Survey on available methods and techniques for the inactivation of pathogens in animal manure

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Introduction

Animal manures are a valuable source of plant available nutrients and organic matter, contributing to soil quality and fertility. However, manures can contain pathogenic microorganisms, parasites and viruses which creates the potential for the transmission of diseases to susceptible animal populations or spread of zoonotic agents from the farm environment into the food chain via plants or food of animal origin or into drinking water.

In order to interrupt one or more of the ways of transmission compiled below, measures have to be taken in order to reduce or totally inactivate the involved bacteria, viruses or parasites of epidemiological importance. The most important precondition is that the method applied is effective, which can only be assured, if it has shown in a validation procedure with representative test-organisms that it capable to reduce the relevant organisms in sufficient manner. A complete documentation on manure management and related biotic as well as abiotic risks had been edited by Burton and Turner (2003), therefore only some basic informations will be given here.

Epidemiological Background

Besides the indigenous microbiological flora and populations of viruses including protozooc and metazooc organisms, manure may contain a variety of obligate and facultative pathogens for animals including zoonotic agents as well as undesired organisms which shall not be spread in the environment and shall not be introduced into the bioecososis like multi resistant bacteria and seeds of weeds (Strauch, 1991). The indigenous flora as well as the pathogens and undesired contaminants are depending in species and amount on type of manure, the epidemiological situation in the animal population and the antimicrobial drugs used in the epidemiological unit as well as on storing and already given treatment conditions. Mainly three hygienic risks do generally exist in handling biological wastes, occupational health risks, environmental risks and risks concerning product safety. In this framework only treatment and measures preventing risks caused by the utilization of manure as fertilizer will be regarded, including the environmental risks related to collection, storage and utilization. The spectrum of applicable measures and methods has gradually different effects on the epidemiological risks related to solid and liquid manure. Manure containing pathogenic vegetative bacteria, as well as bacterial spores, viruses with different chemo- and thermoresistance, as well as infectious stages of different parasites is representing the highest degree of epidemiological risk in this context. A validated treatment capable to inactivate vegetative bacteria and viruses with moderate resistance like enteroviruses results in a manure representing the next lower level in a risk assessment, the remaining parasitic stages, highly resistant viruses and bacterial spores are defining the remaining risk. A validated treatment capable to inactivate vegetative bacteria, viruses of moderate resistance and infectious parasitic stages results in a manure containing only the remaining highly resistant viruses (e.g parvo- and caliciviruses) and spores of sporeformers. A validated treatment capable to inactivate vegetative bacteria, viruses of high resistance and infectious parasitic stages will result in a manure containing only the remaining spores of aerobic and anaerobic sporeformers and represents the hygienic level with the lowest epidemiological risk from the practical point of view, since sterilization resulting in a material free of thermostressive spores seems not to be a desirable aim to reach for a material spread on soil inhabited with the same or related sporeformers, belonging to its indigenous flora.
Treatment Options for Inactivation of Pathogens

The methods which can be applied for inactivation of the different pathogens can be chemical, physical and biological treatment. Except in aerobic or anaerobic biotechnological treatment, the applied micobicidal process may have more or less adverse effects to substantial properties of the treated material deemed to be used as a fertilizer (e.g. loss of nutrients, losses of organic matter). This is of secondary importance if the treatment is done in the case of eradication of notifiable epidemics in animal husbandry, but if the treatment is done in the context of general preventive measures or of voluntary quality assurance systems those factors have to be taken into account. Each process has once to be validated in order to be sure that it will be effective for the intended purpose, the involved type of manure and the related epidemiological situation.

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Physical Treatment

For physical treatment in general the application of heat or irradiation is possible, but not very common. Only drying of poultry manure as well as exposure of spread manure to sunlight have a certain importance in this context. In a certain epidemiological situation pasteurization prior further biotechnological treatment or other utilization is required as well applied in some countries in the case of notifiable diseases as Foot and Mouth Disease.. In this case liquid manure can be pasteurized by direct or indirect heating of the intended volume in a batch process or by microwaves in a continuous mode. If in a batch process a temperature of at least 70 °C is kept for at least 60 min vegetative bacteria, viruses of moderate heat resistance and all infectious parasitic stages will be inactivated if the temperature is even distributed in the material with a high probability. Pasteurization at 90 °C for 60 min will lead to an inactivation of vegetative bacteria, heat resistant viruses and infective parasitic stages in a sufficient level in order to achieve hygienic safety and some heat sensitive bacterial spores will also be affected. Other combinations of temperature and exposure time may be effective as well, if the relevant process data had been confirmed in a suitable validation procedure.

However, this technology is not applied in full scale plants, microwave heating in a continuous flow mode has also shown to be effective to vegetative bacteria, moderate heat-resistant viruses as well as to parasites at a temperature of 80 °C and an exposure time of less than one second in the microwave field.
Thermal drying of manure is mainly done at ambient or moderate temperatures and therefore no significant inactivation of pathogens can be expected. If additional heat is applied and temperatures above 60 °C are achieved inactivation of certain pathogens can be expected, but it must be kept in mind that the main factors influencing the inactivation of the relevant pathogens are the temperature, the exposure time and the water activity in the material. The latter is of special importance, low water activity increases the heat resistance of microorganisms in a way that even vegetative bacteria can survive temperatures of above 100 °C. Therefore the drying procedure must be designed, that the inactivation of the target organisms is complete before the aw-value of the material drops below 0.9. Since the drying procedure is a complex one, those processes have to be validated on a type related basis in order to fix the relevant effective process data.

Chemical Treatment

Chemical disinfection of liquid manure may be done with several substances as could be seen from Table 1. Generally it will be applies in the case of an epidemic and not routinely after every service period. The cheapest way is using 40% lime-wash which is commercially available in most places in Europe and will be distributed directly to the farm. Chemical disinfection of liquid manure will only be successful if the slurry had been carefully mixed up prior adding the chemicals and subsequently stirred every day for at least 1h during the exposure time which is normally 4 days. Solid manure can be disinfected by setting up a pile with manure mixed with quicklime in the following way:

- Selection of a place with solid ground (e.g. concrete floor) a safe distance form buildings and inflammable materials.
- Distribution of a 25 cm high straw-layer on which 10 kg /m² lime is spread;
- Loading of a solid manure spreader layer by layer solid manure and granulated quicklime in a ratio of 100 kg lime per 1 m² of solid manure (at least two layers of each)
- Setting up a pile by turning down the material from the manure spreader to the prepared straw layer up to 1.5 m high under steady moistening with water.
- Covering the pile with black silo foil fixed by stones;
- Turning the pile after 5 weeks;
- Spreading the disinfected manure after a total exposure time of 10 weeks preferable on arable land followed by ploughing in.

Biotechnological Treatment

Aerobic and anaerobic biotechnological treatment can be done in the mesophilic or in the thermophilic range. In such processes a more or less rapid inactivation of pathogens occurs due to different factors like antibiosis, pH-shift, redox-potential, antagonism, nutrient deficiencies and exothermic metabolism. The most effective factor in this context is the elevated temperature (above 50 °C). In the broadest sense, long time storage can be regarded as biotechnological treatment. Generally it can be stated that only a thermophilic process run in a semi batch manner keeping a defined exposure time without adding or taking out manure is suitable for an inactivation of pathogens within a reasonable time frame.

The aerobic process applied may be either Aerobic thermophilic stabilization (ATS) of liquid manure or composting of solid manure or solids separated from liquid manure. The ATS process should be operated in two-stage reactors (two vessels connected in series) at least, to achieve a sufficient exposure time and avoiding hydraulic short-circuits in filling in and taking out of sludge during the operation (Meyer, 2001). Based on the semi batch type of operation (e.g. one hour feeding per day) and 23 hours stabilization (exposure time) and of the temporary decrease of temperature inevitable connected with this type of operation, the following reaction times and temperatures are recommended for inactivation
of vegetative bacteria, viruses of moderate resistance and infectious parasitic stages: 23h at 50 °C or 10h at 55 °C or 4h at 60 °C. Composting of manure can be done with different techniques in windrows and reactors, under open air conditions and under roof.

Table 1: Chemical disinfection of liquid manure for different types of pathogens

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Vegetative Bacteria</th>
<th>Nake viruses</th>
<th>Enveloped viruses</th>
<th>Myco-bacteria</th>
<th>Bacterial spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% lime wash</td>
<td>Dose (kg/m³) 60</td>
<td>60</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposure time (days)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Caustic Soda 50%</td>
<td>Dose (kg/m³) 30</td>
<td>60</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5% NaOH</td>
<td>60</td>
<td>0.8%NaOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposure time (days)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Formalin (37% Formaldehyde)</td>
<td>Dose (kg/m³) 15</td>
<td>15</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5% Formalin</td>
<td>15</td>
<td>1.0% Formalin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposure time (days)</td>
<td>4</td>
<td>4</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Peracetic acid*</td>
<td>Dose (kg/m³) 25 (15%)</td>
<td>40</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0,37 %Per acetic acid)</td>
<td>40 (0.6% Per acetic acid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposure time (days)</td>
<td>0.04</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium cyanamide **</td>
<td>Dose (kg/m³) 20</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposure time (days)</td>
<td>7</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Formation of foam only applicable with small volumes of slurry
** Vigorous stirring necessary

Recommended process parameters for hygienization in composting cannot be given here, because every technique applied has different properties concerning the process parameters to be kept for hygienization. Therefore every type of composting equipment has to be validated by an adequate technique in order to find out the relevant process data to be kept in order to achieve hygienic safety in the intended range. Nevertheless some experience based general data can be given for orientation, 55 °C must be kept for two weeks or 65 °C for one week in windrow composting with at least one turning of the pile and one week in container composting at least 60 °C in the whole material.

Mesophylic anaerobic digestion of manure alone or in codigestion with other substrates is not capable to inactivate pathogens in the exposure time given by the standard procedure of operation, therefore additional physical or chemical treatment is necessary in order to reach the intended level of hygienization. Thermophilic anaerobic treatment at temperatures between 53 °C and 55 °C can be effective in inactivation of vegetative bacteria, viruses with moderate resistance and infectious stages of parasites if in a semi batch procedure an exposure time of at least 20 h can be kept without filling in and without taking out material.
Conclusions

Since manure is classified in regulation 1774/2002 as category 2 material which underlies certain restrictions if it is put on the market as well biotechnological treatment, as physical treatment including thermal drying as chemical treatment e.g. with lime can be applied in order to achieve hygienic safety of the product according to the epidemiological situation as long as the process had been validated according to regulation 185/2007. Routinely on farm level only thermophilic anaerobic treatment or composting can be applied as preventive measure. In the case of notifiable diseases like FMD or other epidemics chemical disinfection of manure will be the favourite procedure in order to avoid spreading of the diseases via environment.

References

Bendixen, H.G. (1999) Hygienic safety - Results of scientific investigations in Denmark (Sanitation requirements in Danish biogas plants), pp 27-47. IEA Bioenergy Workshop (Proceedings), Deutsche Veterinärmedizinische Gesellschaft e.V., Frankfurter Str. 89, D-35392 Giessen