

Monitoring of ABPR 1774 requirements
in anaerobic plants for organic waste
- inspection, process validation,
product control and experiences -

European Compost Network ECN

The Future of Anaerobic Digestion of Organic Waste in Europa
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Introduction I

- Untreated biowastes and animal by-products may contain different types and species of pathogens for human, animals and plants.
- This applies not only to animal by-products but also to wastewater, biogas residues, sewage sludge, and other organic fertilizers and composts of human, animal and plant origin.

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Introduction II

- Untreated biowastes and animal by products may contain a variety of pathogens for humans and animals with a long survival time as well as undesired organisms which should not be spread in the environment, such as like multi-resistant bacteria and seeds of weeds.

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Introduction III

| Primary Pathogens | Secondary Pathogens |
|---|--|
| <i>Salmonella</i> spp. <i>Shigella</i> spp. <i>Escherichia coli</i> O157 <i>Yersinia enterocolitica</i> <i>Clostridium perfringens</i> <i>Brucella abortus</i> <i>Listeria monocytogenes</i> <i>Streptococcus agalactiae</i> <i>Mycobacterium bovis</i> <i>Leptospira</i> spp. <i>Campylobacter</i> spp. <i>Staphylococcus</i> <i>Pasteurella multocida</i> | <i>Klebsiella</i> sp. <i>Enterobacter</i> <i>Serratia</i> <i>Citrobacter</i> <i>Proteus</i> <i>Providencia</i> multiresistant bacteria |

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Introduction IV

Survival of selected pathogens in livestock slurry and bio-waste

| Pathogen | Slurry type | Typical survival time |
|------------------------------|-------------------------|-----------------------|
| <i>Salmonella</i> | Cattle | 200-300 d |
| | Poultry manure (layers) | 90-120 d |
| <i>B. abortus</i> | Cattle (10 °C) | 47-70 d |
| | Cattle (20 °C) | 20 d |
| <i>E. coli</i> | Cattle (winter) | 85-130 d |
| | Cattle (summer) | 30-120 d |
| Foot-and-mouth disease virus | (summer) | 25-32 d |
| | (winter) | < 60 |

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Requirements I

Regulation (EC) No. 1774/2002

The requirements for the treatment (hygienization) of Category 3 material are:

12 mm, 70°C, 60 minutes

but:

the competent authority may authorize the use of other standardized process parameters...

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Requirements II

Regulation (EC) No. 1774/2002 and No. 208/2006

Process validation is generally carried out:
(since 1st of January 2007)

- by variations of standardized process parameters...
(for example 60 °C/1h; 20 mm instead of **70 °C/1 h; 12 mm**; or treatment in thermophilic anaerobic plants)
- Through process validation the anaerobic treatment plant can demonstrate that it is able to minimize biological hazards
- Regulation (EC) No. 185/2007 from 20 February 2007
(This provides the legal basis for the validation of alternative process parameters)

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Requirements III

From an hygienic point of view the following parameters are necessary for the validation and process control in anaerobic treatment plants :

- 1 Openings at the reactor and/or in the pre –or post heating system for introducing and removing suitable germ-carriers.
- 2 Measurement of the temperature at the components of facility which are responsible for thermal inactivation (pre-or post heating, thermophilic reactor (pasteurization 70 °C/1 h)
- 3 Measurement of temperature and pH value near the germ carrier
- 4 Calculated and/or measured real retention time of the material (particle) in one and two-stage plants (for „drying fermentation“ exact knowledge about the flow situation of the aqueous phase).

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Requirements IV

Openings at the reactor and/or in pre-or post heating system for introducing und removing the suitable germ-carrier in the stages of the anaerobic treatment plant



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Requirements V

Temperature measurement at the components of facility which are responsible for thermal inactivation (pre-or post heating, thermophilic reactor (pasteurization 70 °C/1 h)



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Requirements VI

- Temperatures must be recorded at regular intervals. Where possible, these measurements should be continuous.
- They must be taken at least once on every working day and should be automatically recorded.
- Temperatures measurements should be taken in at least three representative zones in the process stages or components of the facility which are responsible for thermal inactivation.

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Regulations and Inspection I

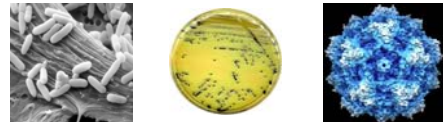
- EC regulation No. 208/2006 describes, in principle, two approaches for process validation and control programs of biotechnological plants. However, these regulations only apply if residual materials from animals are used.

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Regulations and Inspection II

1. Germ carrier system

One principle for the validation of such systems is to use a well-characterized test organism or virus, introduced in a suitable test body in the starting material



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Regulations and Inspection III

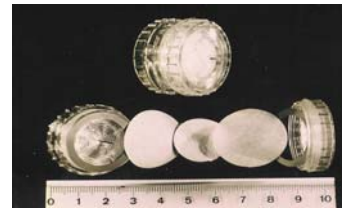
1.1 Suitable test body for test organisms in the starting material for anaerobic treatment plants (germ carrier system).



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Regulations and Inspection IV

1.2 Suitable test body for viruses in the starting material for anaerobic treatment plants (germ carrier system).



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Regulations and Inspection V

The validation of the intended process must demonstrate that the process achieves the following overall risk reduction: (EC No. 208/2006)

- for thermal and chemical processes:
 - reduction of $5 \log_{10}$ *Enterococcus faecalis* or *Salmonella* Senftenberg (775 W, H₂S negative),
 - reduction of infectivity titre of thermo-resistant viruses such as parvovirus by at least $3 \log_{10}$, wherever they are identified as a relevant hazard;
- and for chemical processes:
 - reduction of resistant parasites such as eggs of *Ascaris* sp. by at least 99,9 % ($3 \log_{10}$) of viable stages.

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Regulations and Inspection VI

2. Reduction of endogenous organisms

Another principle for the validation of the intended process is measurement of the reduction of viability/ infectivity of: (EU 208/2006) endogenous indicator organisms during the process, where the indicator is:

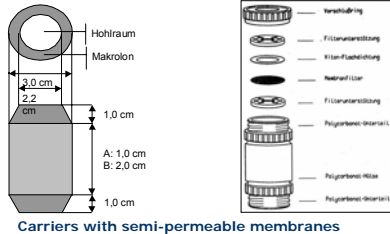
- consistently present in the raw material in high numbers,
- no less resistant to the lethal aspects of the treatment process, but also not significantly more resistant than the pathogens which it is being used to monitor,
- relatively easy to quantify and relatively easy to identify and to confirm;

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Validation I

1. Germ carrier system

Measuring the reduction of viability/infectivity of well-characterized test organisms which are introduced in a suitable test body in the starting material during expose



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Carriers with semi-permeable membranes

Validation II

1. Germ carrier system

In fermentation facilities quantities of 1 ml of *Salmonella* Senftenberg _{w 775} (H₂S negative) are introduced to the process, either with the help of plastic ampoules (contents 2 ml) or on carriers with semi-permeable membranes, depending on the method used in the facility.



Carriers with semi-permeable membranes

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Validation III

1. Germ carrier system



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Carriers with semi-permeable membranes on a rope

Validation IV

1. Germ carrier system

- The carriers with semi-permeable membranes, which are filled with 1 ml of suspension as well as 9 ml of fermentation residue, must be placed into the process stages or components of a facility which are responsible for thermal inactivation.
- After passing through the process, each sample of 1 ml is briefly shaken in 9 ml buffered peptone water (pre-enrichment) and incubated at 37 °C for over 20 hours. The suspension thus gained is tested for the presence of salmonella.

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Validation V

2. Reduction of endogenous organisms

- Not possible in anaerobic treatment plants in practice because endogenous indicator organisms in most cases not present in the raw material in high numbers
- Contamination of the raw material with, for example, *E. coli* and enterococci to determine the reduction of viability/infectivity in practice is not possible and not allowed
- For the validation of anaerobic treatment plants the only feasible and reasonable possibility is therefore the application of the germ carrier systems in conjunction with the product control

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Product control I

- Product analyses (final product controls) must be carried out by external supervisors.
- The operator of an anaerobic treatment plant has samples of the end-product tested for *E. coli* or enterococci. The sampling is carried out immediately after the pasteurization or hygienization process.

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Product control II

Representative samples from anaerobic treatment plants that are taken during or immediately after treatment for the purpose of process control must fulfill the following standards:

- *Escherichia coli*: n = 5, c = 1, m = 1 000, M = 5 000 in 1 g ¹⁾
- or
- *Enterococcaceae*: n = 5, c = 1, m = 1 000, M = 5 000 in 1 g;¹⁾ and
- *Salmonella*: Not detectable in 25 g

¹⁾ 4 samples < 1.000 cfu/g;
 1 sample between 1000 und < 5.000 cfu/g

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Product control III

The number of final samples which can be examined is calculated by the square root of the number within the yearly pasteurization loads, rounded-up to whole numbers, however no more than **20 final samples** per year.

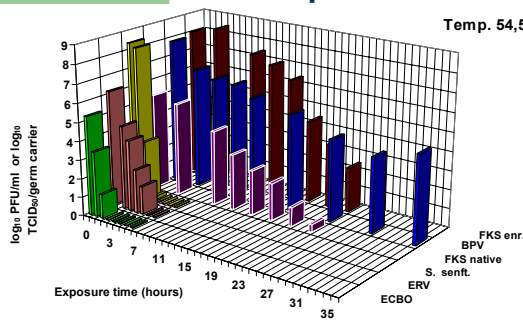


for members in a quality assurance = max. 12 samples/a are necessary

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Experiences I

Temp. 54,5

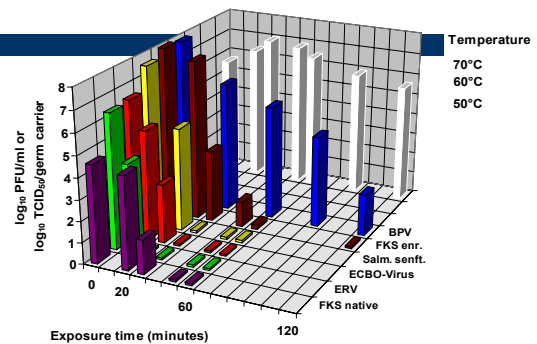


Reduction of different test germs in a thermophilic biogas plant (cattle slurry, 54,5°C; FKS: Fecal streptococci; BPV: Bovine Parvovirus; S. senft.: Salmonella senftenberg; ERV: Equine Rhinovirus; ECBO: Bovine Enterovirus)

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Experiences II

Inactivation of test germs during the hygienisation step of a mesophilic slurry treatment plant (pre-heating, cattle slurry)



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Experiences III

D-values (in h) of **bacteria** in cofermentation-plant (75 % slurry) (BÖHM et al., 2000)

(D-value: = the decimal reduction time (time for the organisms to reduce by 90 %. The decimal reduction time is used as an indicator of the death (decay) of the organisms.

| Temperature | 30 °C | 55 °C |
|-------------------------------|--------|-------|
| EHEC | 78,48 | 0,08 |
| <i>Salmonella</i> Senftenberg | 56,40 | 0,11 |
| <i>Enterococcus faecalis</i> | 186,24 | 1,70 |

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Experiences IV

D-values (in h) of different **viruses** in cofermentations-plant (75 % slurry) (BÖHM et al., 2000)

| Temperature | 55 °C |
|----------------------------------|-------------------|
| Parvovirus (swine parvovirus) | ca. 6 h |
| Swine vesicular disease virus | ca. 3 min |
| Foot and mouth disease virus | ca. 12 min |
| Classical swine fever virus | ca. 12 min |
| Aujeszky`s disease virus | ca. 6 min |
| <u>African swine fever virus</u> | <u>ca. 12 min</u> |

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Conclusions I

- Processing and fermentation in anaerobic treatment plants (co-fermentation) have to eliminate human and animal pathogens and phytopathogenic microorganisms.
- Validation of processes for the treatment of biowastes is principally possible both for simple composting technique and for technically complicated anaerobic treatment plants.
- Additionally, it must be possible to introduce the germ carrier into the anaerobic treatment plant.

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Conclusions II

- Validation of treatment plants should be done by exposure of representative test-organisms, followed by the determination of the inactivation rate after the exposition time, so that the technical parameters which must be kept during the constant process control can be defined.
- The validation with representative test organisms defines the technical and microbiological parameters which must be kept and helps to measure the residual risk during the application of the treated material in the areas.

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Conclusions III

- Monitoring of the end products can be meaningful, if validation is not possible by exposition of test organisms for technical reasons and if the organisms (e.g. *E. coli* or enterococci) in the raw material can be found in a high number
- Monitoring of the final products for naturally occurring test organisms is easily feasible and no additional installations are necessary
- However, the resistance characteristics of the naturally occurring test organisms are not defined and therefore always different.

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THANK YOU FOR YOUR
ATTENTION

