ORAL PRESENTATIONS

STRATEGIES FOR HYGIENIC SAFE RECYCLING OF ORGANIC WASTES AND RESIDUALS TO AGRICULTURE

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SUMMARY AND RECOMMENDATIONS

Certain health-risks for man, animals and plants due to pathogens and other microorganisms with undesired properties are connected with the utilization of wastewater, sewage sludge, organic wastes and residuals as well as animal by-products. Those risks can be minimized by adequate measures in collection, transport, storage and treatment, best in the framework of a HACCP-concept. The first step in the proposed HACCP concept is the analysis of the epidemiological situation concerning diseases of man, livestock and plants in the area the raw materials that are originating from (hazard analysis). This has to be followed by validation of the intended treatment process with test organisms covering the resistance of the "key-pathogens" identified in the hazard analysis accompanied by measuring the technical data in the process that are relevant for the inactivation of the pathogens and test organisms at representative places in the equipment (critical control points). Keeping of the data set in the validation process is verified by continuously measuring the relevant parameters at the critical control points and by filing the data for at least two years. Finally, the treated product has to be examined for presence and absence of raw material dependent relevant indicators also identified by taking the results of the validation procedure into account. If the necessary degree of hygienic safety can't be reached in the treatment, additional use restrictions may be helpful in minimizing the risks of direct transmission via food and feed to man and animals. Since use restrictions are ineffective in minimizing the environmental risks and very limited in avoiding the phytohygienic risks, safe treatment must have the priority before applying the tool of use restrictions.

Keywords: hygienic safety, HACCP, biowastes, sewage sludge, manure, process validation

INTRODUCTION

Recycling of biological wastes by aerobic or anaerobic biotechnological treatment as well as by physical or chemical stabilizing treatment mainly results in the production of organic fertilizers, soil improvers, growth media or comparative products deemed to be used mainly in agriculture or gardening. Municipal wastes, animal by-products, sewage sludge and other organic sludges may contain pathogens of different nature being infectious for several species of animals and plants as well as for humans. Depending upon the type on the type of pathogen and on the type of wastes and residuals, the epidemiological importance for possibly exposed populations of animals,

humans and plants differs depending on origin, storage and treatment of the relevant materials and types of organisms causing the risks (Table 1).

Strategies for minimizing the risks

Hazard Analysis Critical Control Point (HACCP) quality assurance systems are used internationally in the food industry to ensure product quality standards are met and with some modifications, it is part of EU legislation on animal by-products and of several EFSA opinions in 2005. However, the system can be adapted to the treatment of other organic wastes and residuals including sewage sludge and energy crops, particularly to guarantee hygienic standards. The three key actions to be taken to establish an effective HACCP concept are; process validation, process supervision, and product supervision. In the case when a remaining risk has been identified, restrictions on usage may be the fourth measure to be taken. First the risks related to the amount and type of pathogens as well as microorganisms with undesired properties to be attended in the raw material must be considered as well as the intended field of application for the final product, because safe utilization can avoid certain hygienic risks. The initial stage of establishing a HACCP system in the treatment plant itself is to undertake a hazard analysis, which identifies points in the treatment process, which are critical to delivering the final product standards. At these points, the relevant process parameters related to the inactivation of pathogens shall be continuously measured such as temperature, time, concentration, pH value etc. For those Critical Control Points (CCPs) control data for supervision of the safe inactivation of the relevant pathogens can only be fixed reliable by a validation procedure testing the degree of inactivation of such pathogens using representative test organisms in an experimental approach (process validation). Once validated, the HACCP system operating limits are set, and by operating the system within those limits the end product quality is assured. This means that end product testing which is always critical due to the inability to define a representative sample size and number as well as due to of the inhomogeneity of the bulk material, can be reduced to a level which limits microbiological end-product control to the monitoring of a reasonable amount of samples. For each process a contingency programme is required, which details the plan of action if any CCP goes outside its limits and ensures that failed product cannot contaminate assured product.

Table 1. Epidemiological importance of organic wastes and residuals as well as of the resulting fertilizer during transport, treatment and utilization

A.	**Direct transmission to farm animals**
	➪ Contamination of meadows
	➪ Introduction of pathogens by storage and processing close to susceptible animals
	➪ Aerogenic transmission by spreading the materials onto farm land
B.	**Direct transmission to humans**
	➪ Handling of contaminated fertilizers in the household
	➪ Occupational exposure to contaminated products
	➪ Accidental transmission to immuno-compromised persons
C.	**Indirect transmission to farm animals**
	➪ Via feed from contaminated sites
	➪ Via living vectors
D.	**Indirect transmission to humans**
	➪ Via introduction of zoonotic agents into the food-chain
	➪ Via food contaminated by living vectors

E.	**Introduction into the environment**
	➪ Generation of carriers in the fauna
	➪ Introduction of organisms with undesired properties into the biocoenosis and persistence in soil and water

Process Validation is a key tool

The capability of a process to inactivate pathogens causing raw-material dependent risks cannot be judged by analysis of presence or absence of indicators (Bacterial, viral, fungal or parasitic) in the final product. Absence of all or one of the mentioned pathogens or indicators in the final product may be caused by several reasons: They may not be present at this time in the raw material, or they are present in the raw material but in a low count (less than 5 log), the recovery of the involved pathogens may be insufficient due to ineffective enrichment or resuscitation procedures (bacteria) or there may be a failure of isolation due to effects of the complex matrix (viruses). Therefore validation strategies must be followed taking two basic approaches into account. The easiest way is to perform an input-output analysis measuring the inactivation rate of one or more organisms present in the raw material during the treatment process, the other is direct validation of the treatment process by exposing test organisms with defined patterns of thermo- and/or chemo resistance for assessment of the inactivation rate. Validating a process by input-output analysis of a certain indicator is generally possible but under practical conditions of limited importance. In most cases, depending on the microbiological properties of the input materials processed, other strategies must be followed, e.g. process validation with one or more representative test–organism. Either if the thermophilic process itself or if a thermal treatment shall provide an inactivation of pathogens belonging to the indicated level of thermo- and chemo resistance representative test-organisms must be exposed in a similar matrix as treated in a suitable test-body in a defined validation experiment. The relevant process parameters must be recorded during the exposure in order to define the technical conditions to be maintained for effective inactivation according to the results of the survival experiments.

The question of how validation shall be performed and what test containment system can be applied is not easy to answer. In biogas plants two main types of test containments may be applied depending on the test organisms. For those test organisms which can be retained in a test containment system filled with liquid by a membrane filter like bacteria, fungi and parasites type 1 test containments could be used (Rapp, 1995). Exposure of viruses to a process requires a different test containment systems. In such a type 2 system the virus material is adsorbed to a special filter material and released after exposure by desorption due to washing with a special solution according to Traub et al. (1986) and Hoferer, (2002). In composting different approaches are described for bacteria, because a representative amount of raw material can directly be contaminated with the test strain, put into textile sacks protected by a perforated metal basket from mechanical destruction Viruses may be exposed also surrounded by the material deemed to be composted, but in a type 2 test containment system as described above.

Process validation with vegetative Bacteria

Several different test organisms had been discussed in the European context, the most promising are: *Escherichia coli, Salmonella Senftenberg* W775 W, H2S negative and *Enterococci*, e.g. *Enterococcus faecalis*. Table 2 gives some thermoresistance data in a matrix representative in co-fermentation of liquid manure together with catering wastes.

Every of the indicated test organisms have advantages and disadvantages. Escherichia coli is generally less fit in biogas-plants and in composting as *Salmonella Senftenberg* W775 H2S negative and there is no scientific background for the selection of a certain test strain. Moreover enrichment from environmental samples is not as sensitive and effective as for Salmonella.

Table 2. Thermoresistance data (maximal D – values) for selected vegetative bacteria in co-digestion of slurry with catering wastes

SPECIES	TEMPERATURE	50 °C	50 °C	55 °C	55 °C
	Slurry	Pig	Cattle	Pig	Cattle
E. coli		0,43 h	0,40 h	0,08 h	0,03 h
S. Senftenberg W 775, H2S negative		0,60 h	0,53 h	0,11 h	0,06 h
Enterococcus faecium		7.48 h	11,2 h	1,7 h	1,64 h

Even when the test containments are hermetically sealed, accidental contamination from outside cannot totally be excluded under mechanical action in the reactor. In this case, differentiation from contaminants with other E. coli of faecal origin is not so easy to achieve. This may be overcome by the use of mutants of E. coli, but K12 mutants are less fit than field strains. The use of *Salmonella Senftenberg* W775, H2S negative has some advantages. First a reliable quantitative enrichment, also from a contaminated test body, is easy to achieve. Methods are described in CEN WI 308. 049 1–3. *Salmonella* are really representative test organisms with epidemiological importance and not a surrogate. The thermo resistant mutant (H2S negative) used for validation allow a easy differentiation from native Salmonellas (H2S negative) and there are many data available from various survival studies and validation experiments. But there are also some disadvantages, one of the often raised points is, that according to the nomenclature it is still regarded as a pathogen this seems to limit the application (e.g. in Scandinavian countries). For the thermophilic biogas process does not cover the relevant viral pathogens totally in resistance. Hoferer (2002).

Finally *Enterococci* have also some advantages, especially as there are fewer concerns with regard to pathogenicity. As for *Salmonella Senftenberg* there is also a lot of existing data for *Enterococcus faecalis* concerning validation of thermal processes, but mainly in other application fields (hospital hygiene). Since it is more resistant than Salmonella, a quantitative analysis of the results from the validation of pasteurization units is possible, while Salmonella are inactivated too rapidly and bacterial spores are too resistant. The application of *Enterococci* for this purpose also has some disadvantages. First the quantitative enrichment is less effective than in Salmonella, if contaminant flora shall be excluded. In this case contamination from the substrate cannot be detected in an easy and reliable way. Finally it must be kept in mind, since *Enterococci* do not have any epidemiological importance and since they are more chemo- and thermo resistant than most relevant pathogens in this field their application may set a much too high barrier for passing such a validation in certain situations. This means, that the application of test organisms must be strictly related to the process to be evaluated. Therefore if only a process of thermal inactivation like a pasteurization-unit is being validated, *Enterococcus faecalis* is the suitable test organism. If a thermophilic aerobic or anaerobic process shall be validated, it is more realistic to use the above characterized strain of Salmonella Senftenberg, because Enterococci will be a too hard criteria for this purpose, since its chemo-resistance differs substantially from most of the relevant pathogens.

Validation with thermoresistant viruses

In certain epidemiological situation e.g. if animal by-products shall be processed validation with thermoresistant viruses is necessary. It is known from comparative heat inactivation studies, that the Bovine Parvo Virus (BPV)is much more resistant than enteroviruses and will survive treatment at 70°C for 60 min (Stöcklein, 2005). Since recently it was stated by Emmoth et al. (2004) that heating of animal by-products to 70°C for 60 min is not enough for the inactivation of circoviruses and it had been demonstrated that the plant pathogenic tobacco mosaic virus withstands such treatment without any significant reduction, there is a necessity to validate the treatment if such viruses may be present in involved materials. The following viruses may be used in principle as test-viruses in process validation: Parvovirus (bovine, feline) or Circovirus (porcine, avian). Limited results are available concerning the application of thermoresistant viruses in validation procedures. Most data is available with the application of bovine parvovirus in validation of composting and biogas plants. Table 3 gives some D-values in mesophilic and thermophilic cofermentation units. The given data are demonstrating, that not only the temperature but also the type of substrate is influencing their survival.

Process validation with parasites

Due to their lower thermoresistance most parasites eggs and oocysts give no additional information in the validation of thermal processes. But in validation of chemical treatment they may be useful with certain substrates. The exposure techniques are the same as for bacteria. Test organisms which may be used are eggs of *Ascaris suum.* Oocysts of *Cryptosporidium parvum* may also be used, but there is no reliable technique available to judge definitely over inactivation, viability and infectivity, Mayer (2001).

Table 3. Decimal destruction rates as Dt – values in hours (T90 – values) found for several viruses in co-digestion (catering waste and slurry) at different temperatures according to Hoferer (2002)

Dt in hours	Temperature	30°C	30°C	30°C	35°C	50°C	50°C	55°C	55°C
Type of virus	Type of slurry	CS	PS	CS*	PS	CS	PS	CS	PS
ECBO	A	43,44	24,72	25,20	17,36	0,61	0,12	0,24	0,07
ERV	A	34,08	25,92	N.	N.	0,96	0,72	0,54	0,20
Polio	A	N.	32,16	N.	N.	0,63	0,18	0,07	0,03
BPV	A	N.	N.	180,24	N.	20,41 10,48	14,27	4,67	5,47

N = not investigated
* = long time stored pig slurry without catering wastes
A = test organisms absorbed to membranes
PS = pig slurry
CS = cattle slurry

Process validation with bacterial spores

Process validation with bacterial spores is only useful if sterilization processes have to be evaluated. The exposure techniques have to be different from those described above. CEN TC 102 deals with such subjects, the relevant standard is EN 556. Biotechnological treatment will not inactivate bacterial spores neither of Bacillus species nor of Clostridia, as well as pasteurization at

70°C will fail for this purpose (Stöcklein, 2005). Test organisms which may be used in this context are spores of *Bacillus stearothermophilus, Bacillus subtilis* and *Clostridium sporogenes.*

Process validation with prions

If processes deemed to treat category 1 material according to EU regulation 1774 /2002 need to be validated, this have to be done with PrPres. The most convenient way to do this is validation with the hamster adapted strain like 263 K which can be propagated to high titres in a hamster brain and rapidly titrated back after exposure in a bioassay within 90 days. But it must be kept in mind, that BSE-PrPres will not be covered by all scrapie strains in heat-resistance (FAIR, 2001). Comparative studies are needed between representative BSE-strains and the scrapie-strain 263 K. Until then it may be discussed, if a hypothetical six log reduction of strain 263 K in a validation procedure may be regarded as sufficient to cover a five log reduction of a resistant BSE-strain under practical conditions.

Process Supervision

The critical control points identified in the basic analysis and in the validation of the treatment have to be continuously supervised, the relevant data have to be recorded and filed for at least 2 years. There are two types of control points, those which had been identified in the basic analysis of the flow of material in the plant and which are mainly of an organisational nature on the one and those in which technical parameters have to be monitored which are directly related to the treatment process and the associated results of the validation procedure. The organisational control points are mainly related to any form of documentation necessary to assure the keeping of standard operation procedures including the fixation of responsibilities which are common in any HACCP – concept.

With relation to process supervision the technical parameters to be kept are of primary importance. Those parameters are in general dependent on the type of treatment procedure. This means that, for example in a composting process, very simple parameters have to be measured like exposure time related to temperature, moisture and frequency of turning the material, while more complicated technical parameters have to be recorded as in animal by-product treatment like feed screw revolutions per minute (rev. /min.), electric power (amps at given voltage), evaporation/condensation rate, number of pump strokes per unit time. All measuring and monitoring equipment must be calibrated at least once a year. The definition of such control points and the values to be kept are basically related to the results of process validation.

Product Supervision

As mentioned above, the investigation of the final product in order to detect every pathogen which may be present in the material is impossible, therefore representative indicator organisms have to be determined from the point of view of human and animal health as well as for the purpose of safe plant-breeding and production. Those indicator organisms must fulfil several requirements: they have to be present with a high probability in the raw materials, the transmission via the final product must be a factor in epidemiology, the indicator should not be involved in the biotechnological process itself, the indicator should not be an organism which is generally present in soil and soil related materials and the method for isolation and identification must be simple, definitely and reliable if applied to a substrate with a complex microbiological matrix such as compost or digested material.

With respect to public health and veterinary requirements several indicators and parameters are in discussion: *Salmonella enterica, Enterococci* (*Streptococci* of group E), *Staphylococcus aureus, Enterobacteriaceae, Escherichia coli,Campylobacter, Yersinia spp, Listeria spp, Clostridium perfringens,* Sulfite reducing *Clostridia,* Enteroviruses, Rotavirus, eggs of nematodes and larvae of nematodes Materials coming out of processes such as composting or anaerobic digestion are products of a microbial degradation and the knowledge about the microbiological ecology of such materials is still limited. Consequently it is important that, if analysis methods based on clinical microbiology or drinking water examination are used for isolation and identification, a careful validation in combination with all the involved sample matrices is essential.

The variety of species present in environmental sample and in such complex matrices as compost by far exceeds the limited number of species to be taken into account in excreta as well as in body fluids. The variability in species in compost-like materials is very high and not yet fully understood. Moreover, microbial parameters which are used in the field of water hygiene and food inspection are not applicable to substrates like compost or sludges from anaerobic digestion, because most of those indicators belong to the indigenous flora of agricultural soils (Böhm, 1995). It must be taken into account, that methods used in clinical microbiology and in drinking water supervision will often fail if applied to complex matrices like compost or digested sludge. Same applies for the selection of so called indicators. Since most of the materials are of faecal origin, faecal indicators have to be present in the material. The intended field of use has so to be taken into account in this context, therefore the exclusion of organisms which generally may be found in normal soils makes no sense for a substrate and fertilizer as e.g. compost. This means that the following microbial parameters are, with some exceptions in certain situations, inappropriate: *Staphylococcus aureus, Enterobacteriaceae, Clostridium perfringens,* sulphite reducing *Clostridia* and *Listeria.*

One parameter which seems to be very useful and reliable in this connection is the absence or presence of *Salmonella.* There is a high probability of finding *Salmonella* (at a range of levels) in fresh biowastes or untreated sewage sludge. Since it is known that the probability of identifying a positive sample is basically related to the amount of investigated material a compromise between feasibility and reliability has to be found. It is proposed to take 50 g or 100 g (2x50 g) of material to determine presence or absence of *Salmonella.* The approach of the European animal by-product regulations is to use only 25 g of material as has been the practice 15 years ago (ATV 1988) and gives less sensitive performance data as if 50g of material were to be used. Some other parameters are still in discussion with respect to sewage sludge treatment and composting in the framework of EU – directives. *Enterococci* for example cannot be used as indicator in the examination of compost and compost related products, but for the thermophilic anaerobic treatment in biogas plants as well as for pure thermal treatment they are very valuable (Bendixen, 1999). For E. coli, Campylobacter and *Yersinia* beside the lack of reliable re-isolation techniques it must be stated that their thermal resistance and with minor exceptions chemo-resistance is lower than that of *Salmonella*. This means it will make no sense if they are used as additional microbial parameters for describing a hygienically safe product. Enteroviruses are generally present in sludge of faecal origin but not regularly in sludges coming from other sources. In principle Enteroviruses may be used as an additional indicator but the re-isolation procedures are, as for all viruses from environmental samples, both labour and cost intensive. Their resistance in the involved treatment processes is not higher than that of Salmonella, this means, that the additional information resulting from using this indicator organisms are of little value. The same applies for rotavirus;

even it is of special environmental importance according to Metzler et al. (1996) and Pesaro et al. (1999).

The question whether nematodes or nematode eggs are a useful indicator in this connection is not easy to answer. With respect to nematodes pathogenic for man and/or animals the experience shows, that even eggs of *Ascaris suum* are less thermo resistant than *Salmonella*, but behave differently in chemical treatment, this means that if *Salmonella* would have not survived e.g. the (thermal) composting process *Ascaris* eggs and with them all other nematodes eggs would not have done either. This does not apply for treatment with slaked lime or long-term storage. This means that *Ascaris* eggs will not be a necessary indicator in all processes in which the thermal effect is the predominant one but they will give valuable additional information if used in the supervision of all other treatment processes. Nematodes may also be an indicator for insufficient storage conditions for a final product like compost by which plant pathogenic nematodes may have invaded the material. In order to identify this situation eggs or larvae of such species have to be properly identified. This requires special expertise, which is generally not available in most of the relevant laboratories. This means that a general parameter defined as free of nematode eggs and/or larvae" will not be easy to realize in this connection. Another situation in which the investigation of the final products for the presence of nematode eggs makes sense is in co-digestion with liquid manure or sewage sludge if this feeding material had not been heated before entering the reactor. No plant pathogenic virus, fungus or bacterium has been found so far which is comparable in importance to Salmonella for the above-mentioned purpose. The only indicator, which is widely distributed in biological wastes from households and in wastewater, is tomato seed. Even this indicator will not totally cover all requirements, additionally assessment of all reproducible parts of plant materials from biowastes have also to be taken into account. Therefore it seems reasonable and feasible to define the term "phytohygienic safety" of the product as done in the German Biowaste Ordinance: "The final product may not contain more than two tomato seeds per litre product that are capable of germinating and/or reproducible plants parts". A suitable test-method is described by Bundesgütegemeinschaft Kompost (1994).

Use Restrictions

Restriction in the use of fertilizers and substrates resulting from biotechnological waste-treatment should either prevent introduction of undesired chemical residuals by contaminated crops into the food chain of direct transmission of pathogens to susceptible animals via feed. This has been practised in the past especially with sewage sludge. Such a strategy alone does not prevent the environmental risks or introduction of pathogens into vector populations, which will lead to indirect transmission cycles. Several authors have given examples how birds can become carriers of *Salmonella* (Hellmann 1977). One of the sources of infection in sea gulls has been found to be a sewage treatment plants. The further ways of introduction of a certain lysotype of Salmonella enteritidis could be demonstrated by Köhler (1993). He identified the waste delivered from West Berlin to a waste disposal site in the former GDR and followed the introduction of this pathogen via birds into the chicken populations and finally to humans via products containing eggs. (Williams et al., 1977) as well as several other authors like Coulsen et al. (1983), Mayr (1983) described the importance of vectors in the transmission of *Salmonella* to farm animals and humans. Foster and Spector (1995) described specific molecular mechanisms responsible for the ability of *Salmonella* to survive the environmental stress. This means even if the fertilizers containing pathogens are immediately ploughed into the soil or injected by special devices they may generate carriers (e.g. sea-gulls attracted by ploughing) or prolonged survival in sub surface

soil layers. Thus restrictions in use are a tool with limited effects from the point of view of epidemiology and should be avoided if possible and feasible. Moreover concerning plant pathogens and seeds this strategy is ineffective if the products are to be used in agriculture.

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