balances in the digester grow until steady states are reached which remain clearly above the hourly input. Nevertheless these steady states are still significantly below the total sum of infected material fed to the digester. In the case of a D_{90}-value of 68 h, the steady state is almost reached after 4 days. If the input is switched off, the mass balances in the digester decrease according to the reduction curve determined in the simulation with singular feeding of infected material. At low D_{90}-values, they decrease immediately to almost null and within a period of 2–4 days for higher D_{90}-values.

In addition to the inactivation the concentration of infected material in the output is several magnitudes smaller than the magnitude of input due to dilution. The magnitude of the effect of dilution is comparable to the one obtained by the single feeding simulation. As can be seen in Table 3 the concentrations in the digester range between $1.4 \cdot 10^{-6}$ to $8.4 \cdot 10^{-5}$ [Mg m^{-3}] (D_{90}-values = 0.63 h) and $2.9 \cdot 10^{-5}$ to $1.7 \cdot 10^{-3}$ [Mg$\cdot m^{-3}$] at D_{90}-values = 68.17 h while the input has a concentration of 0.1 (10%). The immediate dilution of the infected material also diminishes the risk of short circuit flow-through. If a two-step digestion process is

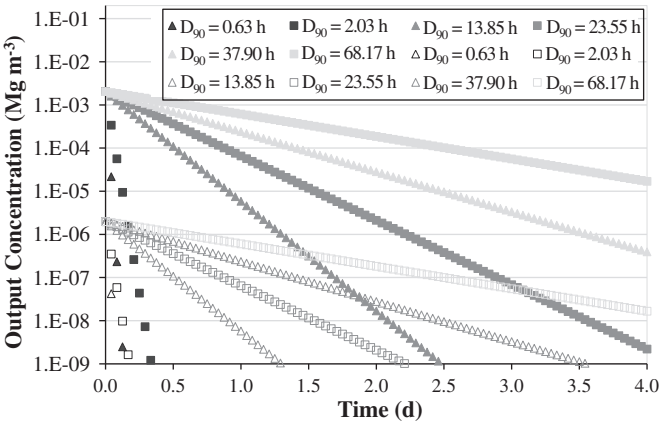

Fig. 2. Decrease of output concentrations (first-step filled markers, second-step unfilled markers) of infected material dependent on D_{90}-values after singular feeding of 2 Mg.

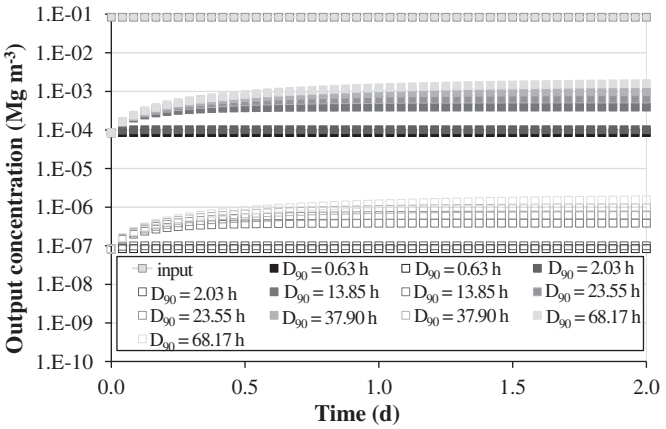

Fig. 4. Steady state output concentrations (first-step filled markers, second-step unfilled markers) of infected material dependent on D_{90}-values with hourly feeding compared to input rate.

Table 3
Output concentrations of infected material of first and second digester depending on D_{90}-values, input concentration of infected material is in all cases 0.1 (Mg m^{-3}).

D_{90}-value (h)	Concentration (1st digester) (Mg m^{-3})	Concentration (2nd digester) (Mg m^{-3})
0.63	$8.56 \cdot 10^{-5}$	$8.40 \cdot 10^{-8}$
2.03	$1.02 \cdot 10^{-4}$	$9.98 \cdot 10^{-8}$
13.85	$3.92 \cdot 10^{-4}$	$3.84 \cdot 10^{-7}$
23.55	$6.36 \cdot 10^{-4}$	$6.24 \cdot 10^{-7}$
37.90	$9.94 \cdot 10^{-4}$	$9.75 \cdot 10^{-7}$
68.17	$1.71 \cdot 10^{-3}$	$1.68 \cdot 10^{-6}$

assumed in the second digester, the infected material is even more diluted to concentrations (another 3 magnitudes smaller).

Up to now most published work dealt mainly with the inactivation of human pathogens in anaerobic digestion (Pandey and Soupir, 2011; Lang and Smith, 2008; Wagner et al., 2008). Thermorshuizen et al. (2003) also investigated bacterial phytopathogens. In the large-scale experiments performed by these authors one can see that the temporal pattern of inactivation of most pathogens match the pattern obtained in this study. The kinetics of the inactivation of *E. coli* calculated by Pandey and Soupir (2011) perform a strongly comparable behavior as the kinetics of inactivation of the phytopathogens considered here.

In contrast to other investigations, this study included also the effect of dilution which leads to a combined impact on the concentration of pathogenic propagules both in the digesters and the digestate.

4. Conclusions

D_{90}-values are helpful to categorize the sanitizing impact of anaerobic digestion on phytopathogen host combinations. The simulation of inactivation and dilution of infected material can be related to these D_{90}-values. If the D_{90}-value of a particular pathogen host combination is known and within the range of the values used here, one can interpolate its behavior. It is obvious that errors in the determination of D_{90}-values lead to disproportional error propagation, i.e. at small D_{90}-values error propagation is less than 1 and at D_{90}-values larger than approx. 11 h error propagation becomes larger than 1.

The performed simulation approach demonstrates that the risk of spreading phytopathogens with digestate is very small as long as the D_{90}-value is less than 2 h and still small with increasing D_{90}-values due to dilution. This effect can be magnified by a two-step digestion.

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