INVESTIGATIONS ON MICROBIAL INDICATORS AND/OR TEST-ORGANISMS IN SUPERVISION OF HYGIENIC SAFETY IN CO-DIGESTION OF ANIMAL SLURRY, BIOWASTES AND/OR ANIMAL BY-PRODUCTS

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Introduction

Recycling of organic material containing tissues and wastes of animal origin is connected with the risk of spreading pathogens of man and animals, especially if animal by-products are involved. Zoonotic agents of bacterial, viral, fungal and parasitic nature can often be found in raw materials as well as under certain epidemiological conditions agents of special veterinary importance as e. g. Foot and Mouth Disease Virus too. Since the aerobic or anaerobic thermophilic process is in principle effective in inactivating most pathogens (sporeformers and TSE – agents excluded) criteria have to be set up to ensure the safety of the applied treatment processes or technologies. In this context a three step procedure is demanded which has proven to be effective and administrable in certain member countries. First only technologies shall be applied which had been validated in a legally fixed procedure. The second step is the steady process supervision by continuous recording of relevant process data and the third step is the regularly supervision of the final product. Recently EU – regulations had been fixed which are dealing mainly with TSE-risks due to animal by products including those of faecal origin. Those regulations have to be regarded very carefully if they are fulfilling the intended purpose concerning risks due to other causative agents of notifiable and other infectious animal diseases as well as other zoonotic agents. The hygienic parameters given there are originating from experiences with products of rendering plants fixed 20 years ago and are only defining simple process requirements and microbiological threshold values for the final products. The given microbiological parameters and the recommended supervision strategies are up to a certain extend conflicting with actual scientific experiences and facts. This was the background for carrying out this investigations mainly for reviewing microbial indicators recommended for the supervision of the final products as well as for giving data concerning tenacity of different test organisms suitable for process-validation before an epidemiological background.

Material and Methods

To compare the inactivation kinetics of bacteria and animal viruses causing infectious diseases with those from other infectious or saprophytic agents, which may act as possible indicator organisms for investigating the hygienisation efficiency of composting and anaerobic digestion plants under practical (field) conditions, two identical composting containers as well as two anaerobic fermenters in a half technical scale were built and driven in parallel, filled with the same materials. In one of them, the inactivation of Classical and African Swine Fever Virus (ASFV), Swine Vesicular Disease Virus (SVDV), Foot and Mouth Disease Virus and Aujeszky Virus was investigated by use of different germ carrier techniques, which are described in more details by Moss and Haas (4) In the second the inactivation of Salmonellae, Fecal Streptococci, Equine Rhinovirus (ERV), Enteriic Cytopathogenic Bovine Orphan (ECBO) Virus and Bovine Parvovirus was studied in parallel. The composting containers were filled with biowastes, the anaerobic fermenters with pig or cattle slurry in a mixture with biowastes (co-fermentation), more detailed descriptions may be found at Hoferer (3). Since only the anaerobic process regarded here the following type of reactor had been used in the experiments for survival studies under mesophilic and thermophilic conditions. His set up of experiments had

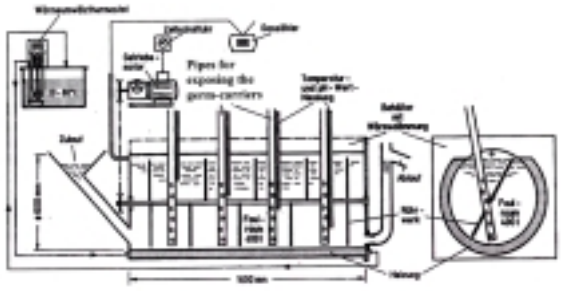

Fig. 1: Scematic representation of the semi-technical biogas reactor

been completed by bacteriological input-output analysis in co-fermentation units using different substrates, described in more details by Beyer et al. (1) and comparative experiments with plant pathogenic fungi and viruses as well as weed-seeds in pasteurization units and biogas reactors for which further details may be taken from Böhm et al. (2).

Results and Discussion

The results concerning survival of different pathogenic and indicator organisms in the anaerobic process are as follows:

- The bacteria and viruses were inactivated under both mesophilic and thermophilic temperature conditions. In the mesophilic environment, the D-value (the time necessary to reduce the number of micro-organisms by one $\log_{10}$ unit at a specific temperature) ranged from 11 hours (ECBO-Virus, 35°C, cattle slurry, volume germ-carrier) to 13 days (Bovine Parvovirus, 30°C, stored pig slurry without addition of leftovers, volume germ-carrier). This shows that the anaerobic codigestion under mesophilic temperature conditions cannot ensure a sufficient inactivation of potential pathogenic epidemic germs in the biogas substrate. Under thermophilic conditions all examined organisms and viruses were inactivated considerably faster. At a temperature of 50°C the D-values were between 7,2 minutes (ECBO-Virus, volume and sandwich germ-carrier, pig slurry) and around 30 hours (Bovine Parvovirus, volume germ-carrier, cattle and pig slurry). At 55°C D-values of 1,2 minutes (*E. coli* O157, pig slurry) and up to 8 hours

(Bovine Parvovirus, cattle slurry, volume germ-carrier) were found.
- With the exception of *E. faecium* and Bovine Parvovirus under thermophilic conditions, all of the analysed bacteria and viruses were reduced by more than four $\log_{10}$ units during a period of six hours.
- After comparing the D-values of the bacteria and viruses analysed at the University of Hohenheim with those of the viruses which where examined at the Federal Research Centre for Virus Diseases of Animals in Tübingen (0) in parallel, the Equine Rhinovirus represents a suitable test organism especially with respect to Foot and Mouth Disease Virus. However, since the differences in the D-values of both viruses were small and other viruses which are relevant for animal epidemics (SVDV, ASFV) had, in some cases, higher D-values than the Equine Rhinovirus under specific temperature conditions, ERV as sole indicator organism would not guarantee sufficient security for the validation of biogas plants. Comparing the D-values and z-values (temperature difference, which corresponds to a reduction of the D-value to 1/10) of all examined germs the faecal streptococci as well as the Bovine Parvovirus can be favoured as test organisms in validation procedures. Due to their high thermo-resistance the Bovine Parvoviruses seem more appropriate for validation of reactors and pasteurization devices at temperatures above 50°C.

Input and output analysis of different substrates used in cofermentation gave the following results:
- No representative indicator could be found which could be used for all types of substrates. Organism used in analysis of drinking water like *E.coli* as indicators for faecal pollution are not generally present in the input material, and if their quantity is high variable no decision on the hygienic status of the final product could be based on.
- Such general parameters like *Enterococci* or *Enterobacteriaceae* are not applicable for input-output analysis if biotechnological processes are involved, because organisms belonging to such groups are part of the process microflora and their propagation in the processed substrates does not necessarily correlate with those of pathogens of epidemiological relevance.
- If *Enterococci* shall be used in this context for input/output analysis in processing certain substrates of faecal origin defined species like *Enteroccus faecalis* shall be used as parameter. Suitable PCR-methods on species level are available (0).

The results concerning relationship between selected indicator and test-organisms of veterinary and public health importance to phytopathogenic organisms and weed seeds in thermophilic biogas processes and in pasteurization devices are as follows:
- The data indicate that an inactivation of *Plasmodiophora. brassicae* takes place during an exposure time of 23 hours at 55 °C in a thermophilic biogas-reactor. Thermal inactivation of tomato seed was also observed under this conditions. This correlates in principle with he behaviour of *Salmonella sp*., *E.coli* and Enteroviruses exposed under the same conditions
- Pasteurization of 1 hour at 70 C inactivates both, *Plasmodiophora brassicae* and tomato seeds as well as *Salmonella sp*., *E.coli* and Enteroviruses
- Inactivation of tobacco mosaic virus was inefficient both after incubation for 24 hours at 55 °C in a biogas-rector or 1 hour at 70 °C in a pasteurization device. Same applies for Bovine Parvovirus.
- Clostridial spores will be totally unaffected, both by pasteurization and thermophilic anaerobic treatment.

Conclusion

The system of process-validation, steady supervision of relevant process data and supervision of the final product for selected bacteriological parameter like Salmonella in 50g cannot be replaced by a simple product supervision, because microbiological properties and the occurrence of pathogens with epidemiological importance in the raw-material are highly variable and material related. If materials with high epidemiological risks concerning the presence of animal and plant pathogen are processed no representative indicator organism in the final product could be identified nor could be found, that the used process recommendations and test-organisms are representative for both groups. With respect to animal by-products this means that processes used for treatment of category 3 materials shall be validated by highly resistant viruses like bovine parvovirus as test organisms.

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