Validation of manure sanitation methods performed at laboratory scale

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Abstract

One use of manure is soil production and use as commercial manure e.g. for garden use. If not sanitized properly, manure can be a health risk for both animals and humans. Commercial use of manure in EU is, since January 2007, controlled by Commission regulation (EC) 208/2006. In this regulation it is stated that the manure should be treated in 70°C for at least 60 minutes or by other processes authorized by the competent authority. To get such authorization of an alternative treatment process a validation of the intended process has to be done by the applicant. We suggest that validation of many suggested processes could be performed in a laboratory scale with control of parameters critical for the sanitation. This article gives an example of validation of heat treatment in 52°C in a laboratory scale process.

Background

Soil production for gardens is one market for manure in Sweden today. When using manure for soil production there is a risk that the final product may contain zoonotic pathogens such as salmonella, verotoxin producing *Escherichia coli* (VTEC), *Mycobacterium* spp. and *Cryptosporidium parvum* oocysts (Pell, 1997; Strauch, 1991). Risk of pathogen content of the manure or contamination during collection, storage and treatment must be taken into consideration. The danger of improper manure handling can be manifested as contamination of products, water supplies, animals or humans (Cotruvo *et al.*, 2004).

The handling of manure to be sold or products thereof is from January 2007 regulated by Commission regulation (EC) No. 208/2006 which is an addition to EC No. 1774/2002. In EC No. 208/2006 it is stated that processed manure products must come from a technical, biogas or composting plant approved by the competent authority e.g. in Sweden the Swedish Board of Agriculture. EC No. 208/2006 states that the manure has to be subjected to a heat treatment of at least 70°C for at least 60 minutes. Although the regulation also opens up for the competent authority to approve on other standardised processed parameters. As sanitation at high temperatures is costly, alternative sanitation methods are highly interesting. To get other process parameters authorized the applicant must be able to demonstrate that such parameters guarantee minimising of the biological risks associated with the manure products (EU, 2006). This demonstration should include a validation of the intended process by measuring the reduction of viability/infectivity of endogenous indicator organisms or a well characterised test organism.

Aim

The objective of this paper is to present a proposed strategy for validation of the sanitary treatment of manure. To do this an example of validation of thermal inactiva inactivation at 52°C is presented.

Validation

alidation is a method of establishing evidence that gives a high degree of assurance that a product lives up to its intended requirements. By validation of the intended manure treatment it can be assured that the process reduces the amount of pathogens in the material to an acceptable level in accordance with EC No. 208/2006 were it is stated that the validation of an alternative treatment process for manure has to show upon a 5 $\log_{10}$ reduction in *Enterococcus faecalis*, if using chemical or thermal treatment processes, and a 3 $\log_{10}$ reduction in the infectivity titter of heat resistant viruses e.g. parvovirus, if viruses have been identified as a hazard. To validate a process the reduction of a specific microorganism can be measured. The microorganism can be an endogenous organism present in manure in sufficient amounts e.g. *E. coli* or enterococci. Validation is also possible through addition of microorganisms to the manure e.g. salmonella.

Analysing endogenous microorganisms repeatedly in manure before and after processing can be the basis for a validation of the process. This validation, however, is only valid for the specific plant where the study was performed. Alternatively, validation can be performed in laboratory scale with the possibility to add representative microorganisms to the whole material. The reductions of these are measured under the surveillance of one or several parameters, critical for pathogen inactivation, which can be controlled during the process. Parameters influencing survival of microorganisms can for example be pH, humidity, time and temperature. In laboratory scale it is possible to measure reduction under given conditions. Statistical analyses can then be used to define pathogen inactivation kinetics and processing conditions to be controlled in the full-scale process, to achieve the reduction targets. A validation based on the control of critical process parameters is more general and the process can be implemented at any plant. The underlying parameters for the determined inactivation need to be fulfilled in the full scale.

Parameters influencing the inactivation of microorganisms

The persistence of microorganisms in the environment has been extensively investigated. Neither protozoa nor virus are able to grow in the environment, thus their numbers will always decrease by time (Feachem *et al.*, 1983). On the other hand some species of bacteria may multiply under favourable environmental conditions (Gibbs *et al.*, 1997). Persistence of microorganism is associated to various biological, physiological and chemical parameters. The inactivation differs between species of microorganisms and can be a synergy effect from several parameters. Some parameters that are recognized as having an effect on the survival of microorganisms in manure are presented in table 1.

Example of validation of thermal inactivation of salmonella and viruses in compost

Composting is a process used for treatment of manure and the sanitation is achieved mainly through thermal inactivation of microorganisms. In the present study validation of inactivation of viruses and salmonella was done at 52°C in a laboratory scale.

Material and method

The material used for the inactivation studies was compost material collected from a compost plant. The material had a dry matter content of approximately 45 %. Inactivation in the material was monitored by analysis of added microorganisms. All media used was supplied by the SVA, Uppsala, Sweden.

Table 1. Parameters influencing the inactivation of microorganisms

Parameter	Effect on survival of microorganisms
Time	The reduction time of microorganisms is under most circumstances exponential. Long time storage has been extensively used as a treatment. Although the inactivation rate of microorganisms is difficult to predict.
Temperature	Temperature is probably the most well known general treatment used for inactivation of microorganisms. Bacteria may grow in a wide span of temperatures, but the growth is better at their optimal growth temperatures, for pathogens this is mostly close to body temperature. Further, many microorganisms survive well in low temperatures and die rapidly at high temperatures due to denaturation of membranes and proteins.
pH	Highly acidic or highly alkaline conditions have an inactivating effect on most microorganisms due to hydrolysation of cell components or denaturation of enzymes.
Humidity	In general humid environments favour survival of microorganisms while a dry environment increases microorganism inactivation. Additionally, humidity helps heat transfer for more rapid thermal inactivation.
Competing microbiota	Lack of nutrients only affects bacteria. Enteric bacteria adapted to a life in the gastro intestinal tract may not always be able to compete for nutrients with the indigenous microbiota in the environment and thus the ability of enteric bacteria to survive and multiply in the environment may be limited.

Sources: Feachem et al., 1983; Mitscherlich & Marth, 1984; Yates & Gerba, 1998

Salmonella Senftenberg (775W) were cultured in nutrient broth over night at 37°C. Bacteriophages was propagated on their host strain to approximately 10^{10} plaque forming units (PFU)/ml. As host strain for bacteriophage ΦX174 *E. coli* strain C (ATCC 13706) was used. *Salmonella* Senftenberg (775W) and bacteriophages were added to the compost material at a final concentration of approximately 10^7 colony forming units (CFU)/g and 10^5 PFU/g respectively. Samples for analysis were taken after 0, 0.5, 1, 2, 7 and 10 hours. The material was diluted in phosphate buffer, pH 7.2, and enumerated using standard methods. Statistical analyses were performed in Sigma Stat (SPSS; Chicago, IL) and the plot was made in Excel (Microsoft Corporation; Redmond, WA).

Results

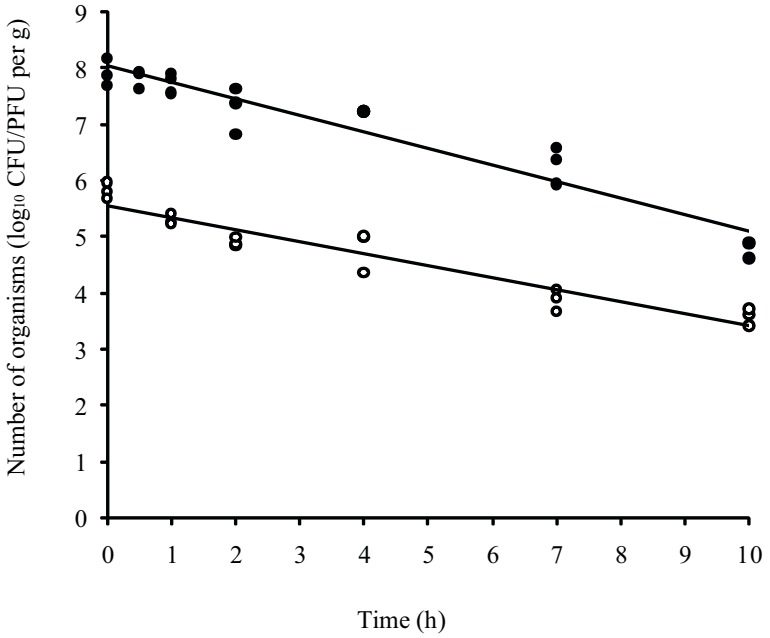
Bacteria and bacteriophages were enumerated and their numbers plotted against time (Figure 1). Linear regression was used to determine the inactivation rate constants for each of the organisms (Table 2).

Table 2. Linear regression models for salmonella and bacteriophage ΦX174 in compost material at 52.0°C

Organism	Equation	SE t*	n	R^2	p
Salmonella	= 8.03-0.29t	0.020	21	0.92	< 0.001
ΦX174	= 5.57-0.21t	0.017	18	0.91	< 0.001

* Standard deviation for the inactivation constant

Figure 1. Salmonella (●) and bacteriophage ΦX174 (○) numbers expressed as log CFU/PFU g^{-1} compost material as a function of time (h) at 52°C.



To resolve if the reduction rates of the tested indicator organisms is sufficient enough to cope with the EU regulations one has to compare the pathogenic reduction times with the retention time (RT) of the reactor. Processes in a reactor can either be continuous or batch wise. In a continuous process, material is continuously feed into reactor at the same time as material is taken out from the reactor. The feeding frequency, corresponding to the minimal retention time (MRT), varies between different reactors. The MRT and also the hydraulic retention time (HRT), *i.e*. the time needed in order to exchange the drum volume, will significantly influence the residual pathogen concentration. In a batch process the material is added in batches and has to keep a certain temperature in the reactor during the RT. In between these (continuous processes with total mixing of the material and batch processes) a plug flow can be possible, for example with compost in a drum reactor.

Inactivation rates were used to calculate the RT needed to achieve 5 $\log_{10}$ reduction of salmonella and 3 $\log_{10}$ of viruses in continuous and batch process (Table 3). RT given in the table represent the 95th percentile (= upper level of a 90% confidence interval).

Table 3. The time needed to achieve sufficient inactivation of microorganisms to meet the requirements in EC No 208/2006 in compost material at 52°C

Temperature (°C)	Continuous treatment		Batch treatment RT (h)
	MRT[1] (h)	HRT[2] (days)	
52.0	24	10	36

[1]MRT = minimal retention time

[2]HRT = hydraulic retention time

Discussion

Heating of material to 70°C is costly in terms of energy consumption. In compost processes part of the material will reach this temperature, however, there will always be cold spots. At temperatures above 50°C salmonella cannot grow in numbers. Therefore, if the whole material reaches a temperature above 50°C a secure reduction of salmonella can be achieved. In the present study we measured the inactivation of salmonella at 52°C. Bacteriophage ΦX174 was used as an indicator for virus removal, however, salmonella reduction was limiting. Inactivation rate was used to propose for how long time composting at this temperature must be performed to reach the reduction target of 5 $\log_{10}$ with a high level of security (95th percentile). By using a confidence interval a satisfactory limit of security is reach so that one can suggest that a compost process operated at a certain temperature will meet the requirements of EC No 208/2006. In this example a compost process operated batch wise at 52°C during 36 h, or in a continuous process with a MRT of 24 h and HRT of 10 days (complete mixing of the material) is sufficient to meet the requirements. For a drum reactor with a plug flow, it is important to assure that all the material is subjected to treatment for at least 36 h.

For a general validation of specific parameters a laboratory scale process with added microorganisms might be used, as in the example given. If done correctly it is important that all parameters are the same in the full-scale process. It has to be pointed out that in the full-scale process one must be able to control the parameters validated in the laboratory scale process. Important scaling effects to consider are un-even distribution of temperature, pH and moisture. A specific technical plant on the other hand can validate their process through continuous sampling during the operation in accordance with the EC No. 208/2006. When performing a validation of a specific technical plant one can rely on endogenous organisms e.g. *E. coli*, enterococci and bacteriophages in manure.

Acknowledgement
This study was financed by The Swedish Board of Agriculture and SVA.

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