Sanitary requirements of biological waste treatment

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1 Introduction

Sustainable production of food can only be achieved through careful treatment of the environment and protection of the natural resources of soil, water, air and biological diversity. In order to fulfil these requirements untreated wastes and organic fertilizers, among others, have to be subjected to treatment making them suited for environmentally-friendly agricultural use as fertilizers of crop plants.

Many of the untreated plant and animal wastes contain a number of pathogens of different pathogenicity and resistance to antibiotics, i.e. human pathogens, animal pathogens, and zoonotic agents.

Land application of untreated organic fertilizers thus constitutes a permanent potential for epidemiological risks to plants, soils and groundwater and thus an infection risk to humans and animals through feeding stuff and food of plant origin. As this infection risk is to be assessed very differently for different pathogens and fertilizers there is a relatively high uncertainty in the risk assessment of possible contaminations of plants with pathogenic bacteria in the context of organic fertilizers produced from wastes (BML, 2015).

The risk of introducing pathogens of faecal origin into the food chain of humans or animals is reduced if fertilizers produced from wastes are properly utilized and used under observance of the existing land application prohibitions and waiting periods. However, an increased epidemiological risk is existent if organic fertilizers are used across different facilities and thus new infection chains are created that are relevant in terms of epidemiology. The reduction or inactivation of pathogens in waste can be achieved by various treatment measures (aerobic (composting) and anaerobic (biogas) treatment, storage etc.).

A phytosanitary risk is existent if source materials are infested or contaminated on the surface with substances harmful to plants and these relevant harmful substances survive in the source materials, possibly withstand a treatment and are able to infest again host plants after land application of the organic fertilizer.

Plant materials with high risk levels are to be subjected to a sanitising treatment before being utilized as fertilizer. Plant material that is known to be infested with quarantine pests of the potato must not be used as source material of fertilizers, i.e. specific phytosanitary regulations take effect (e.g. German Plant Inspection Ordinance). Furthermore, the unintended introduction of quarantine pests of the potato into fertilizers should be prevented by e.g. prohibiting wastewaters and wastes from industrial potato and root processing as source materials for fertilizers (BMEL, 2015; KTBL, 2015).

Closed Substance Cycle and Waste Management Act

Already in 1994 the Closed Substance Cycle and Waste Management Act (German abbreviation: KrW-/AbfG) on the avoidance, utilization and disposal of wastes was announced in Germany; and on 7 October 1994 the included authorizations to issue regulations became effective. This act was modified several times, and was replaced by the Act to Promote Closed Cycle Waste Management

and Environmentally Sustainable Waste Management (Closed Cycle and Waste Management Law – KrWG) on 24 February 2012, which was most recently amended by Article 4 of the act of 04.04.2016 (Federal Law Gazette I p. 569).

Act Reorganising the Law on Closed Cycle Management and Waste
Section 12
Quality assurance in the field of bio-waste and sewage sludge

(1) The quality assurance institutions and the quality seal holder may establish regular quality assurance in order to promote circular economy and ensure the protection of human health and the environment in the production and management of bio-waste and sewage sludge in accordance with the legal provisions applicable hereto.

(2) A quality seal holder shall be a natural or legal person that
1. generates, treats or recovers bio-waste or sewage sludge on a professional basis, in the context of economic enterprises or public installations, and
2. with regard to bio-waste or sewage sludge that has been generated, treated or recovered, also in mixtures with other waste, substances or material, has a quality seal of an institution of quality assurance.

(3) The quality seal may only be awarded if the quality seal holder
1. complies with the requirements as to the organisation and staffing as well as technical and other equipment, and to the reliability and knowledge and expertise of its staff necessary to ensure the quality of the bio-waste or sewage sludge,
2. complies with the requirements as to quality assurance, in particular in order to reduce contaminants, to guarantee safety from disease as well as phytohygienic safety, and
3. undertakes to demonstrate compliance with the requirements in accordance with numbers 1 and 2 in the context of ongoing supervision vis-à-vis the quality assurance institution.

(4) The quality seal holder may only use the quality seal insofar as and to the extent that it has been awarded to him/her by the quality assurance institution.

(5) A quality assurance institution is a grouping of producers or waste management operators of bio-waste or sewage sludge, associations, as well as of facilities, institutions or persons with knowledge and legal capacity. The quality assurance institution shall require recognition by the competent authority. The award of the quality seal shall be effected on the basis of a set of statutes, of a supervision contract or of another binding regulation for the quality seal holder, which in particular determines the requirements of the quality seal holder, of the bio-waste or sewage sludge generated, treated or recovered by the latter and of its supervision.

(6) The quality assurance institution shall avail itself for the supervision of the quality seal holders of experts who have the reliability, independence, as well as the knowledge and expertise which are necessary for the implementation of the supervision.

(7) The Federal Government shall be empowered, after consulting the parties concerned (section 68), by means of a statutory ordinance with the consent of the *Bundesrat*, to prescribe requirements as to the quality assurance of bio-waste and sewage sludge. The statutory ordinance may in particular
1. determine requirements as to the quality assurance activities, including their scope,

2. determine requirements as to the organisation and staffing, as well as technical and other equipment, and the activity of a quality seal holder, as well as demanding adequate liability insurance coverage,
3. determine requirements as to the quality seal holder and the persons employed therewith, in particular minimum requirements as to knowledge and expertise and reliability, as well as to their documentation,
4. determine requirements as to the activity of the quality assurance institutions, in particular as to their establishment, dissolution, organisation and modus operandi, including the appointment, tasks and powers of the supervisory bodies, as well as minimum requirements as to the members of these supervisory bodies,
5. determine minimum requirements as to the experts working for the quality assurance institutions, as well as their appointment, activities and control,
6. determine requirements as to the quality seal, in particular as to the form and the content, as well as to its award, its withdrawal, its expiry and its deprivation,
7. regulate the special prerequisites, the procedure, the award and the withdrawal of recognition of the quality assurance institution by the competent authority,
8. order for the necessary declarations, proof documents, notifications or other data that documents be kept electronically and submitted in electronic form in accordance with section 3a subsection (2) second and third sentences of the Administrative Procedure Act.

Section 15
Basic obligations of waste disposal

(1) Producers or holders of waste that is not recovered shall be obliged to dispose of such waste unless provided otherwise in section 17. The amount and noxiousness of waste shall be reduced through treatment. Any energy or waste occurring in connection with disposal shall be exploited to the maximum extent possible; section 8 subsection (1) third sentence shall apply mutatis mutandis.

(2) Waste shall be disposed of in such a manner that the public interest is not impaired. An impairment shall be deemed to have occurred in particular if

1. human health is impaired,
2. animals and plants are endangered,
3. water bodies and soil are harmfully influenced,
4. harmful influences on the environment are caused by air pollution or noise,
5. the aims, principles and other requirements of regional planning, and the interests of nature conservation, landscape management and urban development, are not considered, or
6. public safety and public order are otherwise threatened or disturbed.

(3) Insofar as this is necessary to meet the requirements in accordance with subsections (1) and (2), waste shall be kept and treated separately. Section 9 subsection (2) shall apply mutatis mutandis.

Section 33

Waste prevention programme

(1) The Federation shall draw up a waste prevention programme. The *Länder* may take part in the preparation of the waste prevention programme. In this case, they shall draw up contributions for their respective remit for which they shall take individual responsibility; these contributions shall be included in the waste prevention programme of the Federation.

(2) Where the *Länder* do not participate in a waste prevention programme of the Federation, they shall draw up their own waste prevention programmes.

(3) The waste prevention programme

1. shall define the waste prevention goals; the goals shall aim to decouple economic growth from the impact on human health and the environment caused by the generation of waste,
2. shall describe the existing waste prevention measures and evaluate the expedience of the waste prevention activities stated in Annex 4 or other suitable ones,
3. shall establish, where necessary, further waste prevention measures, and
4. shall define expedient, specific, qualitative or quantitative standards for established waste prevention activities, by means of which the progress made in the activities shall be monitored and evaluated; indicators or other suitable specific qualitative or quantitative goals may be used as a standard.

(4) Contributions from the *Länder* in accordance with subsection (1) or waste prevention programmes of the *Länder* in accordance with subsection (2) may be included in the waste management plans in accordance with section 30 or drawn up as a separate environmental policy programme or included in such a programme. If a contribution or a waste prevention programme is included in the waste management plan or in another programme, the waste prevention activities shall be clearly identified.

(5) The waste prevention programmes shall be drawn up for the first time as per 12 December 2013, shall be evaluated every six years and updated where necessary. When drawing up or altering waste prevention programmes, the public shall be involved by the competent authority in accordance with section 32 subsections (1) to (4). The Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety, or an authority to be designated by the latter, shall be competent for the preparation of the waste prevention programme of the Federation. The waste prevention programme of the Federation shall be drawn up in agreement with the other Federal Ministries with competence in this area.

Utilization in agricultural fertilization – Germany

In Germany there is still an increasing tendency of using biogenic wastes in recycling management. The goal is to produce valuable fertilizers that can only be used in consideration of the following legal requirements.

Fertilizer Ordinance (DüV)

Article 1 of the ordinance regulates the codes of good practice in the application of fertilizers, soil improvers, culture media and plant aids on agriculturally used areas, and the reduction of risks

presented by certain substances caused by the application of fertilizers, soil improvers, culture media and plant aids on agriculturally used areas and other areas, as far as this ordinance explicitly specifies this.

Beside the basic principles of the application of fertilizers, application limitations and application prohibitions are specified in Article 8.

(1) Fertilizers except farm fertilizers are allowed to be applied only if they conform to a type authorized by the Fertilizers Regulation or by Regulation (EC) 2003/2003 of the European Parliament and of the Council of 13 October 2003 on fertilizers (Official Journal of the European Communities No. L 304, p. 1). Farm fertilizers, soil improvers, culture media and plant aids are allowed to be applied only if they conform to the requirements of the Fertilizers Regulation in terms of composition and proper specification of contents. Farm fertilizers, soil improvers, culture media and plant aids that were produced exclusively from material that accrued at the own facility are exempt from sentence 2. The relevant authority according to Federal state law can issue exceptions from sentence 2 upon request.

(2) The application of fertilizers, soil improvers, culture media or plant aids produced by using bone meal, meat-and-bone meal or meat meal is prohibited on agriculturally used grasslands and for top dressing in the cultivation of vegetables or arable forage crops. If the substances specified in sentence 1 are applied to other agriculturally used areas they are to be immediately dug in.

(3) The application of fertilizers, soil improvers, culture media or plant aids that were produced by using diatomaceous earth is prohibited on cultivated farmland, grasslands, with arable forage crops and on areas that are intended for the cultivation of vegetables or ground-near fruit cultivation. If the substances specified in sentence 1 are applied to other agriculturally used areas they are to be immediately dug in. The application of dry fertilizers, soil improvers, culture media or plant aids that were produced by using diatomaceous earth is prohibited. The application of the substances specified in sentences 1 and 3 outside agriculturally used areas is prohibited.

(4) Fertilizers with the specification “for fertilization of lawns” or “for fertilization of ornamental plants” according to Appendix 1 Section 5 of Fertilizers Regulation are allowed to be used only for the fertilization of these plants.

The Fertilizer Ordinance is currently amended and falls under the notification process of the European Commission. The EU Commission issued a so-called “detailed opinion” which means that considerable modifications of the draft of the ordinance as presented by the German government are requested. The modification points are not known in detail but the draft has to be revised. Thus, the amendment of the Fertilizer Ordinance is not to be expected before the second semester of 2016.

Fertilizers Regulation

The Fertilizers Regulation regulates the authorization of fertilizer types, the placing on the market, the labelling of fertilizers and in Article 5 specific requirements of epidemic and phytosanitary safety.

Article 5 Requirements of epidemic and phytosanitary safety

(1) The fulfilment of the requirements of Article 3(1)(1) requires that no pathogens, toxins, or harmful substances are contained that cause risks to the health of humans, animals and agricultural crops.

(2) The requirements of paragraph 1 are said to be not met:

1. regarding characteristics of epidemic safety, if salmonella are detected in 50 grams of sample material,

2. regarding characteristics of phytosanitary safety, if source materials of plant origin, also in mixtures, are used that are infested with resilient harmful organisms especially

   a) one of the harmful organism listed in Directive 2000/29/EC,

   b) thermoresistant viruses especially those of the tobamovirus group, or

   c) fungal pathogens with resilient permanent organs, especially *Synchytrium endobioticum*, sclerotinia species, *Rhizoctonia solani, Plasmodiophora brassicae,* and were not subjected to an appropriate sanitizing treatment.

(3) In case of delivery to persons who use fertilizers, soil improvers, culture media and plant aids as part of their professional activities, the requirements of epidemic safety are said to be met by derogation of section 2 (1) if

1. the existing contamination has been indicated in the notifications on proper application and the following references, specified as application requirements, are given:

   a) application on arable land is permitted exclusively on uncultivated arable land and with immediate digging in, unless application is done with winter crop and winter rape until stem elongation (GS 30) with application methods close to the ground,

   b) land application on uncultivated arable land with subsequent cultivation of vegetables or potatoes or the subsequent cultivation of medicinal, aromatic and seasoning herbs is not permitted,

   c) a temporal gap of 6 weeks until the next use is to be observed on grasslands and lands used for animal feeding, and

   d) land application is not permitted in zones I and II of water protection areas, and

2. in the event of using sewage sludge as source material the delivery is only used for application on areas that fall under the jurisdiction of the agricultural specialist authority responsible for enforcing the Fertilizer Ordinance at the location of the sewage plant unless the supplier is a member of an agency that regularly carries out quality controls that ensure proper application.

(4) Section 2 (1) and section 3 do not apply to farm fertilizers, except those farm fertilizers that are stored in a storage tank that is jointly used by several farmers. In this case the requirements of epidemic safety are said to be met if it is ensured that the farm fertilizers have accrued exclusively in the facilities of those farmers who participate in the use of the storage tank and that are applied exclusively on the land of these farmers.

Utilization in agricultural fertilization – Europe

The current EU "Fertilizers Regulation", the REGULATION (EC) 2003/2003 OF THE EUROPEAN PARLIAMENT AND THE EUROPEAN COUNCIL of 13. October 2003, revealed that in particular innovative fertilizer products, that often contain nutrients or other organic substances that were recycled from bio-waste or other secondary resources in accordance with the model of circular economy, have difficulties in gaining access to the European domestic market due to various national provisions and standards.

That is why there is a proposal, brought forward on 17 March 2016, concerning the field of agricultural fertilization suggesting a regulation of the European Parliament and the European Council with provisions on making fertilizers with CE labels available on the market and on the change of Regulations (EC) No 1069/2009 and (EC) No 1107/2009.

However, about 50 % of fertilizers that are currently available on the market do not fall into the field of application of the still effective regulation. This is true for some inorganic fertilizers and virtually all fertilizers made of organic substances, like animal or other agricultural by-products or recycled bio-wastes from the food chain. Research, innovation and investment are currently rapidly developing and contribute to circular economy by creating local jobs and utilizing secondary resources obtained in the EU that otherwise would directly by applied to land areas or disposed of in landfills which would have caused unnecessarily eutrophication and greenhouse gases. There is also the trend towards servitization in the economy; products are increasingly better tailored to the customer based on an analysis of the soils to be fertilized. SMEs and other enterprises in all of Europe are increasingly interested in contributing to this development. Currently, the access of customized products containing organic fertilizers to the domestic market depends on the mutual approval and is thus often hindered.

The current Fertilizers Regulation of the EU does not address the fact that contamination of soils, inland waters, oceans and ultimately of foods caused by EC fertilizers results in environmental problems. A widely recognized problem is the presence of cadmium in inorganic phosphate fertilizers. As there are no EU limit values some member states unilaterally defined maximum contents of cadmium in EC fertilizers with reference to Article 114 TFEU, thus creating single markets within the harmonized area. The presence of contaminants in fertilizers that are currently nationally regulated (e.g. in nutrients produced from sewage sludge through recycling) raises similar concerns.

Thus, a further goal is to address this problem and establish uniform limit values for cadmium in phosphate fertilizers. Such limit values, which are intended to minimize the negative effects of the use of fertilizers on the environment and human health, contribute to the decrease of cadmium accumulation in the soil and of cadmium concentration in foods and water. Simultaneously, market obstacles will be removed that were created by the cadmium limit values specified in some worried member states.

The proposal of a new regulation is supposed to improve the functioning of the domestic market for fertilizers. Thus, legal base is Article 114 of the Treaty on the Functioning of the European Union that is also the foundation of the current Fertilizers Regulation.

2 Requirements of Bio-waste Ordinance (BioAbfV)

In Germany the Bio-waste Ordinance is the most important specification concerning the treatment of bio-wastes. In the following the essential sections are presented. The complete text is attached in Appendix 1.

Ordinance on the Recovery of Bio-Waste on Land used for Agricultural, Silvicultural and Horticultural Purposes (Bio-waste Ordinance – BioAbfV)

§ 1 Scope

(1)

This ordinance shall apply to

1. both treated and untreated bio-waste and mixtures that are applied as fertiliser on land used for agricultural, silvicultural or horticultural purposes or handed over for the purpose of application, as well as
2. treatment and investigation of such bio-waste and mixtures.

(2)

This ordinance shall apply to

1. public-law p arties responsible for waste management and to any third parties, associations or self-regulatory public bodies to which obligations to recover bio-waste have been a signed in accordance with Section 1 6 (2), Section 1 7 (3) or Section 1 8 (2) of the Closed Substance Cycle and Waste Management Act of 2 7 September 1994 (Federal Law Gazette Part I p . 2705), most recently amended by Article 5 of the Act of 6 October 2011 (Federal Law Gazette Part I p. 1986) (parties responsible for waste management),
2. producers or holders of bio-waste or mixtures, insofar as they do not entrust this waste to a p arty responsible for waste management,

2a. any party which collects and transports bio-waste (collectors),

3. any party which treats bio-waste (bio-waste handler
4. any producers of mixtures using bio-waste (mixture producers);

4a any party which accepts bio-waste or mixtures for application and releases it with no further change (interim distributor), as well as

5. parties managing land used for agricultural, horticultural or silvicultural purposes on which any treated or untreated bio-waste or mixtures are, or are intended to be, applied.

(3)

This ordinance shall not apply

1. To house and kitchen gardens as well as allotments,
2. To on-site utilisation of bio-waste of plant origin in agricultural holdings or horticultural and landscape gardening businesses, in cases in which utilisation in accordance with Sections 6 – 8 is guaranteed on se lf-farmed land,
3. Where the Sewage Sludge Ordinance is applicable,

3a. for animal by-products which are to be picked up, collected, dispatched, stored, treated, processed, used or marketed in accordance with Regulation (EC) No 1069/2009 of

the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (OJ L 300, 14.11.2009, p. 1), most recently amended by Directive 2010/63/EU of 22 September 2010 (OJ L 276, 20.10.2010, p. 33) in its current version, in accordance with the legislation adopted for implementation purposes by the European Union, in accordance with the Animal By-Products Disposal Act of 25 January 2004 (Federal Law Gazette Part I p. 82), most recently amended by Article 2 (91) of the Act of 22 December 2011 (Federal Law Gazette Part I p. 3044) in its current version, or in accordance with legal ordinances enacted on the basis of the Animal By-Products Disposal Act, or

4. To any substances the management of which is subject to other legal provisions.

(4)
The provisions of legislation on fertilisers and plant protection products shall remain unaffected. If bio-waste and animal by-products within the meaning of paragraph (3) number 3a are treated together or used for manufacturing mixtures and applied to soils, the provisions of this ordinance shall apply in addition to the provisions designated in paragraph
(3) number 3a.

(5)
The parties referred to in paragraph (2) shall ensure that the pollutants are dropping below the limit values for treated and untreated bio-waste and mixtures referred to in this ordinance as far as possible. The fact that any soil values in accordance with Section 9 (2) have been reached or exceeded shall not in itself give rise to any general restrictions on cropping or any other restrictions not referred to in this ordinance.

§ 2 Definitions

For the purposes of this ordinance,

1. Bio-waste shall mean: waste of animal or plant origin or from fungal materials for recovery purposes, which can be degraded by microorganisms, soil-borne organisms or enzymes, including wastes for recovery purposes with high organic content of animal or plant origin or fungal materials; deemed to be bio-waste is waste which is stated in Annex 1 number 1 in Column 1, further specified in Column 2 and labelled more specifically based on the supplementary provisions in Column 3; soil material with no significant bio-waste content shall not be deemed to be bio-waste; plant residues that occur on forestry or agricultural land and remain on these types of land shall not be deemed to be bio-waste;
2. Sanitising treatment shall mean: biotechnological treatment of biodegradable materials for sanitation purposes thanks to
   a) pasteurisation in accordance with Annex 2 number 2.2.1,
   b) aerobic sanitising treatment in accordance with Annex 2 number 2.2.2 (thermophilic composting),
   c) anaerobic sanitising treatment in accordance with Annex 2 number 2.2.3 (thermophilic anaerobic digestion), or
   d) any other form of sanitising treatment in accordance with Annex 2 number 2.2.4;

2a. Biologically stabilising treatment shall mean: biotechnological treatment of biodegradable materials for the biodegradation of organic matter under aerobic conditions (composting) or anaerobic conditions (anaerobic digestion), or other measures for the biological stabilisation of organic matter; any form of sanitising treatment in accordance with number 2 (b) and (c) shall also be deemed to be biologically stabilising treatment;

3. Untreated bio-waste shall mean: bio-waste that has not been consigned to any treatment;

4. Treated bio-waste shall mean: bio-waste that has been subjected to sanitising and biologically stabilising treatment, including treated waste stated in Annex 1 number 2 in Column 1, further specified in Column 2 and labelled more specifically thanks to the supplementary provisions in Column 3, or treated biodegradable materials stated in Column 2 and labelled more specifically thanks to the supplementary provisions in Column 3;

5. Mixtures shall mean: mixture of treated bio-waste and untreated, sanitised or biologically stabilised bio-waste in accordance with Section 10 and waste stated in Annex 1 number 2 in Column 1, further specified in Column 2 and labelled more specifically based on the supplementary provisions in Column 3, or treated biodegradable materials and mineral substances stated in Column 2 and labelled more specifically based on the supplementary provisions in Column 3, as well as the combination of a mixture which contains one of the above named constituents together with lime within the framework of the application process; the mixing together of bio-waste within the framework of common sanitising or biologically stabilising treatment, as well as the mixing together with materials stated in Annex 1 number 2 shall not be deemed to be a mixture;

6. On-site utilisation shall mean: application of bio-waste of plant origin arising from self-cultivated farmland on self cultivated farmland. On-site utilisation shall also include the application of
   a) bio-waste of plant origin arising during horticultural services in third party areas on self-cultivated farmland as part of the service,
   b) untreated bio-waste of plant origin taken back on a pro rata basis by members of producer associations in wine-growing, as well as fruit and vegetable cropping, on self-cultivated farmland, provided that the plant raw materials have been produced in areas belonging to members of the respective producer association.

§ 3 Requirements concerning the sanitising treatment

(1)
Where not covered by an exemption in accordance with Section 10 (1) or (2), parties responsible for waste management, producers and owners must supply bio-waste for sanitised treatment prior to application or production of mixtures in order to ensure epidemic and phytosanitary safety.

(2)
Epidemic and phytosanitary safety in accordance with paragraph (1) shall be deemed to be satisfied if no damage is caused to the health of humans or animals as a result of the release or transfer of pathogens, and no damage is caused to plants, plant products or soil
as a result of the spread of harmful organisms. The various sanitising treatment requirements to be complied with in detail, and the materials, are specified in Annex 2.

(3)

The bio-waste handler must carry out the sanitising treatment of bio-waste in accordance with the provisions stipulated in Annex 2 in order to ensure the epidemic and phytosanitary safety of bio-waste following treatment as well as during any such release or application on self-cultivated farmland. With respect to the aerobic or anaerobic sanitising treatment of bio-waste in plants with an annual capacity of up to 3,000 tonnes of input materials, in individual cases the competent authority may, in agreement with the competent agricultural and veterinary technical authorities, permit a number of exceptions to the requirements contained in paragraph (4), first sentence, number 1 and Annex 2 for the process inspection. The prerequisite for this is that epidemic and phytosanitary safety is guaranteed by way of compensatory measures, or that there should not be any impact on epidemic and phytosanitary safety issues based on the type, nature, and origin of the bio-waste. In individual cases, the competent authority may, in agreement with the competent agricultural and veterinary technical authorities, permit another form of sanitising treatment in accordance with Section 2 number 2 (d) provided that an equivalent level of sanitation can be demonstrated in line with the requirements of Annex 2. Bio-waste treated in accordance with other provisions shall be deemed to have been subjected to a different form of sanitising treatment in accordance with Section 2 number 2 (d), provided this other bio -waste treatment method is listed in Annex 1 number 1, Column 3, with a reference to this sentence.

(4)
Unless covered by an exemption in accordance with Section 10 (1) or (2), bio-waste handlers must allow examinations to be carried out in accordance with paragraphs (5) to (9) on

1. the effectiveness of the sanitation process thanks to a process inspection which varies for pasteurisation systems by way of technical acceptance,
2. compliance with the required temperature for the necessary duration of treatment during the sanitising treatment by way of monitoring the process, and
3. compliance with the maximum permitted limit values for pathogens, viable seeds and budding plant parts following the sanitising treatment of releasable material by way of examining the sanitised bio-waste.

The methods stipulated in Annex 1 number 4 must be applied for the examinations.

(5)
The bio-waste handler must allow the process inspection to be carried out in accordance with paragraph (4), first sentence, Number 1 within twelve months following the commissioning of a newly constructed treatment plant for sanitising purposes in accordance with the provisions of Annex 2 number 3.1. This shall apply analogous to plants that have already been examined when using new procedures or in the event that significant technical changes are made to the procedures or to process control. With respect to newly constructed pasteurisation plants, the bio-waste handler must allow technical acceptance to be carried out in place of the process inspection prior to commissioning in accordance with the provisions of Annex 2 number 2.2.1.2, in place of the process inspection, by the competent authority for the plant, which shall issue a certificate of acceptance on this. With respect to newly constructed plants used for other forms of sanitising treatment, the process control and process inspection requirements must be defined in agreement with the authority which is responsible for the plant prior to the implementation of process inspection. Until the process inspection is successfully completed, the bio-waste handler may release materials originating from the treatment plant, and which are used for sanitation purposes, to be used for

recovery purposes with the consent of the competent authority if process monitoring requirements in accordance with paragraph (4), first sentence, number 2, and the requirements for inspection of the sanitised waste in accordance with paragraph (4), first sentence, number 3, are met and there is no evidence to suggest that the hygienic safety of these materials has been compromised.

(6)
The bio-waste handler must carry out the process monitoring in accordance with paragraph 4, first sentence, number 2 in accordance with the requirements of Annex 2 number 3.2, and must keep the following records:

1. The temperature profile during pasteurisation,
2. The temperature profile and the implementation dates for aerobic sanitising treatment (thermophilic composting),
3. The temperature profile and the feeding and discharging intervals for anaerobic sanitising treatment (thermophilic anaerobic digestion), and
4. The procedure-specific parameters determined in agreement with the competent authority for other forms of sanitising treatment.

The temperature profile during sanitising treatment must be recorded with a permanent and contact-free direct temperature measurement in the material treated and the temperature must be automatically recorded. With respect to enclosed aerobic sanitising treatment, the competent authority may allow the treatment temperature to be measured in the exhaust air stream o f t he compost material instead of direct temperature measurements. Deviating from the second sentence the competent authority may allow, in the case of o pen aerobic sanitising treatment that the treatment temperature has to be measured and documented at regular intervals, at least once per working day. Devices for temperature measurement must be calibrated regularly, at least once per year; the calibration must be documented. If the bio-waste handler ascertains during the process monitoring that the respective process control requirements have not been met, he has to inform the competent authority promptly about this and about the initiated measures. The competent authority shall assign measures to retain any bio-waste that has not been subjected to sufficient sanitising treatment and to remedy any defects if the measures initiated by the bio-waste handlers are insufficient or inappropriate.

(7)
The bio-waste handler must allow examinations to be carried out on sanitised bio-waste in accordance with paragraph (4), first sentence, number 3 for every 2,000 tonnes of fresh weight commenced within the framework of the sanitising treatment of used bio-waste, including any materials stated in Annex 1 number 2 in accordance with the provisions of Annex 2 number 3.3. In agreement with the competent agricultural authorities, the competent authority may allow examinations to be carried out only on volumes of sanitised bio-waste exceeding 2,000 tonnes if the composition has not changed or has barely changed according to the type, nature and origin of the bio-waste used. In the event that there is a significant change to the composition according to the type, nature or origin of the bio-waste used, the competent authority may order examinations to be carried out on volumes of sanitised bio-waste which are less than 2,000 tonnes. Without prejudice to the first to third sentences, the bio-waste handler must carry out an examination of the sanitised bio-waste within an interval not exceeding three months. In the event that the limit values stated in accordance with Annex 2 number 4.2.2 or 4.3.2 are exceeded when examining sanitised bio-waste,

the bio-waste handler must inform the competent authority promptly of the examination result and of any measures that have been initiated. If the repetition of the examination leads to the same result, or if the limit values are exceeded again in a number of different samples taken, the competent authority shall order measures in order to remedy the defects.

(7a)
By way of derogation from paragraph (7), first sentence, bio-waste handlers who handle more than 24,000 tonnes of fresh bio-waste, including any such materials stated in Annex 1 number 2, and who are exempt from providing examination results or proof of documentation in accordance with Section 11 (3), first sentence, may allow examinations to be carried out on sanitised bio-waste once a month. Paragraph (7), second to sixth sentences, shall apply accordingly.

(8)
The examinations during the process inspection in accordance with paragraph (4), first sentence, number 1 and during the tests on sanitised bio-waste in accordance with paragraph (4), first sentence, number 3 must be carried out by independent investigative bodies selected by the competent authority. The bio-waste handler must submit the examination results to the competent authority within four weeks of the implementation of the examination and must retain them for a period of ten years. The records regarding process monitoring and documentation regarding the calibration of temperature measuring devices in accordance with paragraph (6) must be retained by the bio-waste handler for a period of three years and must be submitted to the competent authority on request. If it is ascertained in the examination of the sanitised bio-waste that the limit values for pathogens, viable seeds and budding plant parts have been exceeded, the examination results shall be transmitted by the investigative body promptly to the bio-waste handler, who shall transmit them promptly to the competent authority. The latter shall promptly forward the examination results to the competent agricultural and veterinary technical authority.

(8a)
An investigative body in accordance with paragraph (8), first sentence, shall be determined if the applicant has the requisite expertise, independence, reliability and equipment and submits the necessary documents. The determination shall be made by the competent authority of the federal state in which the applicant has its place of business, and shall apply to the entire federal state territory; if there is no domestic place of business, the federal state shall be competent in which the activity in accordance with paragraph (4) is to be primarily exercised. The determination can be subjected to a reserve of revocation, to a sunset clause, to conditions, to instructions and to a reservation of imposing instructions. The competent authority may require from an applicant operating nationally to submit a valid accreditation of compliance with the requirements of DIN EN ISO/IEC 17025:2005 (available from Beuth-Verlag GmbH, 10772 Berlin, and archived in a secure manner at the German National Library in Leipzig) referring to the parameters and examination procedures in accordance with Annexes 2 and 3. Procedures in accordance with this paragraph can be effected via a single agency. The examination of the application to determine an investigative body must be completed within three months; Section 42a (2), second to fourth sentences, of the Administrative Procedure Act shall apply.

(8b)
Equivalent recognition from another Member State of the European Union or from another State

which is a party to the Agreement on the European Economic Area shall be deemed to be equivalent to determinations in accordance with paragraph (8), first sentence. When examining the application for a determination in accordance with paragraph (8), first sentence, documentation from another Member State of the European Union or from another State which is a party to the Agreement on the European Economic Area shall be deemed to be equivalent to domestic documentation if it reveals that the applicant satisfies the pertinent requirements of paragraph (8a), first sentence, or the requirements of the issuing state which are essentially comparable in terms of their objectives. The original or a copy of the documentation shall be submitted to the competent authority prior to taking up the activity. Certification of the copy, as well as a certified German translation, may be required.

(9)
The additional provisions specified in Annex 1 number 1 Column 3 with regard to separate storage, treatment and application of bio-waste shall be observed.

(10)
Paragraphs (1) to (9) shall apply analogously to all materials with respect to the common sanitising treatment of bio-waste with materials stated in Annex 1 number 2. If bio-waste which has already been subjected to sanitising treatment with materials stated in Annex 1 number 2 is subjected to any subsequent biologically stabilising treatment, paragraph (4), first sentence, number 3 shall apply provided that any examinations of sanitised bio-waste are only carried out following the biologically stabilising treatment of releasable material. By way of derogation from the second sentence, the examinations of sanitised bio-waste may be carried out following the sanitising treatment of releasable material if the subsequent biologically stabilising treatment of bio-waste which has already been subjected to sanitising treatment is carried out on an agricultural holding together with any biodegradable materials arising there, and the treated materials are applied on self cultivated farmland.

3 Issues of epidemic safety

3.1 Pathogens in bio-waste

3.1.1 Historical development

Turn of the century

It is known that pathogens can be existent in solid and liquid municipal wastes. As early as at the beginning of the last century (1908) people were interested in the issue of microbial threats to humans by pathogenic germs in sweepings and waste and conducted according experiments with typhoid bacteria, paratyphus B and pseudodysentery bacteria as well as anthrax bacteria under different temperature conditions in house sweepings. In these experiments typhoid bacteria remained viable for more than 40 days, the other three bacteria types for more than 80 days. Destruction times of the used pathogens were not determined. Dysentery bacteria dried in fabric pieces died after 19 days in sweepings, vibrio cholerae were already inactive after 24 hours. If the waste contained coal ash and briquette ash typhoid bacteria would remain alive for 115 days, paratyphus B bacteria for 136 days, dysentery bacteria for 48 days and pseudodysentery bacteria for 69 days. The respective destruction times in garbage consisting of kitchen waste were 4, 24, 5 and 20 days. Typhoid bacteria were still detectable after 44 days in the surroundings of fabric pieces infected with them. These data were tested and confirmed by another researcher in 1928 determining in addition that flies were able to collect typhoid bacteria from kitchen wastes. This leads to the conclusion that domestic and kitchen wastes as well as sweepings in the surroundings of humans always pose a threat, be it through direct transfer of infectious substances or through spreading of infectious pathogens through insects (STRAUCH [80]).

First composting plants for domestic waste

When after World War II the first composting plants for domestic waste were erected (e.g. in Baden-Baden, Bad Kreuznach, Heidelberg) the discussion about the health threats to humans and animals posed by domestic waste was revived. Even more so as municipal sewage sludge was used in these composting plants about which it was known that it contained in concentrated form all pathogens excreted by infected humans into the wastewater. In order to provide clarification from the viewpoint of human and veterinary hygiene, amongst others, the then Federal Ministry of the Interior issued research assignments to the working group for municipal waste management (Baden-Baden) and to the working group Gießen University Institutes for Waste Management. The results from the areas of human and veterinary hygiene are published in “Handbuch der Müll- und Abfallbeseitigung” (Erich Schmidt-Verlag, Berlin, Handbook of garbage and waste disposal) under index numbers 5000-5165 and 6618-6620 in the years 1964-1968. Special sanitary studies on the reutilization of solid and liquid municipal wastes were conducted using anthrax bacteria and their spores, Salmonella enteritidis, erysipelas bacteria *Erysipelothrix rhusiopathia*, and psittacosis pathogen. Furthermore, the international state of knowledge concerning questions on the sanitation of waste disposal was comprehensively presented (STRAUCH [80], [81]).

Introduction of separate collection

After composting of unsorted domestic waste was largely abandoned in Germany and instead the separate collection and composting of bio-waste was to be introduced nation-wide the discussion about the occurrence of pathogens in raw materials and compost was revived again. The major focus is put here on domestic kitchen wastes that might be contaminated with pathogens and thus pose an infection risk not only to humans but also to animals. It has to be noted in this context that compost is not always dug into the soil but is often only applied on the surface of the soil. Thus, dogs and cats can get in contact with it in gardens but also wild birds and rodents. If the compost still contains pathogens these animals are susceptible to infection when exposed to the material. That is why in addition to human hygiene also veterinary hygiene is interested in bio-waste compost being unobjectionable in terms of epidemic safety.

3.1.2 Bacteria, viruses, fungi and parasites as pathogens

Main source - bio-waste

The main source of infectious agents in bio-waste is currently seen in kitchen waste. This is confirmed by the results of studies on food hygiene. According to information by the Federal Institute for health-related consumer protection and veterinary science in Berlin (BgVV) a global increase in food-borne infections can be determined attributing the introduction of various germs to the global movement of goods and changed eating habits [34].

Bacteria, viruses, fungi and parasites as pathogens

Various

- bacteria,
- viruses,
- fungi and
- parasites

are listed. Among them are Salmonella, *E. coli*, Yersinia, Streptococcus, Staphylococcus, Enteroviruses, ascaris, *Aspergillus fumigatus.* In addition, enterococcus, pseudomonas, klebsiella, enterobacteriacea, Proteus, Serratia and Citrobacter species were detected in individual studies.

Salmonella as bacterial pathogens

The increasing infections of food of animal origin caused by salmonella play a not insignificant role. This concerns especially poultry and pork but also eggs and raw sausage products. Salmonella have also been detected in nearly 6 % of raw animal organs in dog food. Furthermore, salmonella have also been found in untreated dry products such as herbs, spices, teas, dried vegetables, dried mushrooms and asparagus. Additional sources of pathogens are also contaminated paper tissues, napkins and disposable towels from households where sick people live (Roth [71]).

Thus, it has to be expected that pathogens can always be present in bio-waste. This is also confirmed by own studies that detected 17 different serovars of salmonella (S. anatum, blockley, derby-5, enteritidis, gaminara, hadar, infantis, livingston, london, mbandaka, newport, orion var. 3, 15, saint-paul, schleissheim, thompson, typhimurium, virchow) in 43 of 58 examined samples from the bio-waste delivered to three composting plants, which is 74 % of raw material. Salmonella concentration was in the range of $2.0 \cdot 10^{-1}$ to $1.6 \cdot 10^{4}$ CFU/g DS (ROTH [71]).

Other sources

Other sources of pathogens are the faeces in the litter in pet cages that are disposed of in the bio-waste bin as well as dog and cat faeces that arrive in the bio-waste bin with lawn cuttings or directly at a bio-waste composting plant with park wastes. This situation might increase if, as proposed in e.g. Switzerland, baby diapers were also approved as bio-waste and composted. This would expand the range of pathogenic microorganisms by the germs of infant infections excreted through the intestinal system.

Viral pathogens

Beside bacterial pathogens, viral pathogens that can reach bio-waste through food also play a role. Out of the specifically human pathogens the following have been detected in particular in milk, butter, cheese, meat, fish, oysters and clams: poliomyelitis virus, hepatitis A virus, coxsackie and ECHO viruses, reovirus, adenovirus. Viruses also enter a household and thus the bio-waste via clinically ill or inapparently infected members of family, visitors and pets who might carry or excrete viruses. Entero, reo, rota, adeno or influenza viruses have to be expected almost constantly, often also rhino, orthomyxo and paramyxo viruses as well as herpes viruses. Calici and retro viruses are of minor importance (see Appendix 11 B).

Own studies detected for the first time the occurrence of viruses in the air of three bio-waste composting plants. These were entero viruses of the Picornaviridae family: ECHO 7 and 11, coxsackie B 4, B 5 and B6. In addition, herpes simplex viruses were detected in the delivery area of two of the three bio-waste composting plants. Even if all isolated viruses came from the air of different workstations of the composting plant it can be assumed that they had arrived in the composting plants primarily with the delivered bio-waste and had reached the air surrounding the examined workstations with dust particles in the course of the individual process steps (PFIRRMANN [67], [68]).

Fungi as pathogens

Another group of microorganisms that play a role in composting are fungi. Their main task in the ecosystem is the decomposition of biologically inactive material in interaction with other microorganisms. There is an extraordinarily high number of fungi types that occur both in bio-waste and in the composting process. A prominent example of these is *Aspergillus fumigatus* that on the one hand is of importance also in composting and subsequently in the soil for the decomposition of organic material but that is on the other hand accused of being the worst pathogenic microorganism for the personnel of composting plants and the residents of the surrounding areas of composting plants.

In connection with the public discussion triggered by the former Federal Public Health Office under the motto of "Threats from the bio-waste bin?" (13.11.1991), this lead to the question being raised about involving a mycological monitoring of the composting process up to the final product conducted by a representative of clinical mycology. The distance of the lab researcher from the daily practice of waste and environment microbiology even lead to the assertion that the press release by the Public Health Office of 13.11.1991 had "shocked especially waste microbiologists", which was definitely not the case as they had decades of practical experience and a huge amount of research material and did not have to refer to single findings like the initiator of the press release of the Public Health Office.

The Federal Public Health Office itself pointed out in its "explanations" of 06.12.1992 concerning the above mentioned press release that "*the fungi type mentioned in the press release, Aspergillus fumigatus, can be detected in all composts, amongst others, independently from the source material but in different germ density and depending on the composting stage. Consequently, A. fumigatus is widespread in our environment which explains the possible occurrence of such spores in inhalation air".* This quite correct understanding of the Public Health Office is also the reason for the fact that the omnipresent fungi and their spores are not taken into account in the assessment of newly developed or already operated composting processes in terms of epidemic safety because it is long known that it is impossible to produce compost free of fungi, unless it is sterilized after the composting process is finished. Apart from that, Robert Koch Institute points out in its press service edition 19/95 of 19.07.1995 that the discussion about the bio-waste bin as a source of scattering of mould fungus spores and thus as health risk for severely immunocompromised patients often overlooks "*that the normal household offers much more sources of fungi that might lead to considerably higher exposure as is to be expected when opening the bio-waste bin*".

The composting of municipal waste dates back to almost 1,000 BC when the waste of the city of Jerusalem was collected at a spot in the Valley of Hinnom. The organic material was composted; the inorganic part was incinerated in a permanent fire (ERHARD in [32] and [33]). Since that time and probably also before humans have dealt with composted material. Even medicinal mycologists point out that "*the task of fungi in the natural ecosystem is to decompose biologically inactive or dead material in interaction with other microorganisms, to compost it. Each of the many fungi types is able and specialized to decompose various materials of plants, animals and humans due to special enzyme compositions*". This scientifically underpinned insight then raises the question why the so helpful microorganisms should be killed during the composting process if the composted material is subsequently returned again to the soil in which the fungis, that were previously destroyed with high technological and financial efforts, are already present in high concentrations. As the same fungi also occur in considerable amounts in the ambient air, in street dust, in the forest and thus virtually everywhere the compost would be repopulated by fungi during its storage due to external contamination even if all fungi in the compost were destroyed, which would re-establish the original condition.

Measures against infections

The well-known Italian compost research group also agrees with this and opposes the complete sterilization of compost as it is not economic and, very importantly, as the elimination of the competitive potential of the compost microflora would foster the reinfection of the compost through

external contamination and, linked to this, the excessive growth of pathogens, such as salmonella, in the initially decontaminated material. Furthermore, the Italian colleagues stress the fact that the soil as the almost invariable destination of compost products also contains certain pathogens such as *Clostridium tetani*, *Cl. botulinum*, *Aspergillus fumigatus* making the attempt of eliminating pathogenic microorganisms in the compost that occur naturally in the soil a superfluous undertaking and a waste of money (DE BERTOLDI et al. [9]). That is why it seems to be unnecessary to routinely include representatives of various fungus genera as test germs in the assessment of composting technologies in terms of epidemic safety as this would only confirm the old finding that these fungi are still present in the well-rotted compost.

Here the suggestion of the Federal Public Health Office takes effect that calls for the population to be informed about the fact that the content of the bio-waste bin might become the infection source of immunocompromised people and that for this reason this group of people – in contrast to healthy people – has to be especially careful in the handling of bio-waste, be it the bio-waste bin or the compost heap. Furthermore, it should be pointed out in general to not put up bio-waste bins and home composters in the living area (e.g. kitchen, balcony, basements).

Apart from that, the explanations in the reference book “Mykologie” (mycology) should be noted that read in the section “Pathogenese” (pathogenesis), among others: „*The development of almost all mycoses is probably fostered by predisposing factors … The following conditions are worth considering as predisposing underlying diseases: hypo- and agammaglobulinemia, Diabetes mellitus, long-term treatment with broad-spectrum antibiotics and other chemotherapeutics, immune-suppressive treatment of allergies, asthma, organ transplantations etc. and corticosteroid therapy in general, contraceptives, consuming diseases such as tuberculosis, alcoholism and other weak conditions. The typical infection occurs through inhaling germs with subsequent colonization of bronchial tubes or lung tissue …. The vast majority of pathogens do not find conditions suitable for them, even if they reach the “right” spot of a host that is receptive in principle. Either germ count is insufficient for infection (single germs are sufficient only in case of coccidioides) or the germs fall prey to the general infection defence (cellular defence, ciliated epithelium); infections then are abortive or silent, they “do not start”. Later, immune reactions prevent persisting infections from spreading. But still, humans and animals contract mycoses. The reason must be that mechanisms of resistance or immunity of the host have failed or were “overridden” by the pathogen. Opportunities for infection are probably existent almost constantly so that every human could contract Candida albicans, Aspergillus fumigatus or a pathogenic representative of mucoraceae several times a day. In the frame of these permanent contacts even an otherwise healthy person can acquire a mycosis at some point but it would probably progress inconspicuously, subclinically and add to the development of the person’s immunoreactivity”* (MÜLLER und LÖFFLER [28]).

Summing up it can be noted that most of the pathogens in bio-waste originate from the households themselves with kitchen waste generally playing the leading role. From the viewpoint of veterinary science it thus has to be ensured in the composting of bio-waste that possibly existing pathogens are inactivated or reduced in numbers to such a degree that they can cause no infections anymore.

3.2 Problems of sanitation in the collection and removal of bio-waste

3.2.1 System bio-waste bin

Collection containers in living area

Bio-waste should be collected in households in special, tightly closing containers that are often emptied into the bio-waste bin if possible. These collection containers in the living area should be thoroughly cleaned after each emptying.

Designs of bio-waste bins

Bio-waste bins should be put up outside in the shade if possible. In general, bio-waste containers are placed at special waste collection locations within apartment building areas. Bio-waste bins and containers have to close tightly to prevent contamination with vermin and harmful organisms.

The attempt has been made to minimize the disadvantages of the standard municipal waste bin for storing bio-waste (anaerobic processes, bad smells, leachates) by changing the constructional design of the bio-waste bins. A bin with a mesh grid shelf, openings for aeration and ventilation in the side walls and in the lid, and bars in the interior has proved most successful. The authors conclude from this that the bio-waste should be stored until removal in such a way that the same decomposition processes can start already in the bins that are desired in the subsequent composting. Another advantage is said to be the low bad smell during the dumping periods until collection and during the emptying of the bin into the collection vehicle (KOWALD und MÜLLER [52]). But what seems to be very important here is to keep the gaps of the mesh grid at < 6 mm (mice) or < 12mm (rats) in order to avoid making the fact that the bio-waste attracts the mentioned rodents a partial problem in the area where the bio-waste bins are located. Furthermore, it has to be expected that an increase in the aired surface inside the bin might lead to changes in the bioaerosol emissions discharged from the bin.

Dumping periods until collection of bio-waste bins

The issue of the dumping periods until the collection of bio-waste bins has been discussed fiercely for years. On 17 June 1992 an expert talk on questions of the sanitation of bio-waste bins took place at the then Federal Public Health Office during which also these dumping periods were addressed. The Public Health Office favoured a weekly collection rhythm while numerous other participants thought a collection with an interval of two weeks sufficient due to research results and economic considerations (LUKASSOWITZ [54]). Comprehensive and very thorough studies taking into consideration aspects of bacteriology, insect contamination and odour nuisance came to the result that "*the attracting effect of waste containers on insects and the development of insects in waste containers, the microbial contamination and the odour development in the waste bins do not necessitate a weekly collection of the different waste types*" (SCHERER [76]).

Since that time further studies on this topic have been conducted by different workgroups that in part also generated results that contradicted each other. That is why the Federal Public Health Office

hosted again a work meeting in Berlin on 07.11.1995. 8 short presentations were given there as introduction to the topic areas of

- Collection of bio-waste and residual waste in dependence from territory structure, collection intervals and composition of waste,
- Problems of odours and sanitation,
- Influence of bin design,
- Health-related risks from flies, rats and nuisance pests,
- Home composting.

The subsequent, very lively discussion was subdivided into the areas of

- Importance of bacteria,
- Importance of fungi,
- Importance of vermin and nuisance pests,
- Recommendations for immunocompromised people,
- Acceptance problems due to odour nuisance and the occurrence of maggots, technical solutions.

Bacteria in bio-waste bin

There is agreement to a large extent that the occurrence of bacteria in the waste does not pose a health risk to the users of bio-waste bins, especially since the pathogens existing in the waste previously passed through the household. But in order to make final reliable statements statistical data or epidemiological studies are still missing. The situation is slightly different in the case of garbage collectors as there have been individual cases in which certain symptoms were determined that could generally be traced back to bacterial endotoxins, as well as a significantly increased sensitization against thermophilic actinomycetes. Additional research of this issue is required.

Fungi in bio-waste bin

The importance of fungi as a threat to human health has been discussed very controversially, particularly as a case study revealed that apparently fungal infestation of living spaces also is of importance, though not quantifiable yet. Increasing the collection intervals from 7 to 14 days did not seem to significantly increase the concentration of mould fungus spores when opening the bio-waste bin.

Vermin and nuisance pests in bio-waste bin

The issue of vermin and nuisance pests was also intensively addressed but without leading to a uniform opinion especially since some debaters referred to contradictory results and/or alleged short-comings of the methodological approach. But apparently this issue seems to be connected to the territory structure. In areas of single-family or two-family houses fewer complaints were registered than in areas with apartment buildings let alone in commercial areas. It also seems that the users of bio-waste bins exhibit a learning effect as the problem decreased with longer usage,

which also might be explained with critical waste being increasingly disposed of into the residual waste bin.

Concerning rats there was general agreement on making a distinction between bio-waste bin and home composting. It is important that bio-waste bins are always tightly closed which is not possible in the case of outside home composting in compost heaps.

The discussion of the topic of immunocompromised people did not provide any new insights. There was agreement on continuing recommendations that people with weakened immune status should avoid any contact with bio-waste bins. But it was also pointed out that the collection containers for bio-waste in households and the bio-waste bins are only one of many possible sources of scattering of mould fungi. It was stressed as important that affected people were specifically advised by doctors. But in this context the question has to be allowed about what has been done so far by the responsible institutions to inform the medical profession about this problem area and to provide them with relevant information materials.

Problem: acceptance

The acceptance problem of the bio-waste bin due to odour nuisance and the occurrence of maggots was also discussed very controversially. Sufficient knowledge of the molecules involved in the odour formation were lacking and thus of their effects and possible toxicity. So far there have been no indications supporting the notion that the emission of odorous substances from bio-waste is connected to a health-related risk to the users of bio-waste bins. On the other hand, revulsion-provoking reactions culminating in nausea and hysterical behaviour were reported to have been observed.

Practical implementation

Well-closable containers were favoured as technical solutions for the collection containers in households and the bio-waste bin. Container volume was to be sufficiently high to avoid over-filling and thus lids that stand open. In order to help avoiding anaerobic conditions structural materials are to be used (green waste and garden waste) as well as screwed-up paper and newspapers but not glossy paper. The bio-waste bins should not be exposed to solar radiation to avoid temperature increase inside the bin. One workgroup also recommended a bio-waste bin with a biofilter installed in the lid whereas other participants were of the opinion that the same effect could also be achieved by using a tightly closed bin. Furthermore, the addition of slaked lime, possibly also mixed with bentonite, was recommended to decrease odour formation.

3.2.2 Collection of bio-waste

Emptying

There was no agreement on the topic of regular cleaning of the bio-waste bin with especially the issue of costs playing a role. Also no agreement was reached between the proponents of a 7-day or 14-day collection rhythm respectively although figures were presented that e.g. in the federal state

of North Rhine-Westphalia in almost 70% of all cases the 14-day collection was the norm. Concerning the federal state of Saarland one waste disposal company reported that already today only 60 % of bio-waste bin owners put their containers out regularly to be emptied while 40 % already practiced an interval that considerably exceeded 14 days. Among others, the suggestion was made to collect every 7 days during the summer months and every 14 days during the rest of the year. This was countered by the statement that a weekly collection would be up to 40 % more expensive than a biweekly one at which the chairman of the meeting pointed out that the costs of disposal were not the subject of the work meeting but that mainly aspects of sanitation should be considered in corresponding recommendations.

Occupational safety/ health protection

Bio-waste is collected in special purpose vehicles intended for this use. The employed personnel wear protection gear and work gloves that simultaneously provide a protective function against the direct contact with the waste bins and their contents. As far as it is possible the garbage collectors should clean their hands with soap during their breaks before eating, drinking, smoking, and should get the opportunity of thoroughly showering before putting on their own clothes after their shifts.

Odorous emissions

One city in the federal state of Baden-Württemberg experienced complaints from their residents about the foul smell coming from the collection vehicles during bio-waste collection in hot weather in the summer. Upon consultation with the vehicle manufacturer a technical solution could be found with which all collection vehicles were retrofitted after which the complaints stopped.

The detailed minutes of this work meeting can be requested from the Federal Public Health Office (HOFMANN and SZEWZYK [45]).

Bioburden emission

In the mean time studies were conducted that focused especially on aspects of occupational safety and the occupational exposure of garbage collectors during the collection of waste (BIDLINGMAIER et al. 1996; MARTENS et al. 1998; BECKER et al. 1999; NEUMANN and BALFANZ 1999; SENKPIEL et al. 1999). At this point it should be exemplary referred to the results of a study on bioburden emissions occurring during the collection of paper, bio-waste, residual waste and total waste that was conducted in a German metropolis (MARTENS et al., 1998; MARTENS et al., 1999). In this study, the concentrations of air-borne microorganisms, amongst others, were determined as they occur at the rear of the collection vehicle immediately in the area of bin emptying during the collection of the listed waste fractions.

In total the measured concentrations of air-borne microorganisms during the collection of waste paper were slightly (app. 1 power of 10) higher than the normal background concentrations. In contrast, higher immission values occurred during the collection of the fractions of total and residual waste and bio-waste with no indications of fundamental differences being recognizable between the three types of waste. There were indications that an increase of dumping periods before collection (implemented in the course of waste separation) might lead to a change in the spectrum of emitted

mould fungus spores, namely to an increase of spores of the thermotolerant *A. fumigatus*, probably due to a longer rotting period taking place due to self-heating of the material in the bins before collection. There were also indications of location-dependent differences (single-house areas versus high-density inner-city areas) that need further studies for clarification. Details on this are published elsewhere (LUKASSOWITZ [54]). The basic principle of these results was confirmed by other studies (BIDLINGMAIER et al., 1996; MARTENS et al., 1998; BECKER et al., 1999; NEUMANN and BALFANZ, 1999).

3.3 Issues of sanitation concerning animal by-products and catering waste in anaerobic treatment (biogas) and composting

The requirements are defined in APPENDIX V of Commission Regulation (EU) No 142/2011 from 25 February 2011 on the implementation of Regulation (EC) No 1069/2009 of the European Parliament and of the Council with sanitary requirements for certain animal by-products not intended for human consumption, and on the implementation of Directive 97/78/EC of the Council regarding certain samples and goods that are exempted from veterinary controls at the borders according to the mentioned directive. In addition, the "Animal By-Product Disposal Act" from 25 January 2004 (Federal Law Gazette I p. 82) and the "Animal By-Product Disposal Regulation" from 27 July 2006 (Federal Law Gazette I p. 1735) are to be observed.

Requirements for plants
Section 1
Biogas plants

1. A biogas plant must have an imperative pasteurization section for animal by-products or subsequent products that are fed into the plant with a particle size of 12 mm at the most before entering the plant while having the following installations available:
   a) monitoring devices that ensure that a temperature of 70 °C is guaranteed for one hour;
   b) recording devices for the continuous recording of the monitoring results stated in 1(a), and
   c) an appropriate system to prevent insufficient heating.
2. By derogation from Section 1 (1) a pasteurization section is not obligatory for biogas plants if they exclusively process the following materials:
   a) materials of category 2 that were processed following method 1 according to Appendix IV chapter III;
   b) materials of category 3 that were processed following one of the methods 1 to 5 or following method 7, or in case of materials from aquatic animals following one of the methods 1 to 7 according to Appendix IV Chapter III;
   c) materials of category 3 that were subjected to pasteurization in another approved plant;
   d) animal by-products that are permitted to be used as raw material without processing according to Article 13 (e)(ii) of Regulation (EC) No 1069/2009 and according to the present regulation;
   e) animal by-products that were subjected to alkaline hydrolysis according to Appendix IV Chapter IV Section 2 (A);

f) the following animal by-products as far as they are approved by the responsible authority:

i) animal by-products according to Article 10 (f) of Regulation (EU) No 1069/2009 that were subjected to processing according to Article 2 (1)(m) of Regulation (EU) No 852/2004 if they are intended for other purposes than human consumption;

ii) animal by-products according to Article 10 (g) of Regulation (EU) No 1069/2009 or

iii) animal by-products to be processed into biogas if fermentation residuals are subsequently composted, processed or disposed of according to the provisions of the present regulation.

3. If the biogas plant is located in or close to a facility that keeps livestock and if it processes not only manure, milk or colostrum of these animals the plants is to be erected with sufficient distance to the area where the animals are kept.
This distance is to be established in such a way that the biogas plant poses no unacceptable risk of transferring diseases infectious to humans or animals. In any case a complete physical separation of biogas plant and livestock as well as feed and litter has to be ensured, if necessary through a fence.

4. Every biogas plant must have an in-house laboratory available or make use of the services of an external laboratory. The laboratory must be equipped for conducting the necessary analyses and be approved by the responsible authority, accredited according to international standards, or subjected to regular controls conducted by the responsible authority.

Section 2
Composting plants

1. A composting plant must have an imperative closed composting reactor or area for the animal by-products or subsequent products to be fed while the following installations have to be available:
   a) devices for monitoring temperature;
   b) recording devices for – if necessary continuous – recording of the monitoring results stated in 1 (a);
   c) an appropriate system to prevent insufficient heating.
2. By derogation from Section 2 (1) other types of composting systems might be permitted as long as they
   a) are operated in such a way that the complete material in the system reaches the required parameters of time and temperature while ensuring the, if necessary continuous, monitoring of the parameters, or
   b) process exclusively material according to Section 1 (2), and
   c) fulfil all other relevant requirements of the present regulation.
3. If the composting plant is located in or close to a facility that keeps livestock and if it processes not only manure, milk or colostrum of these animals the plants is to be erected with sufficient distance to the area where the animals are kept.
This distance is to be established in such a way that the composting plant poses no unacceptable risk of transferring diseases infectious to humans or animals. In any case a complete physical separation of biogas plant and livestock as well as feed and litter has to be ensured, if necessary through a fence.

4. Every composting plant must have an in-house laboratory available or make use of the services of an external laboratory. The laboratory must be equipped for conducting the necessary analyses and be approved by the responsible authority, accredited according to international standards, or subjected to regular controls conducted by the responsible authority.

Chapter II

SANITARY REQUIREMENTS FOR BIOGAS AND COMPOSTING PLANTS

1. Animal by-products are to be processed as fast as possible after their delivery to the biogas or composting plant. They are to be properly stored until processing.
2. Containers, tanks and vehicles that deliver untreated materials have to be cleaned and disinfected at an accordingly designated area.
   This area has to be located and designed in such a way that any risk of contamination of treated products is prevented.
3. Based on a documented pest control plan, systematic preventative actions are to be taken against birds, rodents, insects and other pests.
   For this purpose a documented pest control programme is to be conducted.
4. Cleaning methods have to be specified and documented for all areas of the plant. Suitable cleaning tools and detergents are to be made available.
5. Sanitary controls have to include regular inspections of the work environment and the work equipment.
   Time schedules for these inspections and their results have to be documented.
6. Installations and equipment have to be kept in flawless condition and measuring devices are to be calibrated regularly.
7. Fermentation residuals and compost are to be handled and stored in the biogas or compost plant in such a way that recontamination is impossible.

Chapter III

CONVERSION PARAMETERS

Section 1

Standard conversion parameters

1. Materials of category 3 that are used as raw material in a biogas plant with pasteurization section have to fulfil the following minimal requirements:
   a) particle size before entry into pasteurization section: 12 mm at the most,
   b) minimum temperature of the entire material in pasteurization section: 70 °C, and
   c) minimum retention time in pasteurization section without interruption: 60 minutes.

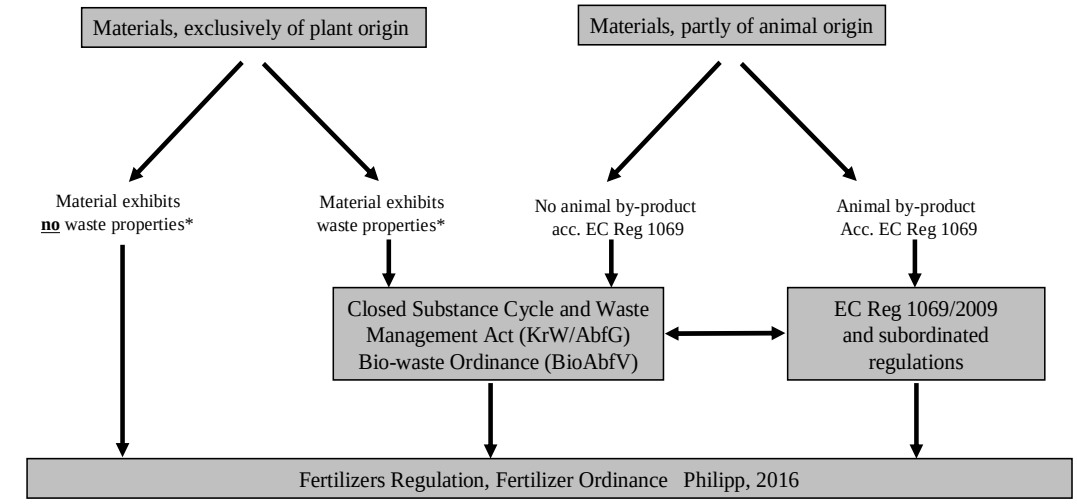
   Milk, milk-based products, products produced from milk, colostrum and colostrum products of category 3 can be used in a biogas plant without pasteurization if the responsible authorities are of the opinion that there is no risk of spreading severe diseases infectious to humans or animals.

   The minimum requirements listed in 1 (b) and 1 (c) also apply to materials of category 2 that are fed into a biogas plant without previous processing according to Article 13 (e)(ii) of Regulation (EC) No 1069/2009.

2. Materials of category 3 that are used as raw materials in composting plants have to fulfil the following minimum requirements:
    a) particle size before entry into composting reactor: 12 mm at the most,
    b) minimum temperature of entire material in reactor: 70 °C, and
    c) minimum retention time without interruption: 60 minutes.

The minimum requirements listed in 2 (b) and 2 (c) also apply to materials of category 2 that are composted without previous processing according to Article 13 (e)(ii) of Regulation (EC) No 1069/2009. The following overviews present the national and European legal areas of the reutilization of various biogenic wastes (Fig. 1, 2 and 3).

Overview of legal areas of input materials



* waste properties according to Section 3(1) KrW/AbfG

Figure 1: Overview of legal divisions of various waste streams

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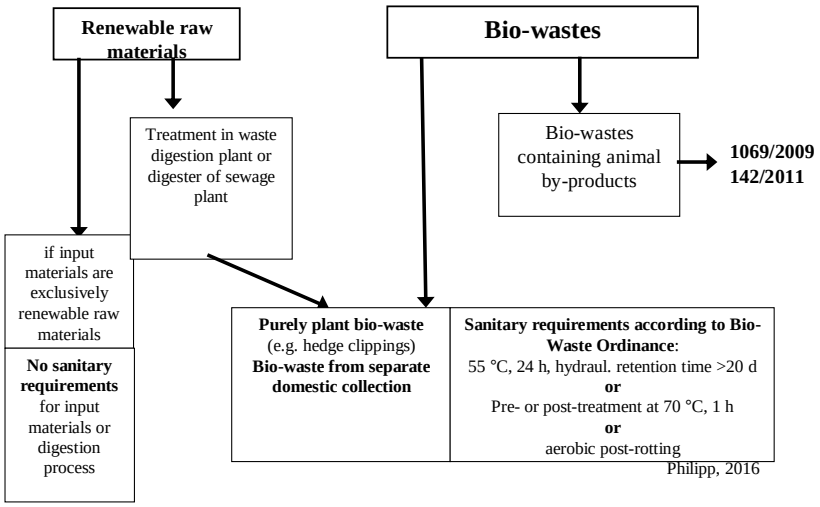
Overview of sanitary requirements of digestion and composting



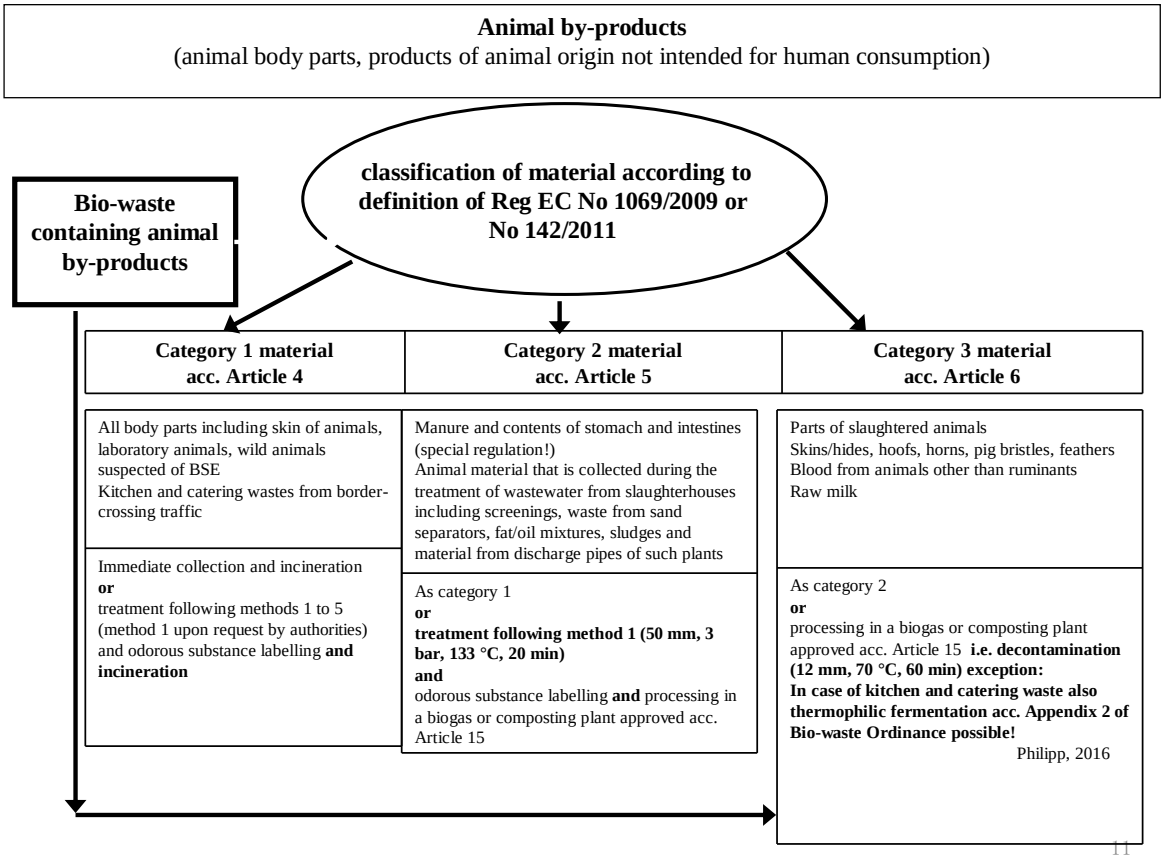


Figure 3: Classification of animal by-products

4 Issues of phytosanitation

Requirements for the use of compost as soil improver

The use of compost as soil improver or component of pot or container plants in gardening implies two preconditions for the control of plant diseases:

- The product should be free of pathogens, and
- Its use must not stimulate pathogens that are already present in the soil or in other substrata treated with compost.

Pathogens in compost

The occurrence of pathogens in compost depends on whether they are present in the source material and whether the sanitising processes during composting were effective. Populations of pathogens often occur in large numbers in old plant tissue where they enter dormant states to survive during the absence of vulnerable plants. They reach the composting system in large numbers if harvest residues constitute the main component of the raw material. Harvest residues contain remains of field and greenhouse crops as well as fruit and vegetable remains from the organic fraction of domestic and garden wastes.

Separate collection

Due to the current trend of collecting organic waste separately from other domestic wastes the amount of compostable material increases in many countries und thus also the possible amount of phytopathogens (BOLLEN and VOLKER [19]). Due to the introduction of separate bio-waste collection and treatment the following substances of plant origin can be found in the organic waste depending on the season:

- lawn cuttings, tree and bush prunings,
- flowers, windfall, rootstalks, foliage, fir sprigs,
- fruit and vegetable waste.

Pathogens of plant diseases and plant pests can occur in all of these waste types (HERMANN et al., 1994 [43]). This problem area attracted comparatively little interest in former discussions on the composting of domestic waste while aspects of epidemic safety were at the centre of attention.

4.1 Pathogens in materials of plant origin

Similar to epidemiological pathogens there are different groups of harmful organisms also in the area of plants (MENKE [62], [19])

- bacteria,
- viruses,
- fungi,

- animal pests (nematodes) and
- weeds

Bacteria

Bacteria as phytopathogens can cause a number of plant diseases with the infection predominantly occurring from the soil. However, they are unable to produce spores like some bacteria pathogenic to humans and animals can. That is why they are more sensitive to external influences. Previous studies on the behaviour of phytopathogenic bacteria in composting revealed that this process effectively sanitises the wastes of plant origin that were infected by these bacteria. *Erwinia amylovora*, the pathogen of fire blight in fruit trees and ornamental plants, was destroyed if infected saplings were subjected to a composting process of 7 days at 40 °C or more but not at lower temperatures.

Two other bacteria were even less resistant, e.g. *Erwinia carotovora* var. *chrysantemi* and *Pseudomonas phaselicola* in chrysanthemum saplings or bean leaves. Anaerobic fermentation of plant material, too, has a negative effect on the survival of pathogens. *Clavibacter michiganense,* the pathogen of bacterial canker in tomatoes, was destroyed in an anaerobic reactor with tomato wastes at 35 °C. The available data makes it highly unlikely that properly produced compost is infested with bacterial phytopathogens (BOLLEN and VOLKER [19], [62]).

Viruses

Viruses infect not only humans and animals but also plants. Their reproduction can only happen in living cells. About one sixth of plant viruses occur in the soil. They infect plants through the roots, bulbs or shoots. Viruses occur in most horticulturally or agriculturally used soils. Even in plant nurseries where more attention is given to plant health than in production facilities the soils are often infected. In case of some heat-resistant plant viruses the literature data on their resistance during composting is contradictory. Tobacco mosaic virus (TMV) was detected in compost produced from remains of infected tobacco plants although composting had been conducted for six weeks at 50 – 70 °C. Extracts from compost very substantially reduced the infectiousness of TMV without, however, causing complete inactivation. On the other hand, other researchers found that TMV had lost its infectiousness completely in compost heaps consisting of a mixture of bio-waste and wood shavings even in samples that had been subjected to maximum temperatures lower than 65 °C (HERMANN et al., 1994 [43]). The infectiousness of the virus was studied in experimental plants. But the authors stressed the fact that it might have been a case of reversible inactivation (adsorption without destroying the virus molecule) instead of an irreversible inactivation through destruction of the virus. There are indications of the occurrence of reversible inactivation of TMV from other sources (BOLLEN and VOLKER [19]).

Fungi

In studies on sanitisation fungi have been given more attention than other pathogens. Of special interest in terms of composting are such species that are able to survive unfavourable environmental conditions through production of resistant resting spores or also so-called sclerotia. Most fungal pathogens become inactivated quickly. This is also true for sclerotia-producing fungi in dormant

states, such as e.g. *Sclerotium rolfsii*, *Sclerotinia trifoliorum, Verticillium dahliae* and *Sclerotium cepivorum* or *slerotiorum*. Despite their known resistance to unfavourable conditions and their longevity in the soil they are unable to endure temperatures as they prevail in composting during high-temperature stages (BOLLEN and VOLKER [19]).

Biotrophic fungi, that produce thick-walled resting spores, are less easily eliminated. They are obligate root-infesting parasites. The resting spores are resistant against drying up and heat and they survive in the soil for many years. In general, temperatures of at least 60 °C at sufficient humidity should prevail for several hours during composting in order to kill off the spores. One example is *Olpidium brassicae*, which is the viral vector of lettuce and other harvested products. Phytosanitary studies have put a lot of attention on the control of *Plasmodiophora brassicae* as this pathogen poses a serious threat to the production of cabbage because compost is used on cabbage fields. Literature data on this issue are quite contradictory. Extremes were survival after composting lasting for 3 weeks at 65 °C and complete eradication after 24 hours in compost at 54 °C. Obviously, other factors beside temperature play a role in the eradication of this fungus. Information on the behaviour during composting of other fungi that produce resting spores is urgently needed. Fungi are also highly sensitive to impacts of anaerobic fermentation as could be demonstrated for *Fusarium oxysporum f. sp. Dianthi* (pathogen of wilt disease in carnations) and *Sclerotium cepivorum* (white rot in onions). It would be important to know if this is also true for biotrophic parasites that produce thick-walled resting spores (BOLLEN and VOLKER [19]).

Nematodes

Nematodes (roundworms) are an animal pest; some of them have a huge range of host plants while others are specialized in only one plant species. Especially those nematodes that form so-called cysts are of great importance here. These cysts are females that are transformed into resting forms. They are filled with packages of eggs or larvae depending on species. These cysts can survive in the soil for many years. If favourable conditions are available, i.e. if host plants are growing nearby, these larvae are induced to hatch and migrate to the roots of the host plants that they subsequently infest (MENKE [62]). The survival data of nematodes during composting reveals that they are sensitive to it. This is also true for those cyst-forming species and root-knot nematodes that are more resistant than most other nematodes to unfavourable conditions in the soil, such as drying up and chemicals. During tests of a highly effective composting method for domestic wastes root-knot nematode *Meloidogyne incognita var. acrita* in tomatoes was killed off in all of the eight conducted experiments. The same was found for *M. incognita* in bell pepper during composting of kitchen and garden wastes. Nematodes are more sensitive to high temperatures than most other phytopathogens. Although only few studies were conducted on the composting of infected crop residues the results support the conclusion that properly produced compost including a stage of high temperature and post-composing is free of phytopathogenic nematodes (BOLLEN and VOLKER [19]).

Weeds

Weeds are not phytophatogens in the narrow sense but they are in competition with cultivated plants for nutrients, light, water and others and are thus undesired in cultivations. Weed seeds remain germinable in the soil for several years. If a garden is dug over or a field is ploughed up these

seeds are brought back to the surface and can germinate subsequently. Such weed seeds can also be present in bio-waste in certain seasons (MENKE [62]).

4.2 Treatment in composting plants

Principle

The inactivation and destruction of pathogens of plant diseases during composting is brought about by various factors:

1. the high temperatures occurring during the first rotting phase,
2. the toxicity of decomposition products,
3. enzymatic degradation and
4. microbial antagonism.

Temperature ranges

It is stated as conclusion that the temperatures occurring during the first aerobic composting phase of crop residues and domestic bio-waste exceed the limit necessary for killing of most pathogens. The temperature necessary for the complete elimination of a phytopathogen depends on the extent of pathogen occurrence.

Humidity content

The influence of material humidity on the thermosensitivity of pathogens is also verified. Increased resistance under dry conditions is the general rule and has been verified several times. The occurrence of dry spots in the compost material is probably the main reason for the survival of pathogens in those compost piles and heaps that were expected to have eliminated all pathogens due to the achieved temperatures. A minimum humidity content of 40 % is thus recommended.

Toxicity of decomposition products

There is only insufficient data on the interactions between heat resistance and pH value of the substrate. The destruction or the loss of infectiousness of phytopathogens in composts whose production did not include the necessary thermal conditions is attributed to the activity of toxic conversion products or the direct decomposition of pathogenic structures by microflora. Evidence for the involvement of toxic compositions is mainly deduced from observations of decomposition under anaerobic conditions.

Enzymatic degradation

Enzymatic degradation is possibly one of the mechanisms for inactivating viruses as they are exposed to the proteolytic activity of the microflora during the decomposition of plant residues. Data on the vulnerability of virus proteins to the conditions prevailing during composting are still missing. There

are indications regarding the tobacco mosaic virus that its decomposition is accompanied by high microbial activity.

Microbial antagonism

Microbial antagonism, too, seems to be a major factor of the pathogen-suppressing properties of mature compost. An increased level of fungistasis as the consequence of the competition between pathogens and compost microflora is described as the main effect, while mycoparasitism and amensalism due to the formation of antifungal substances are involved to a lesser degree. The authors draw the conclusion that genuine evidence of the role of an often claimed microbial antagonism that contributes to the decomposition of phytopathogens during composting has not been given so far (BOLLEN and VOLKER [19]).

Summary

Summing up it can be noted that the vast majority of phytopathogens that have been studied so far do not survive the conditions prevailing during the composting of plant residues, even if some few survive undamaged. The phytopathogens that are critical in this regard include heat-resistant viruses, some biotrophic fungi, and few strains of *Fusarium oxysporum*. Among the factors involved in the inactivating of phytopathogens temperature is regarded as the main factor in aerobic composting and the produced toxic substances in the anaerobic decomposition. Although toxic products contribute to the killing of pathogens in composting, temperature is the best parameter for assessing sanitisation as temperature can be monitored much more easily than the formation of toxic metabolites. The current methods of taking samples and determining phytopathogens in compost do not allow a reliable assessment of the pathogen infestation in the end product compost. The currently best suited way is to specify the conditions that have to be fulfilled by the sanitisation of compost as it is done in the Bio-waste Ordinance. It will not be possible to completely eliminate every risk but the selection of the test organisms *P. brassicae*, tomatoe seeds, and tobacco mosaic virus covers most of the phytopathogens (BOLLEN and VOLKER [62], HERRMANN et al., 1994 [43], POLLMANN and STEINER [69]).

5 Bioburden emissions during composting

5.1 Level of knowledge in the previous century

Bioaerosols

After an accumulation of illnesses of workers probably caused by aerosols were registered in Denmark in the early 90s (MALMROS et al., 1992) [58] bioaerosols that are generated or discharged in connection with composting have also become the focus of national and international attention in recent years.

Statements on bioaerosol emissions

In January 1993, a group of experts was summoned in the USA by the Composting Council, the Environmental Protection Agency (EPA), the Department of Agriculture (USDA), and the National Institute for Occupational Safety and Health (NIOSH) who attempted to summarize the then level of knowledge in this field on the basis of 200 literature references and case studies. This study, published by now more than six years ago, that was concerned with aspects of occupational safety in connection with bioaerosol exposure at composting workplaces as well as the problem of associated immissions into the surrounding areas of composting plants, drew the following conclusions as its results (MILLNER et al., 1994) [64]:

1. The population is not put at risk by
   - systemic or
   - soft tissue infections

   as a result of bioaerosol emissions emanating from composting activities.
2. Immunocompromised individuals face an increased risk of infections from various opportunistic pathogens like *Aspergillus fumigatus* that occurs not only in compost but also in other self-heating organic materials in the natural environment.
3. Individuals with a disposition to asthma and allergies have an increased risk due to reactions to bioaerosols from various environmental influences and organic dust sources including compost.
   - *A. fumigatus* is not the only or even most important bioaerosol to be considered in the risk assessment for Organic Dust Toxic Syndrome (ODTS), Mucous Membrane Irritation (MMI) and Hypersensitivity Pneumonitis (HP), which can occur if individuals are exposed to organic dusts.
   - The sum of air-borne pathogens that sensitize and subsequently cause asthmatic or allergic incidents cannot be defined with the currently available information especially if the range of sensitivity variations of affected individuals, the numerous sources of allergens in the natural environment and the variety of components and bioaerosols are taken into account.

- Due to these factors the prospects of developing such a precise definition are currently limited.

4. Despite the fact that some bioaerosols might cause occupational allergies and diseases and that some of these bioaerosol types are present in the air of bio-waste composting plants, the available epidemiological data do not support the assumption that allergic, asthmatic, acute or chronic respiration diseases occur in the general population living at or close to the different open and in one case closed composting plant that were studied.

   - Consequently, the answer to the question from the beginning of the work meeting is: "Composting plants pose no extraordinary threats to the health and well-being of the public."
   - The most important basis for this conclusion is the fact that attention was paid to the workers as that part of society that is at the highest risk. In the cases where the workers' health had been assessed over time periods of up to ten years in composting plants, no significant health-related consequences were detected. Furthermore, studies of bioaerosols with regard to aerobic bacteria, thermophilic fungi and *A. fumigatus* conducted in residence areas surrounding composting plants revealed in most cases that the air-borne concentrations did not differ significantly from the background concentrations (without composting plant).
   - The fact that the bioaerosol level did not differ significantly from the background level can be reasonably explained by the fact that also the bacteria that naturally grow on organic substances decomposing through self-heating and subsequently aerosolize are spread far into the entire environment.

5. The occupational risks caused by aerosols in composting plants can be significant depending on the conditions that prevail in the plant, the areas of activities and their proximity.

   - The workers in composting plants are exposed to compost bioaerosol to a greater extent than the population in the surrounding area.
   - But as already established above the personnel of such plants did not exhibit significant differences regarding general and respiratory fitness in comparison to unaffected people. On the other hand, negative health-related influences were observed in some workers in mushroom cultivations and facilities that processed wood chips and tree bark.

   This suggests that future studies on occupational health should conduct systematic assessments of MMI, ODTS and HP and similar health problems at low chronic exposure situations, such as e.g. general exposures of 10^4 - 10^5 CFU/m^3 of air.

6. Due to continuing concerns in the public and due to the huge range of potential respiratory reactions to organic dusts, additional studies would be helpful to assess the obvious lack of negative health-related effects of composting plants.

   Two types of studies (epidemiological ones and those concerning nuisances) would be helpful in defining potential effects of bioaerosols from any source – composting or other.

   a) Epidemiological studies could help in determining the relations of dosage and effect.

If they are carefully planned and conducted they might be able to clearly document every negative health-related effect on the population in the surrounding areas of composting plants.

Such epidemiological studies are expensive and difficult and have not been conducted so far.

If they were conducted they had to include objective measuring methods like pulmonary function testing, serology of antigens of compost aerosols and microbial serotypes in the affected environment as well as complete medical histories of the affected people and other suitable measures to quantify irritant reactions to organic dusts.

b) Nuisance studies can be conducted much more easily.

They can provide useful information at much lower costs and have done so.

If they are carefully planned and are conducted in municipalities in the surrounding areas of composting plants, in connection with the determination of current exposure, these studies can serve to verify the nuisance in connection with the occurrence or lack of bioaerosols and other factors such as bad odours, irritations, feelings of discomfort, noise, visual concerns and traffic.

Methods for the assessment of nuisances are available and might be useful for assessing the effects on residents as they offer a systematic mechanism for documenting observations (olfactory or other), confirmations, correlations and interpretations.

Expanding nuisance studies by a limited number of objective measurements might be helpful in breaking down correlations into causes and effects.

Studies on the issue of bioaerosols

The need for research in this field as expressed in these statements was reflected in a number of studies in Germany and abroad that generated in part differing results but also did not provide fundamental answers to the addressed questions. Examples of studies on the problem of workstations come from Scandinavia (Malmros, 1995 [57]; Poulsen et al. 1995 [70]) and one corresponding study from Germany (Schappler-Scheele 1999 [75]). Regarding the problem of immissions, on which studies are currently conducted in the USA (Johanning 2000 [46]) as well as in Germany, the results of the studies of Gerbl-Riger et al. (1999) [36], Hessisches Umweltministerium (1999) [36], and Schilling et al. (1999) [83] serve as examples.

5.2 Aspects of occupational safety

Already before the turn of the century or millennium the level of knowledge on bioaerosol-caused health-related effects in the context of composting was published (Herr et al., 1999) [41].

Against the background of the various aspects of occupational safety the regulations that exist in Germany and are to be adhered to should be pointed out. The Biological Agents Ordinance turned the EU directive on the protection of workers from risks related to biological agents at work (90/6679

EEC) into national law, in the form of the Ordinance on Safety and Health Protection at Workplaces Involving Biological Agents (Biological Agents Ordinance – BioStoffV, 2013). It regulates measures for the protection of the safety and health of workers from threats posed by such activities. It further also regulates measures for the protection of other people who might be endangered by workers or by operators without personnel due to the latter's use of biological agents. The ordinance also applies to activities that are subjected to the regulation of genetic engineering as long as no equivalent or stricter regulations are available for the protection of workers.

Section 2 of Biological Agents Ordinance puts the focus on risk assessment.

In the context of risk assessment according to Article 5 of the Occupational Health and Safety Act, the employer has to assess the risk posed to the workers by activities involving biological agents before beginning these activities. The risk assessment has to be conducted by experts. If the employer does not have the necessary knowledge he is obligated to consult experts.

(2) The employer has to immediately update the risk assessment if

1. relevant changes of work conditions or new information, e.g. accident reports or insights from occupational-medical health examinations, make it necessary or

2. the assessment of the function and efficiency of protection measures revealed that the established protection measures are ineffective. Otherwise the employer has to audit the risk assessment every second year and update it if necessary. If the audit reveals that updating the risk assessment is not necessary the employer is obligated to record this under stating the date of the audit in the documentation according to Article 7.

(3) As part of risk assessment the employer has to identify the following in particular:

1. Identity, risk group classification and transfer paths of biological agents, their possible sensitizing and toxic effects and entry paths as far as this information is available to the employer; in doing so he also has to gather information on the question whether the biological agents might cause other effects that impair health,

2. Type of activity under consideration of operational procedures, work methods and used work equipment including operational facilities,

3. Type, duration and frequency of exposure of workers as far as this information is available to the employer,

4. Possibility of using biological agents, work methods or work equipment that would pose no or little risks to the workers (substitution assessment),

5. Activity-related insights a) on risk and exposure situations including psychological risks, b) on known diseases and countermeasures to be taken, c) from occupational health prevention.

(4) Based on the information determined according to section (3) the employer has to assess the infection risk and the risk posed by sensitizing, toxic or otherwise health-impairing effects independently from each other. These individual assessments are to be combined into an overall

assessment on the basis of which the protection measures have to be defined and implemented. This also applies if several biological agents occur simultaneous or are used in one activity.

In this context the subordinated Technical Regulations for Biological Agents (TRBA)

- 405 “Application of measuring methods for air-borne biological agents”,
- 430 “Procedures of determining mould fungus concentrations in the air at workstations”,
- 450 “Criteria for the classification of biological agents”,
- 460 “Classification of fungi into risk groups”,
- 462 “Classification of viruses into risk groups”,
- 464 “Classification of parasites into risk groups”

as well as TRBA 500 „General measures of sanitation: minimal requirements“ are to be observed.

A handbook of Bundesgütegemeinschaft Kompost (BGK, 2010), a German association concerned with quality assurance of compost, offers help to operators of composting plants (including composting of digestate) to understand the causes of the emissions of climate-relevant gases, to recognize risk factors for increased emissions, and to prevent such emissions with preventing measures and technical means as far as possible. The prevention of odorous emissions, which are given great importance in practical operation, and questions of occupational safety are addressed to a smaller degree. In future editions, fermentation methods are to be added and also emissions are to be included, of odours, germs, dust including prevention measures and limitations.

In January 2015, the Industrial Safety Regulation and the modification of the Ordinance of Hazardous Substances were passed in Germany coming into force on 1 June 2015.

The new Industrial Safety Regulation (BetrSichV) aims to improve occupational safety during the use of work equipment by workers and the protection of third parties during the operation of facilities in need of monitoring. Simultaneously, the new version will make the application of occupational safety regulations concerning work equipment easier for employers, especially of small and medium-sized enterprises (SME). For this purpose the Industrial Safety Regulation as it has been effective since 2002 is redesigned in terms of concept and structure. Additionally, duplicate provisions, among others, regarding the Ordinance on Hazardous Substances or regarding the new Federal Water Protection Regulations (AwSV) are eliminated in certain documentations and assessments. A conceptual and structural adjustment to other modern occupational safety regulations is done, especially the Ordinance on Hazardous Substances.

The new ordinance takes special accident black spots into account (maintenance, special operational statuses, operational disturbances, manipulations) and contains special requirements for age- and aging-appropriate arrangements.

It takes into consideration ergonomical and mental strains resulting from the use of work equipment. This takes into account the request of the Federal government to improve the employability of elder people.

Beside this

- the Ordinance of Workplaces (ArbStättV, especially articles 5 and 14), that is also based on the Working Conditions Act (ArbSchG, in its version of August 1996) and
- the Ordinance on Hazardous Substances (GefStoffV), and
- the subordinated Technical Rules of Hazardous Substances (TRGS)
    - 500 (protection measures – minimum standards),
    - 540 (sensitizing substances),
    - 907 (list of sensitizing substances) and
    - 908 (reasons of assessment of substances in TRGS 907).

are to be observed.

5.3 Measurement strategies for determining bioaerosol emissions from composting plants

In the following current questions regarding bioaerosol emissions from composting plants in the narrower sense will be addresses, i.e. questions in the context of immissions related to the composting plant into the surrounding areas of such plants might be of relevance. The presented level of knowledge is based on three research projects addressing this topic that were conducted in Germany for the first time. Results were presented in the course of a VDI/DIN status seminar in September 1999 in the German city of Langen (Hofmann et al. 1999 [44]). In the following some essential passages from these explanations are paraphrased and literally quoted in closing:

„The measurement strategies that are currently used in Germany for prognoses of the measurement of bioaerosol emissions and immissions exhibit in part considerable differences that include, beside the selection of collectors, also the selection of sample locations, the number of sample repetitions, the studied emission situation (worst case scenario versus average conditions) and other.

Distinct similarities can be seen regarding the parameters determined in current studies (on bioburden emission, author's note). Generally, the concentrations of mesophilic bacteria and mould fungus spores, of thermotolerant mould fungi, including *Aspergillus fumigatus*, and of thermophilic actinomycetes were determined. Among these, thermotolerant mould fungi, namely *A. fumigatus*, and the group of thermophilic actinomycetes seem to be suited indicators for emissions from composting plants.

These groups of microorganisms usually achieve only low concentrations in the unpolluted external air but occur frequently in composting plants, due to the selective advantage of the thermophilic rotting process. Some authors judge the detection of mesophilic mould fungi and bacteria to be far more unspecific with regard to emissions from composting plants.

Further activities were aimed to test different measurement strategies. The strategies have in common that measurements were conducted at several sampling locations leeward of the plant depending on the wind direction at the time of measurement. The sampling locations were situated at different distances from the plant in an angle in whose centre the main emission was to be

expected. One strategy included the taking of samples at all sampling locations simultaneously or in close temporal correspondents and synchronously with emission measurements in the plant with average situations in terms of meteorology and plant construction being studied. Another strategy chose the moment of measurement, with simultaneous sample taking, in such a way that the meteorological situations made maximum emission probable. At the same time the plant was operated in such a way that emissions also reached maximum values (worst case). This measurement strategy is well suited if the maximum burden is to be determined. It has the advantage that it can be conducted within a relatively short measurement campaign. But the measurement can only be conducted under defined meteorological conditions, and especially when using simultaneous measurements great efforts in terms of technology and personnel have to be realized short-term.

Other measurement strategies took samples in the course of a year under normal operating conditions and different atmospheric conditions (different spreading conditions) at all sampling locations in sufficient frequency. Thus, a more comprehensive picture of the different possible immission situations in the surrounding area of a plant is generated. In contrast to the strategy explained before a verified statement requires a huge number of measurement repetitions per sampling location.

All these studies employed filtration collectors to take samples, and sample preparation was conducted following TRBA 430. Therefore, the measured values determined in the studies can be compared to each other at least in sub-areas. Furthermore, comparison with measurement data on microorganisms from the field of occupational safety is possible that were also obtained with filtration collectors (following TRBA 430). It has to be noted that the results from comparative studies under laboratory conditions or from workstation studies can be transferred to the area of immissions only to a limited extent. Filtration collectors are additionally well suited for the verification of air-borne spores. Both thermotolerant mould fungi (including *A. fumigatus*) and thermophilic actinomycetes that have proven to be suitable indicators of bioaerosol immissions in the surroundings of composting plants in studies can be well verified using this method. By combining “direct” and “indirect” methods of sample taking the method can additionally be used for a wide range of concentrations.

Despite the mentioned advantages, which unambiguously speak in favour of the use of filtration methods, there are also situations and problems in which other measuring methods or collectors are better suited to achieve certain measurement goals. For example, impingers might achieve higher yields with some microbiological measurement parameters (especially vegetative forms of microorganisms), and the devices can also be employed with relatively high air humidity. The use of multi-stage impactors allows the fractioning of samples according to aerodynamic particle size.

The comparative assessment of these and other studies reveals on the one hand similarities, on the other hand also differences. In this regard, the parameters of gram-negative bacteria and mesophilic bacteria consistently proved as suited indicators only in individual cases. The data on the distances at which no increased concentrations of spores of mould fungus *A. fumigatus* compared to “standard values” were established vary between 90 meters and more than 1 kilometre (Millner et al. 1977 [63]; ERCO 1980 [31]; Lundholm and Rylander 1980 [55]; Kothary and Chase 1984 [51]; Boutin et al. 1987 [20]; Gerbl-Rieger et al. 1999 [36]; Hessisches Umweltministerium (Ed.) 1999 [83]).

This shows that general statements on the immissions from composting plants cannot be made as all studies consistently came to the result that an assessment of each individual case is necessary that takes into account the concrete topographical conditions, the meteorological frame conditions and the plant concept of each respective plant. On top of that, it can be said that there are no uniform measuring strategies even in the studies conducted in the recent past in Germany. The development of different approaches is feasible here as e.g. annual means as well as worst-case scenarios each offer statements that are qualitatively different.

If the three German studies presented here are compared in this context the most important similarity of the results is that in all cases a distinct concentration profile depending on the distance became evident for important parameters (indicator groups). The values measured on the leeward side of the plants were highly dependent from distance.

All three studies determined distinctly increased measured values at a distance of 200 m (Gerbl-Rieger et al. 1999 [36]; Hessisches Umweltministerium (Ed.) 1999 [83]; Schilling et al. 1999 [77]). A low but still existent influence from the plant could be observed in some cases at a distance of 500 m. At this distance, as already with the values at 200 m distance, there were important differences regarding the height of measured values depending on the fact whether open or closed (enclosed plants) composting plants had been studied. While an influence from the plant was detected almost everywhere in case of open plants this was often not the case with closed plants. The concentration values measured at a distance of 500 m from the plants were additionally strongly dependent from the meteorological frame conditions and the local topography.

At distances greater than 500 m an influence from the plant could only be determined if optimal conditions for spreading (worst case) were prevailing. There is no information on the frequency of such spreading situations. A special problem that has to be mentioned is that with increasing distance the probability of measured values being influenced by other emission sources also increases. Here, the unambiguous identification of sources is necessary.

In order to assign bioaerosol immissions to their respective source, emission measurements in close temporal agreement are necessary. Immission prognoses also can only be calculated if the source strength is known. When conducting emission measurements it has also to be noted that the different source types require different measurement strategies, especially when measuring (immissions) close to the source. There is still only little experience with measurements at emission sources. In general it can be established, however, that emission measurements should be conducted – as far as possible – following the guideline VDI 2066.

Furthermore, correctness and accuracy of the measured values are also determined by other influencing parameters. Daily and annual fluctuations of emission loads and background contamination, tenacity of microorganisms in the ambient air or collection stress during sample taking and transport of samples can be named as examples. As the presented methods exclusively measure living organisms and/or those capable of reproduction it has to be pointed out here that the working steps of cultivation and colony count, that result in the selection of certain organism groups, also pose a considerable influence on the measurement accuracy.

Guidelines that have been worked out in the meantime address standardized measurement strategies and detection methods. The following conditions have to be taken into account in this context:

- conditions of sample taking (meteorological situation (worst case – standard situation), number and position of sampling locations, method of sample taking, number of measurement repetitions, information on storage and transport of samples).
    - determining reference method
    - selection of measurement parameters (organism groups, organism components, dust, size fractioning)
    - sample preparation (media, cultivation duration, cultivation conditions, detection methods etc.)
    - evaluation of measured values (significance, statistical evaluation, performance characteristics etc.)

But there is still a need for research and development in the following areas:

- Comparison of different methods of taking emission and immission samples
- Optimization of methods of taking samples at emission sources, especially at diffuse emission sources
- Validation of measurement methods
- Development of measurement methods for determining total cell count (cultivable and non-cultivable microorganisms)
- Development of continuous measurement methods
- Automation of measurement method
- Assessment of influence of different biological parameters (e.g. different media with the same organism group, cultivation conditions, suitability of collection systems etc.) on the respective measured value
- Consideration of more modern methods (immunological and molecular-biological detection methods, possible suitability for bioaerosol detection)”

6 Issues of sanitation of anaerobic systems

Similar to composting, the anaerobic treatment of waste is also an area in which special attention has to be paid to the fact that the operational conditions in the treatment plants and the specific process-related effects can generate products that are safe in terms of epidemic and phytosanitary safety and suitable for unrestricted agricultural use.

Appendix 2 of Bio-waste Ordinance (BioAbfV) comprehensively presents the requirements of the sanitising treatment of bio-waste to guarantee epidemic and phytosanitary safety (see Appendix 2).

6.1 Requirements for anaerobic systems

Wastes for fermentation

Anaerobic treatment or fermentation of biological wastes is especially suited for wet wastes and wastes poor in structure (e.g. leftovers, food waste; waste from vegetable and fruit processing etc; lawn cuttings, foliage, hedge clippings).

Principle of decomposition

Anaerobic bacteria decompose all organic substances of plant or animal origin except lignin (wood) and produce methane. Little energy is left to the bacteria for metabolization and thus reproduction. That is why little surplus energy is released in the form of heat in fermentation unlike in composting. This basic precondition poses a problem in practice with regard to sanitation or epidemic safety. In order to achieve a successful decontamination effect through the factor “heat”, energy in temperature ranges above 50 °C has to be applied to warm or heat the fermenter. The energy produced by the methane gas (electricity and heat) is used for this.

Comprehensive studies have been conducted in recent years in the context of epidemic safety in the anaerobic treatment of different biological wastes (MARTENS et al., 1999 [60], 2000 [61]).

Process auditing has to be possible

The Bio-waste Ordinance dictates the same auditing modalities for anaerobic systems as for composting with especially the conduction of sanitation assessments in anaerobic systems being more complex (see *Chapter 2 Requirements of Bio-waste Ordinance (BioAbfV)*).

6.2 Examinations of epidemic safety in anaerobic systems

Importance of examinations of epidemic safety for approval

The majority of national and international studies were conducted in anaerobic systems used for the treatment of manure with or without co-fermentation by using bacteria. There are still only few results available concerning the behaviour of selected viruses. But especially with regard to issues of animal health conditions the focus is on the problem of eliminating relevant bacterial and viral animal pathogens in the fermentation of bio-waste and food waste as well as in the fermentation of manure and renewable resources. Only if confirmed data on this and insights on process design and control are available a special approval according to animal health law is acceptable for the reutilization of those substances in anaerobic systems that are subject to regulations on carcass disposal (MARTENS et al., 1999 [60], 2000 [61]).

Current studies established the tenacity of salmonella, listeria and ESBL *Escherichia coli* in laboratory fermenters (bio-wastes and corn silage) in mesophilic and thermophilic temperature ranges (Hälzle et al., 2015).

6.2.1 Results of studies at mesophilic conditions (40°C)

As presented in Table 1 and Figure 4 all of the three added test organisms were reduced by only one Log10 level within the first 12 h. After a retention time of 24 h in the laboratory fermenter reductions by one further Log10 level were determined for *S. Typhimurium* and *E. coli*; *L. monocytogenes* could be reduced by two further Log10 levels. In total, reductions of only about two Log10 levels were verified for *S. Typhimurium* and *E. coli* in the course of the 24 h long experiment, three Log10 levels for *L. monocytogenes*.

Table 1: Summary of results of mesophilic round in CFU/ml[1]

	Temperature	Test organism	Initial concentration	4 h	8 h	12 h	24 h
Fermenter 1	**40.4 °C**	*S. Typhimurium*	7.9×10^6	9.3×10^5	4.6×10^5	2.3×10^5	2.4×10^4
		L. monocytogenes	1.7×10^6	9.3×10^5	1.5×10^5	1.1×10^5	4.6×10^3
		E. coli	6.4×10^6	4.3×10^5	2.4×10^5	1.1×10^5	4.6×10^4
Fermenter 2	**40.4 °C**	*S. Typhimurium*	7.9×10^6	2.4×10^5	2.3×10^5	2.3×10^5	4.6×10^4
		L. monocytogenes	1.7×10^6	4.3×10^5	2.4×10^5	1.1×10^5	4.6×10^3
		E. coli	6.4×10^6	9.3×10^5	4.6×10^5	1.1×10^5	4.6×10^4

Figure 4: Summary of results of mesophilic round at 40 °C

[1] Colony-forming unit per ml of examined fermentation substrate

The results of this round of experiments revealed that even at retention times of 24 h in the fermenter no sanitisation (reduction of > 4 Log10 levels) was achieved. Due to this, spreading of pathogens into the digestate storage and, depending on the use of the digestate, into the environment or animal farming is to be expected. Furthermore, it has to be considered that continuous feeding of the fermenter leads to new pathogens being constantly brought into the fermenter thus causing the reduction to be even lower or to take longer.

6.2.2 Results of studies at low thermophilic conditions

In conducting this round of experiments the laboratory fermenters were operated at temperature ranges between 50 and 53 °C. The results of both rounds are presented in Tables 2 and 3 and Figures 5 and 6. Within the first four hours, a reduction of four Log10 levels was verified for *S. Typhimurium* and *L. monocytigenes*. After eight hours a further reduction of germ count by one Log10 level was determined for *S. Typhimurium*. During the experimental round at 51.4 °C the verifiable germ count of *S. Typhimurium* remained constant until the end of the experiment; at 52.4 °C a further reduction by one Log10 level was detected after twelve hours. Afterwards the germ count remained constant until the end of the experiment. The germ count of *L. monocytogenes* remained constant after 8 and 12 hours, only after 24 h a reduction by one Log10 level was determined at 51.4 °C and two Log10 levels at 52.4°C. *E. coli* was reduced by three to four Log10 levels within the first 4 hours. After 8 h a further reduction by one Log10 level was verified at 51.4 °C. The germ count remained constant after 12 h; at 52.4 °C a further reduction by one Log10 level was determined. After 24 h the germ count was reduced by a further Log10 level at both temperatures. In both experimental rounds all of the added pathogens were still detectable after 24 h.

Table 2: Summary of results of first round at low thermophilic conditions in CFU/ml[2]

	Temperature	Test organism	Initial concentration	4 h	8 h	12 h	24 h
Fermenter 1	**51.4 °C**	*S. Typhimurium*	5.7×10^6	3.6×10^2	1.1×10^1	2.3×10^1	2.4×10^1
		L. monocytogenes	1.3×10^6	4.6×10^2	2.3×10^2	2.1×10^2	4.6×10^1
		E. coli	9×10^6	3.6×10^3	4.6×10^2	3.6×10^2	2.3×10^1
Fermenter 2	**51.4 °C**	*S. Typhimurium*	5.7×10^6	2.1×10^2	1.1×10^1	3.6×10^1	4.6×10^1
		L. monocytogenes	1.3×10^6	4.3×10^2	3.6×10^2	3.6×10^2	4.6×10^1
		E. coli	9×10^6	1.1×10^4	9.2×10^2	1.1×10^2	2.3×10^1

[2] Colony-forming unit per ml of examined fermentation substrate

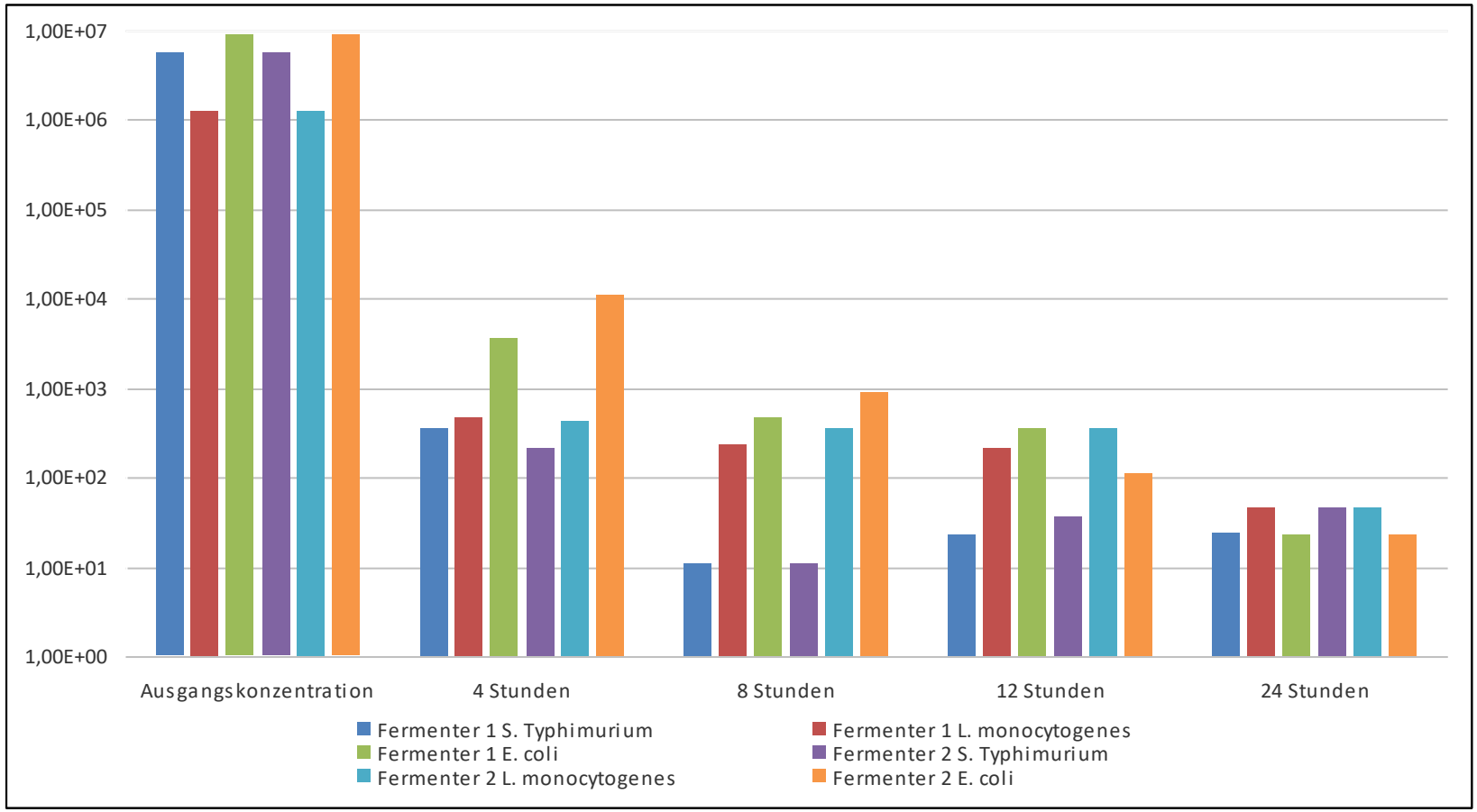


Figure 5: Summary of results of first round at low thermophilic conditions (51.4 °C)

Table 3: Summary of results of second round at low thermophilic conditions in CFU/ml

	Temperature	Test organism	Initial concentration	4 h	8 h	12 h	24 h
		S. Typhimurium	1.6×10^{7}	2.4×10^{2}	2.4×10^{1}	3.6×10^{0}	2.3×10^{0}
Fermenter 1	**52.4 °C**	*L. monocytogenes*	1×10^{7}	9.2×10^{2}	2.3×10^{2}	2.1×10^{2}	2.1×10^{0}
		E. coli	1.2×10^{7}	2.3×10^{3}	2.1×10^{2}	4.3×10^{1}	4.3×10^{0}
		S. Typhimurium	1.6×10^{7}	2.4×10^{2}	2.4×10^{1}	4.3×10^{0}	1.1×10^{0}
Fermenter 2	**52.4 °C**	*L. monocytogenes*	1×10^{7}	3.6×10^{2}	9.2×10^{1}	1.5×10^{2}	4.3×10^{0}
		E. coli	1.2×10^{7}	1.5×10^{4}	4.6×10^{2}	2.4×10^{1}	9.3×10^{0}

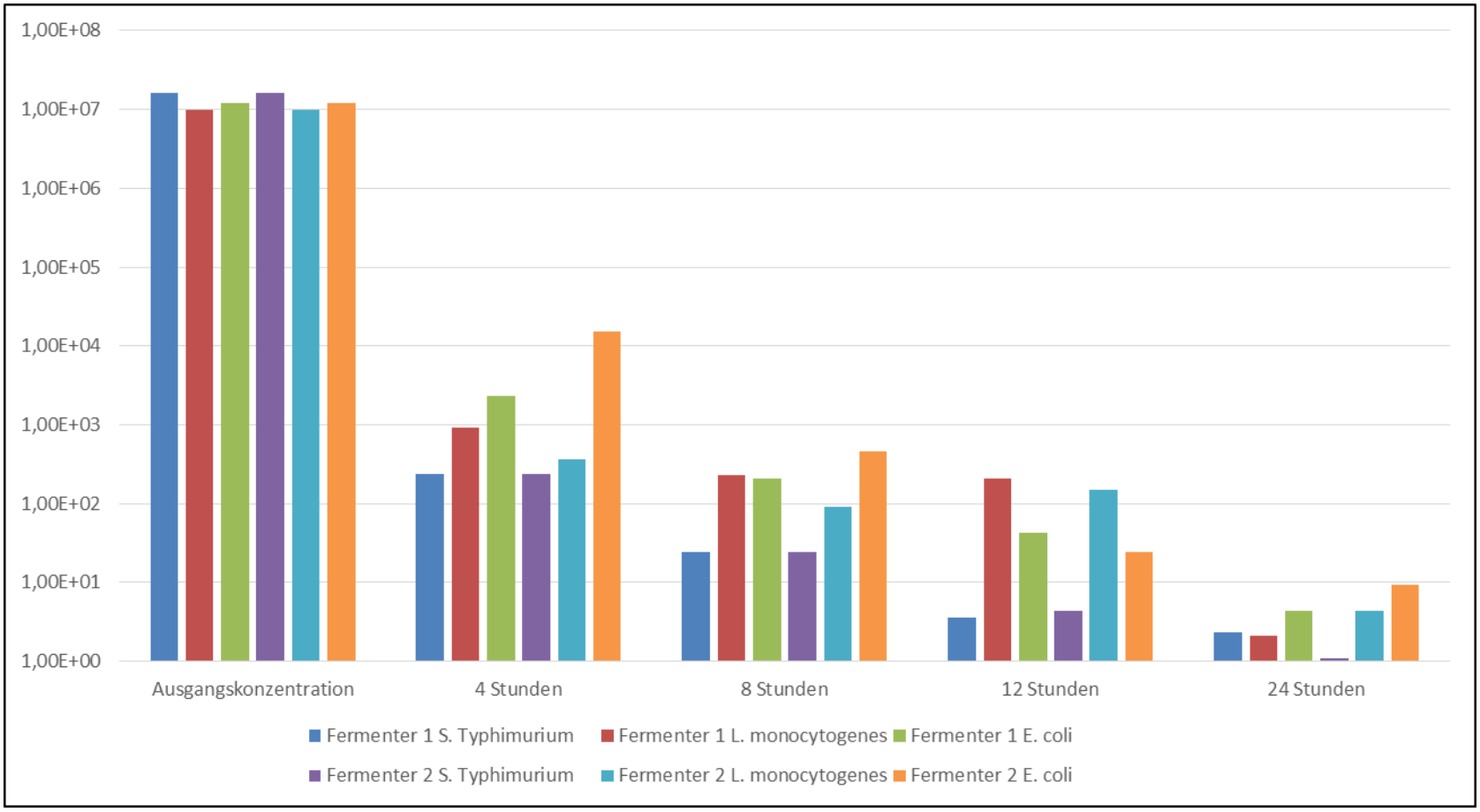


Figure 6: Summary of results of second round at low thermophilic conditions (52.4 °C)

The results of this experimental round reveal that in the selected temperature range of 50 to 53 °C a temperature difference of 1 °C already has an influence on the inactivation of added pathogens. At a fermenter temperature of 51.4 °C sanitisation in the sense of reduction of germ contamination by more than four Log10 levels was achieved for *S. Typhimurium* after eight hours of retention in the fermenter, for *L. monocytogenes* and *E. coli* only after 24 h. At a fermenter temperature of 52.4 °C this goal was achieved for *S. Typhimurium* and *L. monocytogenes* after 4 h and for *E. coli* after 8 h. However, it should be noted that the used fermenters were fed with fresh substrate only once a day. This was done before the examined pathogens were added to the fermenter and after the experiment ended. If the fermenter is fed continuously the existing germ amount can, on the one hand, be decreased which would result in a faster reduction of germ count, but on the other hand new pathogens could enter the fermenter constantly in this way which would result in slower reduction.

6.2.3 Results of studies at high thermophilic conditions

Other studies examined whether an increase of temperature over 55 °C has an improving sanitising effect to the substrate than temperatures below 53 °C. The results are presented in Tables 4 and 5 and in Figures 7 and 8. *S. Typhimurium* was reduced by five Log10 levels already in the first 4 h. After 12 h at the latest a further reduction of the germ count by one Log 10 level was determined and after 24 h no germs were detectable. Regarding *L. monocytogenes* and *E. coli* reductions by three to four Log10 levels were determined after 4 h. After 8 h a further reduction by three to four Log10 levels occurred. After 12 h no significant further reduction of germ counts was detectable. After 24 h neither *L. monocytogenes* nor *E. coli* was detectable in the substrates.

Table 4: Summary of results of first round at high thermophilic conditions in CFU/ml[3]

	Temperature	Test organism	Initial concentration	4 h	8 h	12 h	24 h
Fermenter 1	**57.4 °C**	*S. Typhimurium*	5.7×10^6	4.6×10^1	2.9×10^1	2.1×10^0	n.d.
		L. monocytogenes	1.3×10^6	2.3×10^2	9.3×10^1	3.6×10^1	n.d.
		E. coli	9×10^6	6.2×10^2	2.3×10^2	9.3×10^1	n.d.
Fermenter 2	**57.4 °C**	*S. Typhimurium*	5.7×10^6	2.4×10^1	1.1×10^1	1.2×10^0	n.d.
		L. monocytogenes	1.3×10^6	2.4×10^2	7.2×10^1	2.4×10^1	n.d.
		E. coli	9×10^6	9.3×10^2	4.6×10^2	1.1×10^2	n.d.

n.d. = not detectable

[3] Colony-forming units per ml of examined fermentation substrate

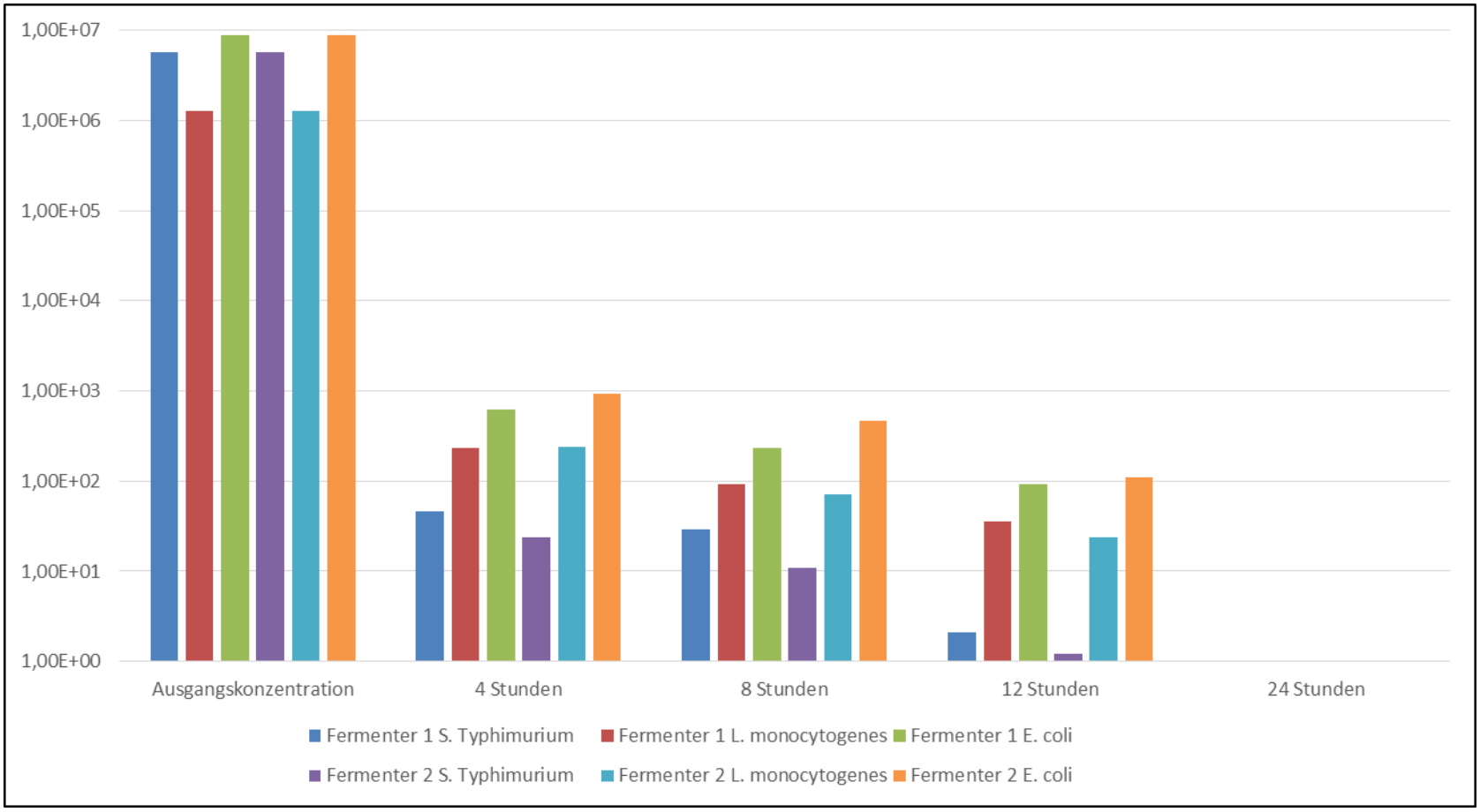


Figure 7: Summary of results of first round at high thermophilic conditions (57.4 °C)

Table 5: Summary of results of second round at high thermophilic conditions in CFU/ml[4]

[4] Colony-forming units per ml of examined fermentation substrate

	Temperature	Test organism	Initial concentration	4 h	8 h	12 h	24 h
Fermenter 1	**57.2 °C**	*S. Typhimurium*	7.9×10^6	2.4×10^1	4.6×10^0	1.1×10^0	n.d.
		L. monocytogenes	1.7×10^6	1.5×10^2	9.2×10^1	2.1×10^1	n.d.
		E. coli	6.4×10^6	4.6×10^2	9.3×10^1	4.6×10^1	n.d.
Fermenter 2	**57.2 °C**	*S. Typhimurium*	7.9×10^6	2.4×10^1	4.3×10^0	1.1×10^0	n.d.
		L. monocytogenes	1.7×10^6	2.4×10^2	2.7×10^2	2.1×10^1	n.d.
		E. coli	6.4×10^6	1.1×10^3	4.3×10^2	2.4×10^2	n.d.

n.d. = not detectable

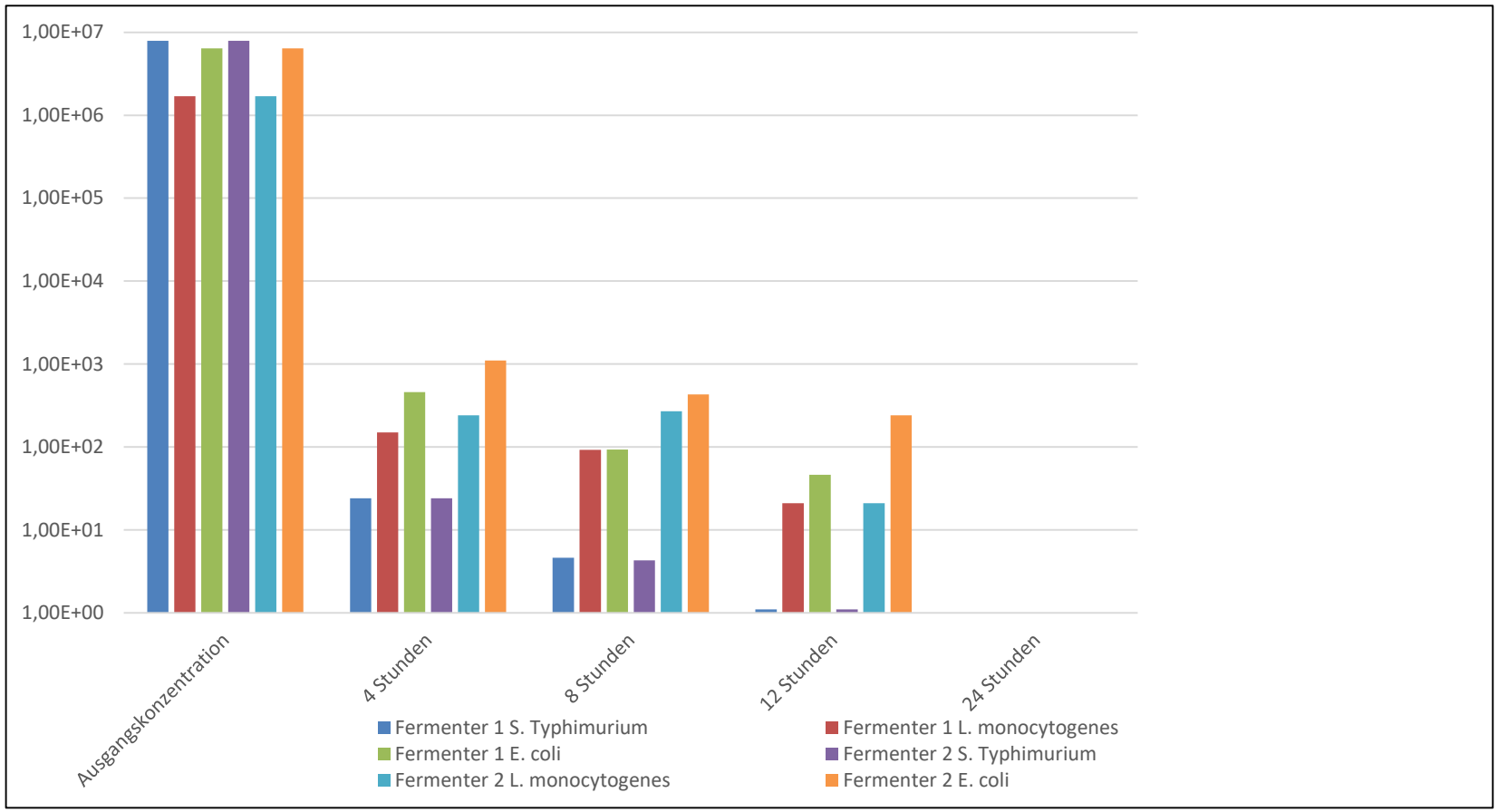


Figure 8: Summary of results of second round at high thermophilic conditions (57.2 °C)

The experiments proved that in the temperature range of about 57 °C sanitisation in the sense of germ count reduction by more than four Log10 levels can be achieved for *S. Typhimurium* after 4 hours and for *L. monocytogenes* after eight hours. Such reduction could be determined for *E. coli* in one sample after 8 h and in another sample after 12 h, in both other samples only after 24 hours. But it became evident that no added pathogens were detectable after 24 hours at these experimental temperatures. Here, too, the fermenters were fed only once daily before the experiment started and after it ended. Here, too, continuous feeding can lead to a decrease of pathogens on the one hand and to constant recontamination of the fermenter on the other hand.

6.2.4 Behaviour of viral pathogens in laboratory fermenters

In order to simulate fermenter conditions for studying the inactivation of viruses the Hohenheim biogas yield test (batch test) was used (Helffrich and Oechsner, 2003)[5]. For this purpose glass syringes are fed with fresh fermenter material from a thermophilically run laboratory fermenter and a small amount of fresh cow manure and silage to achieve a stable biogas process. The ERAV used in this experiment serves as surrogate virus for the MKS virus and the BVD virus as surrogate virus for the KSP virus.

A “germ carrier method” was used to be able to work with small amounts of viruses. Here, the viruses were applied to a virosorb membrane and sealed in polycarbonate foil. The semi-permeability of these foils ensures a material exchange with the environment; but the virus is not released into the environment. These experiments were conducted in triplicate to ensure high accuracy. At every sampling time three germ carriers were taken from each glass syringe and titrated to determine the virus titre. The selected points of time were 15, 30, 60, 120 min and 24 h. Three technical replicates were generated at intervals of three days to make a statistically verified statement. The initial titre of the virus suspension was 10^7 viruses per millilitre.

It became apparent that in the experiments using the BVD virus a high titre loss occurred due to the germ carrier method so that these results could not be evaluated. In the following only the results of ERAV are presented.

The total duration of the thermal inactivation period was 24 hours. During this period samples were taken in regular intervals. The initial titre of the virus suspension was 1.6 x 10^9 viruses per millilitre. A reduction by 7-8 Log10 levels after 24 hours can be seen (Fig. 9).

[5] Helffrich D, Oechsner H (2003): Hohenheimer Biogasertragstest - Vergleich verschiedener Tab. 1: Spezifische Methan- Laborverfahren zur Vergärung von Biomasse. Landtechnik 58 Issue 3, pp. 148 - 149 and Agrartechnische Forschung 9, Issue 1, pp. 27- 30

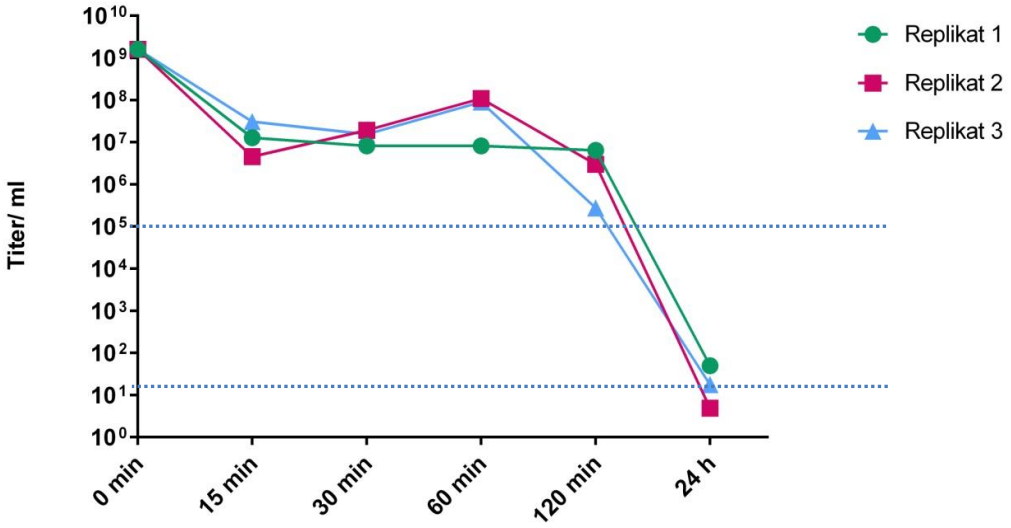


Figure 9: Batch test. Development of virus titre of Equine Rhinitis A Virus over 24 hours. Temperature was 53 °C (+/- 1.5) during the experiment. Each sampling examined three germ carriers from each glass syringe (Hölzle et al., 2015).

6.2.5 Conclusion of microbiological studies on reduction and possible reproduction of selected pathogens in biogas plants on laboratory scale (laboratory fermenter)

The experiments revealed that none of the examined bacterial pathogens reproduced at the operational conditions. But it became apparent that only a very low reduction of germ counts occurred at mesophilic conditions. At temperatures between 50 and 53 °C significant reductions occurred although all of the pathogens were still detectable after 24 hours. Sanitisation in the sense of germ reduction by more than four Log10 levels for all used pathogens was achieved only after 24 hours. At temperatures over 55 °C a sanitisation was also achieved only after 24 hours with the difference that at that time all added pathogens were no longer detectable. Based on the available results it is recommended to operate biogas plants thermophilically at a temperature >55 °C and with a retention time of 24 h. It was verified for non-enveloped viruses that an explicit sanitisation effect can be achieved at a temperature of 53 °C and a retention time of 24 h (Hölzle et al., 2015).

7 Special problems in cofermentation plants with utilization of farm fertilizers

Procedures in salmonella findings

Of the plants that cofermentate bio-waste only mesophilic plants are required to pretreat the bio-wastes (pasteurization ≥ 70 °C / 1 h) but not farm fertilizers (slurry, liquid manure, solid manure) according to respective regulations in the Bio-waste Ordinance. If in such cases salmonella are detected in the substrate in individual cases it has to be investigated if the positive salmonella findings are caused

by improperly pretreated bio-wastes or by the untreated farm fertilizers. If they are clearly influenced by farm manure they can, according to current knowledge, only be tolerated if the concentration of such sporadic findings is below 10^2 CFU/g of substrate.

In these cases the following approaches are recommended as suitable measures for farm fertilizers or for the entire end product employing so-called storage for "self-decontamination".

a) Storage of substrate for at least three months before agricultural use.

b) In case of subsequent application on grass lands used for mowing and grazing an additional one-month waiting-time period is to be observed.

c) If applicated to cropland (recommended are grains and root crops) the substrate should be dug in if the land is not cultivated.

If salmonella concentration is higher than 10^2 CFU/g of substrate suitable measures are to be determined in individual cases for farm manure (e.g. pasteurization ≥ 70 °C / 1 h) or for the entire end product.

Furthermore, it is to be assessed if animal health related consequences ensue. This is of special importance in salmonella findings in substrates from cattle farms. In these cases the Animal Health Law takes effect (TierGesG, 2013).

8 Aspects of environmental sanitation in anaerobic biological waste treatment

German Association for Waste Water (ATV) information sheet 365

This issue still receives huge attention in the public and expert discussion. Not least due to this, an ATV working group developed the information sheet "Hygiene bei der biologischen Abfallbehandlung - Hinweise zu baulichen und organisatorischen Maßnahmen - ATV-M 365" (Sanitation in biological waste treatment – instructions on constructional and organisational measures – ATV-M 365) in which the corresponding risks are pointed out and prevention strategies are explained.

The information sheet first gives an overview of the legal frame in which collection, treatment and utilization of biological waste are carried out. Afterwards the location requirements are presented with regard to the aspects of environmental sanitation and epidemic safety. The requirements of the layout of the facility and its operation are comprehensively presented under consideration of environmental and operational sanitation and occupational safety, and the requirements for the methods from the viewpoint of epidemic safety and phytosanitation according to the level of knowledge and technology valid at the time of printing are described in detail (ATV-1999; quoted from BÖHM et al., 2000 [15]).

On top of that the German Association for Water management, Wastewater and Waste (DWA) developed uniform technical regulations for the fields of water management, land development, soil protection, wastewater and waste technology and published them in the DWA set of rules. This set of rules contains information on planning, construction, operation, maintenance and audits of plants and

on the sustainable use of water and soil. In the following some DWA information sheets are presented in the context of anaerobic utilization of material in biogas plants:

- Information sheet DWA-M 376 - Sicherheitsregeln für Biogasbehälter mit Membrandichtung (Safety regulations for biogas holders with membrane seals) – October 2006
- Information sheet DWA-M 380 - Co-Vergärung in kommunalen Klärschlammfaulbehältern, Abfallvergärungsanlagen und landwirtschaftlichen Biogasanlagen (Cofermentation in municipal sewage sludge digesters, waste fermentation plants and agricultural biogas plants) – June 2009
- Information sheet DWA-M 907 - Erzeugung von Biomasse für die Biogasgewinnung unter Berücksichtigung des Boden- und Gewässerschutzes (Generation of biomass for biogas production under consideration of land and water protection) – April 2010
- Information sheet DWA-M 363 - Herkunft, Aufbereitung und Verwertung von Biogasen (Origin, treatment and utilization of biogases) – corrected version January 2011
- Information sheet DWA-M 361 - Aufbereitung von Biogas (Treatment of biogas) – October 2011

Criteria of acceptance Important criteria contribute to the acceptance of a waste treatment plant

- selection of location,
- layout of facilities and organisation of operation, and
- requirements for the methods (composting and anaerobic treatment).

Aspects of environmental sanitation

Aspects such as emissions of noise, odours, dust and microorganisms are especially important aspects of environmental sanitation, the last one especially in case of aerogenic spreading or of transmission through inanimate vectors. BÖHM et al. (2000 [15]) provide a detailed presentation of this issue.

Climate protection

With regard to climate protection biogas plants make a valuable contribution by preventing diffuse methane discharges from untreated biomass into the atmosphere through goal-oriented fermentation in the anaerobic plants and by substituting fossil fuels through the transfer of the gained biogases into electricity and heating energy. In doing so app. 1.6 to 1.8 tonnes of carbondioxide of fossil origin per tonne of decomposed organic dried mass can be saved in biogas plants.

Decrease of odorous emissions

The fermentation of manure and organic wastes can additionally contribute to a considerable relief of the odour issue during the subsequent agricultural use of substrates through the decomposition of odorous substances by 40 to 60 % (OECHSNER, 2000 [65]).

Annex 1

Bio waste directive Germany

Annex 2
(in relation to Section 2 number 2, Section 3 paragraphs (2)–(7))

Requirements for the sanitising treatment of bio-waste in order to ensure epidemic and phytosanitary safety

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3 Examinations of epidemic and phytosanitary safety

3.1 Process inspection (in relation to Section 3 (4), first sentence, number 1, read inconjunction with Section 3 (5))

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3.1.2 Plants used for aerobic sanitising treatment (thermophilic composting plants)

3.1.2.1 Clamp composting

3.1.2.2 Other composting procedures

3.1.3 Plants used for anaerobic sanitising treatment (thermophilic anaerobic digestionplants)

3.2 Process inspection (in relation to Section 3 (4), first sentence, number 2, read inconjunction with Section 3 (6))

3.3 Examinations of sanitised bio-waste (in relation to Section 3 (4), first sentence,number 3, read in conjunction with Section 3 (7) and (7a))

4 Methods for examining epidemic and phytosanitary safety

4.1 Tracer studies to determine the minimum residence time in anaerobic sanitisingtreatment procedures (thermophilic anaerobic digestion)

4.1.1 Tracer study with spores of Bacillus globigii

4.1.1.1 Preparation

4.1.1.2 Implementation of examination

4.1.1.3 Detection method

4.1.1.4 Minimum residence time

4.1.2 Tracer study with lithium

4.1.2.1 Preparation

4.1.2.2 Implementation of examination

4.1.2.3 Detection method

4.1.2.4 Minimum residence time

4.2 Epidemic sanitation testing

4.2.1 Process inspection

4.2.1.1 Test organism and limit value

4.2.1.2 Deposit samples for aerobic sanitising procedures (thermophilic composting)

4.2.1.3 Deposit samples for anaerobic sanitising procedures (thermophilic anaerobicdigestion)

4.2.1.4 Detection method

4.2.2 Examinations of sanitised bio-waste

4.3 Phytohygienic tests

1 General comments

The requirements and provisions for the sanitising treatment (systems and procedures) and inspection of sanitised bio-waste are described in this Annex.

If bio-waste is supplied for treatment that does not meet the requirements for sanitation purposes (e.g. mesophilic anaerobic digestion), the sanitising treatment of bio-waste must also be carried out in accordance with the provisions of this Annex.

The plant must be run and the treatment must be carried out in such a way so that any recontamination of the materials subjected to sanitising treatment is avoided.

2 Sanitising treatment

2.1 Treatment procedure for sanitation purposes (in r elation t o Section 2 number 2)

The sanitising treatment of b io-waste is carried out through

pasteurisation (number 2.2.1),

aerobic sanitising treatment (thermophilic composting) (number 2.2.2),

aerobic sanitising treatment (thermophilic anaerobic digestion) (number 2.2.3), or

any other form of sanitising treatment (number 2.2.4).

2.2 Requirements for the sanitising treatment

2.2.1 Pasteurisation

Pasteurisation may be carried out either before or after an additional, particularly biologically stabilising, treatment (e.g. mesophilic anaerobic digestion).

2.2.1.1 Process control requirements

Prior to pasteurisation, the bio-waste must be crushed into particles with an edge length (two dimensional) of no more than 12 mm. The material shall be homogenised during heating and must have a water content that ensures a sufficient heat transfer both between as well as within the particles.

The process control in pasteurisation plants must be conducted in such a way for the sanitation of bio-waste that a temperature of at least 70 °C acts on the entire material for a continuous period of at least 1 hour.

2.2.1.2 Process inspection (in relation to Section 3 (4), first sentence, number 1, read in conjunction with Section 3 (5))
A process inspection in a ccordance with n umber 3.1 shall not be required for pasteurisation plants; instead, pasteurisation p lants must obtain technical acceptance from t he competent authority, along with a n expert consultation w here appropriate, prior to commissioning (Section 3 (5), third sentence). The competent authority shall issue a c ertificate of acceptance if it has established that the pasteurisation p lant has satisfied the process control requirements in a ccordance with n umber 2.2.1.1 and is equipped with the necessary facilities and equipment, in particular such as

- devices for temperature monitoring,
- devices used to continuously record measurements, and
- an adequate safety system in order to prevent insufficient heating.

2.2.1.3 Process monitoring (in relation to Section 3 (4), first sentence, number 2, read in conjunction with Section 3 (6))
Process monitoring shall be carried out in accordance with the provisions of number 3.2. - 60 -

-61 -**2.2.1.4 Examinations of sanitised bio-waste** (in relation to Section 3 (4), first sentence, number 3, read in conjunction with Section 3 paragraphs (7) and (7a))
Examinations of sanitised bio-waste must be carried out in accordance with the provisions of number 3.3 and the methods in accordance with number 4.2.2 (epidemic sanitation) and number 4.3.2 (phytosanitation).

2.2.2 Aerobic sanitising treatment (thermophilic composting)

2.2.2.1 Process control requirements
Process control in composting plants must be conducted in such a way for the sanitation of bio-waste that a thermophilic temperature range and a high level of biological activity with favourable moisture and nutrient conditions as well as an optimal structure and airflow can be guaranteed over a period of several weeks. The water content should be at least 40 % and the pH-value should be approximately 7. During aerobic sanitising treatment, a temperature of at least 55 °C must act on the entire rotting material over a maximum continuous period of 2 weeks, or over 6 days at a temperature of 60 °C or over 3 days at a temperature of 65 °C.

2.2.2.2 Process inspection (in relation to Section 3 (4), first sentence, number 1, read in conjunction with Section 3 (5))
For composting plants used for sanitation purposes, the process inspection must be carried out in accordance with the provisions of number 3.1.1 and number 3.1.2.

The following methods shall apply to the use of test organisms (test and indicator organisms) and for checking their mortification or inactivation:

- for epidemic sanitation, the methods in accordance with number 4.2.1 (except number 4.2.1.3), and
- for phytosanitation, the methods in accordance with number 4.3.1 (except number 4.3.1.2.2).

2.2.2.3 Process monitoring (in relation to Section 3 (4), first sentence, number 2, read in conjunction with Section 3 (6))
Process monitoring is carried out in accordance with the provisions of number 3.2.
. . .

2.2.2.4 Examinations of sanitised bio-waste (in relation to Section 3 (4), first sentence, number 3, read in conjunction with Section 3 (7) and (7a))
Examinations of sanitised bio-waste must be carried out in accordance with the provisions of number 3.3 and the methods in accordance with number 4.2.2 (epidemic sanitation) and number 4.3.2 (phytosanitation).

2.2.3 Anaerobic sanitising treatment (thermophilic anaerobic digestion)

2.2.3.1 Process control requirements

Process control in anaerobic digestion plants must be conducted in such a way for the sanitation of bio-waste that the temperature acts on the entire material within the thermophilic range (at least 50 °C) over the consecutive period of the minimum residence time. In this respect, the minimum residence time used in the passed process inspection (see number 2.2.3.3) specified or demonstrated from a technical perspective (see number 2.2.3.2) and the treatment temperature must not be fallen short of.

2.2.3.2 Determination of minimum residence time

If the minimum residence time in the fermenter is not technically specified through the use of a hydraulic barrier within the charging and discharging intervals, it must be demonstrated by a tracer study using a method in accordance with number 4.1 prior to the process inspection (see number 2.2.3.3).

The tracer study allows the time interval at the anaerobic digestion plant used for sanitation purposes to be determined that all substrate parts (solid and liquid) have as the shortest residence time in the fermenter. This means that the substrate to be digested will be marked with indicators (tracers) before it is added to the fermenter. The minimum residence time of the material to be digested in the fermenter is the time frame which was determined up until the last experiment which found no initial evidence of the tracer.

Until the results of the tracer study become available, the minimum residence time calculated by the plant manufacturer and planner must not be fallen short of in the plant. In order to ensure that the minimum residence period is not fallen short of, the maximum daily input quantities determined by the plant manufacturer and planner must not be exceeded on a permanent basis after the desired level in the fermenter relevant for sanitation purposes is reached. It there is no calculation available, it must be made in consultation with the competent authority along with an expert consultation where appropriate.

2.2.3.3 Process inspection (in relation to Section 3 (4), first sentence, number 1, read in conjunction with Section 3 (5))

For composting plants used for sanitation purposes, the process inspection must be carried out in accordance with the provisions of number 3.1.1 and number 3.1.3.

The required treatment temperature within the thermophilic range (at least 50 °C) must be used for the process inspection. The process inspection must be carried out with the minimum residence time specified or demonstrated from a technical perspective (see number 2.2.3.2).

The following methods shall apply to the use of test organisms (test and indicator organisms) and to checking their mortification or inactivation:

- for epidemic sanitation, the methods in accordance with number 4.2.1 (except number 4.2.1.2),
- and for phytosanitation, the methods in accordance with number 4.3.1.1 (except for test organism tobacco mosaic virus in accordance with (c)), number 4.3.1.2 (except number 4.3.1.2.1), and number 4.3.1.3.

If the process inspection is not passed, it must be repeated with a higher treatment temperature or extended minimum residence time.

2.2.3.4 Process monitoring (in relation to Section 3 (4), first sentence, number 2, read in conjunction with Section 3 (6))

Process monitoring is carried out in accordance with the provisions of number 3.2.

2.2.3.5 Examinations of sanitised bio-waste (in relation to Section 3 (4), first sentence, number 3, read in conjunction with Section 3 paragraphs (7) and (7a))

Examinations of sanitised bio-waste must be carried out in accordance with the provisions of number 3.3 and the methods in accordance with number 4.2.2 (epidemic sanitation) and number 4.3.2 (phytosanitation).

2.2.4 Other forms of sanitising treatment

The equivalent effectiveness of the sanitation in line with the requirements of this Annex must be demonstrated (Section 3 (3), fourth sentence) for other forms of sanitising treatment procedure, along with an expert consultation where appropriate.

2.2.4.1 Process control requirements

The process control requirements for the sanitising treatment of bio-waste must be determined and described in such a way in agreement with the competent authority, along with an expert consultation where appropriate, that an equivalent sanitation level is achieved.

2.2.4.2 Process inspection (in relation to Section 3 (4), first sentence, number 1, read in conjunction with Section 3 (5))

The process control requirements must be determined and described in such a way in agreement with the competent authority, along with an expert consultation where appropriate, that an equivalent sanitation level is achieved whilst taking into account the provisions of number 3.1.1 as well as the methods in accordance with number 4.2.1 (epidemic sanitation) and number 4.3.1 (phytosanitation).

2.2.4.3 Process monitoring (in relation to Section 3 (4), first sentence, number 2, read in conjunction with Section 3 (6))

The process control requirements must be determined and described in such a way in agreement with the competent authority, along with an expert consultation where appropriate, that an equivalent sanitation level is achieved whilst taking into account the provisions of number 3.2.

2.2.4.4 Examinations of sanitised bio-waste (in relation to Section 3 (4), first sentence, number 3, read in conjunction with Section 3 paragraphs (7) and (7a))

Examinations of sanitised bio-waste must be carried out in accordance with the provisions of number 3.3 and the methods in accordance with number 4.2.2 (epidemic sanitation) and number 4.3.2 (phytosanitation).

3 Examinations of epidemic and phytosanitary safety

The sanitary safety of bio-waste is determined using the

- process inspection in accordance with Section 3 (4), first sentence, number 1, read in conjunction with Section 3 (5) and in accordance with the descriptions contained in number 3.1,
- process monitoring in accordance with Section 3 (4), first sentence, number 2, read in conjunction with Section 3 (6) and in accordance with the descriptions contained in number 3.2, and
- examinations of sanitised bio-waste in accordance with Section 3 (4), first sentence, number 3, read in conjunction with Section 3 paragraphs (7) and (7a) and in accordance with the descriptions contained in number 3.3.

The epidemic and phytosanitary tests must be performed at the same time where possible.

The treated bio-waste shall only be classified as hygienically safe once all examinations are passed in accordance with numbers 3.1 to 3.3.

3.1 Process inspection (in relation to Section 3 (4), first sentence, number 1, read in conjunction with Section 3 (5))

3.1.1 General requirements

The process inspection is an examination of individual treatment plants used for sanitation purposes which must be carried out once each time when a plant is newly constructed as well as when significant changes are made to the procedure. As a result, this determines the effectiveness of the sanitation procedure. In addition, epidemically and phytosanitary relevant test and indicator organisms are introduced into the plant along with the bio-waste; checks are then made on the basis of examinations carried out on treated materials as to whether the test organisms were destroyed or inactivated as a result of sanitation.

The process control requirements for other forms of sanitising treatment (number 2.2.4) must be determined and described in such a way in agreement with the competent authority, along with an expert consultation where appropriate, that an equivalent sanitation level is achieved whilst taking into account the provisions of this section as well as the methods in accordance with number 4.2.1 (epidemic sanitation) and number 4.3.1 (phytosanitation).

The methods (sampling, preparation, examination and maximum permissible limits to be observed) in epidemic sanitation in accordance with number 4.2.1, in phytosanitation in accordance with number 4.3.1, and in accordance with the following detailed descriptions for the respective plants (see numbers 3.1.2 and 3.1.3), shall apply to the process inspection (Section 3 (4), second sentence).
The process inspection shall be deemed to have been successfully completed if the limit values specified in accordance with number 4.2.1.1 (epidemic sanitation) and number 4.3.1.1 (phytosanitation) are not exceeded in the two consecutive examination stages in accordance with the relevant procedural step for sanitation.

3.1.2 Plants used for aerobic sanitising treatment (thermophilic composting plants)

The process inspection is comprised of two examination stages carried out at separate times within a minimum interval of 3 months, of which one shall take place in the winter.

The test organisms are introduced into characteristic rotting areas or in representative process sections for the thermal inactivation of the test organisms, and are tested for surviving or infectious test organisms following removal.

3.1.2.1 Clamp composting

For each examination stage, a total of 60 individual samples are examined, of which 24 samples are accounted for by epidemic sanitation testing and 36 samples are accounted for by phytosanitation testing. The number of individual samples is calculated as follows:

- When c onducting epidemic sanitation t esting, a single test organism (see number 4.2.1) is introduced in duplicate samples into three different rotting zones (edge, core and base area) as well as at four different clamp points.
- When conducting phytosanitation testing, 3 test organisms (see number 4.3.1) are introduced as individual samples into three different rotting zones (edge, core and base area) as well as at four different clamp points.

The samples from the edge may be covered with 10 cm rotting material. The samples shall remain in their respective areas until the end of the inspection.

For small plants with an annual capacity of up to 3,000 tonnes of materials used, only half of the individual samples need to be examined as part of a reduced examination scope. As a result, the test organisms are only introduced at two different clamp points.

3.1.2.2 Other composting procedures

For each examination stage, a total of 60 individual samples are examined, of which 24 samples are accounted for epidemic sanitation testing (single test organism, see number 4.2.1) and 36 samples are accounted for phytosanitation testing (3 test organisms, see number 4.3.1). The test organisms are inserted in characteristic areas of the rotting body, or channelled into suitable sample containers for dynamic procedures with the material flow through the standard rotting and procedural process. The sample containers used must have sufficient perforations so that the substance transformation conditions within the sample containers correspond to those for the composting process to be inspected for sanitation purposes.

With respect to dynamic procedures, attention must be paid to ensure that all test organisms are added as evenly as possible throughout the insertion process so that they are distributed as homogeneously as possible in the rotting unit. In addition, the type of sample container used must also ensure that they comply with the material to be composted with respect to the behaviour of the material flow and the residence time.

If the specific plant technology does not restrict the size of the sample containers (e.g. clear openings for snails, etc.), a total of 12 sample containers shall be introduced into the rotting unit (guided through); each sample container shall contain

- a single test organism in duplicate samples when conducting epidemic sanitation testing (see number 4.2.1), and
- three test organisms as individual samples when conducting phytosanitation testing (see number 4.3.1).

If the introduction (i.e. guiding through) of correspondingly larger sample containers is not possible, the individual samples must be distributed over a correspondingly larger number of smaller sample containers.

For small plants with an annual capacity of up to 3,000 tonnes of materials used, only half of the individual samples need to be examined as part of a reduced examination scope. As a result, only 6 of the 12 sample containers shall be introduced and guided through.

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3.1.3 Plants used for anaerobic sanitising treatment (thermophilic anaerobic digestion plants)

The process inspection is comprised of two examination stages carried out at separate times within a minimum interval of at least 3 months.

For each examination stage, a total of 24 individual samples are examined, of which 8 samples are accounted for by epidemic sanitation testing and 16 samples are accounted for by phytosanitation testing. The number of individual samples arises as follows:

a) When conducting epidemic sanitation testing, a single test organism (see number 4.2.1) is introduced in duplicate samples as well as at four different points in the fermenter (for standing fermenters in a vertical direction and lying fermenters in a horizontal direction).
b) When conducting phytosanitation testing, 2 test organisms (see number 4.3.1 with the exception of tobacco mosaic virus) are introduced in duplicate samples as well as at four different points in the fermenter (for standing fermenters in a vertical direction and lying fermenters in a horizontal direction).

For small plants with an annual capacity of up to 3,000 tonnes of materials used, only half of the individual samples need to be examined as part of a reduced examination scope. As a result, the test organisms are only introduced at two different points in the fermenter.

The test organisms are introduced into the fermenter in the minimum residence time specified or demonstrated from a technical perspective (see number 2.2.3.2) and are examined after removal.

When carrying out the process inspection, there must be suitable openings in the vats to allow the insertion and removal of samples.

3.2 Process inspection (in relation to Section 3 (4), first sentence, number 2, read in conjunction with Section 3 (6))

The monitoring process is a continuous inspection and recording of temperatures during treatment for sanitation purposes. This is done in order to establish whether the temperature required for sanitation purposes and the required exposure time are both complied with.

The process control requirements for other forms of sanitising treatment (number 2.2.4) must be determined and described in such a way in agreement with the competent authority, along with an expert consultation where appropriate, that an equivalent sanitation level is achieved whilst taking into account the provisions of this section.

If the temperature in the exhaust air stream of the composting clamp is measured and recorded in an enclosed composting plant used for sanitation purposes (Section 3 (6), third sentence), the treatment temperature must be determined through a plant-specific correction factor with respect to the direct temperature measurement of the rotting material. The plant-specific correction factor must be

examined on a regular basis through direct temperature measurements of the rotting material taken in parallel. For the temperature measurement in the exhaust air stream, the requirements must be determined in agreement with the competent authority, along with an expert consultation where appropriate.

The temperature measurements are to be taken in representative areas of the process sections or plant parts that are relevant for sanitation purposes.

Process monitoring is deemed to have been successfully completed if the temperature and exposure time stipulated for the respective procedure (see numbers 2.2.1.1, 2.2.2.1, 2.2.3.1 and 2.2.4.1) were complied with for the sanitising treatment of the material.

3.3 Examinations of sanitised bio-waste (in relation to Section 3 (4), first sentence, number 3, read in conjunction with Section 3 (7) and (7a))

The examinations of sanitised bio-waste are regular examinations of the materials to check for pathogens, viable seeds and viable plant parts following treatment for sanitation purposes.

The examinations of sanitised bio-waste are carried out on releasable material following the sanitising treatment (see number 2). A sample shall be examined from both the epidemic sanitation and phytosanitation for each examination of sanitised bio-waste.

The methods (sampling, preparation, examination and maximum permissible limits to be observed) in epidemic sanitation in accordance with number 4.2.2 and in phytosanitation in accordance with number 4.3.2 shall apply to the examinations (Section 3 (4), second sentence).

The examinations of sanitised bio-waste shall be deemed to have been successfully completed if the limit values specified in accordance with number 4.2.2, last paragraph (epidemic sanitation) and number 4.3.2, last paragraph (phytosanitation) are not exceeded in any of the samples taken.

4 Methods for examining epidemic and phytosanitary safety

4.1 Tracer studies to determine the minimum residence time in anaerobic sanitising treatment procedures (thermophilic anaerobic digestion)

In order to be able to assess the effect of anaerobic treatment procedures, knowing the minimum residence time of the waste slurry in the digester is of importance. If the minimum residence time needs to be determined, a tracer study shall be carried out for this purpose (see number 2.2.3.2). In the tracer study, the waste slurry is marked with indicators (tracers) prior to entering the fermenter, and its first appearance at the outlet is recorded.

Biological tracers with spores of Bacillus globigii (see number 4.1.1) or chemical tracers with lithium (see number 4.1.2) are suitable for the tracer study in anaerobic treatment plants used for the sanitation of biodegradable waste.

4.1.1 Tracer study with spores of Bacillus globigii

The spores of Bacillus globigii are used as a biological tracer. Spores from this test bacterium are not naturally present in the biological substrates, are not pathogenic for humans and animals, are able to withstand any process effects in anaerobic treatment systems, and are easily detectable.

4.1.1.1 Preparation

Required materials and reagents

- Tryptone Glucose Bouillon (TGB), used to produce the inoculum from the Bacillus globigii spores: Yeast extract: 2.5 g, Tryptone: 5.0 g, Glucose: 1.0 g, Water (distilled): 1,000 ml;
- Malt yeast extract agar (MYA), used to produce Bacillus globigii spores: Peptone from meat: 10.0 g, Yeast extract: 2.0 g, Manganese sulphate monohydrate: 0.04 g, Agar: 15 g, Water (distilled): 1,000 ml;
    - Bacillus globigii seed stock, used to produce Bacillus globigii seed stock spore suspension:
        - Bacillus globigii (DSM No 675 [Bac. Atrophaeus]): Deutsche Stammsammlung für Mikroorganismen), Marscheroder Weg 1b, 38124 Braunschweig.) or,
        - Bacillus globigii (DSM No 2277 [Bac. Atrophaeus]), or
        - Bacillus globigii (collection from the University of Hohenheim, Institute for Environmental and Animal Hygiene, Garbenstrasse 30, 70599 Stuttgart.);
- Centrifuge with an acceleration of 10,000 g.

Sample preparation

Tryptone Glucose Bouillon (TGB): The bouillon is given in test tubes in portions of 10 or 100 ml. It is sterilised in autoclaves.When measured at 20 °C, the pH-value of the medium must be 7.2 (± 0.2) followingsterilisation.

Malt yeast extract agar (MYA): The agar is given in Roux bottles or Petri dishes. It is sterilised in autoclaves. Whenmeasured at 20 °C, the pH-value of the medium must be 7.0 (± 0.2) following sterilisation.

Bacillus globigii seed stocks:The Bacillus globigii seed stocks (glycerol culture, storage temperature -80 °C) are thawedand incubated in tryptone glucose bouillon (TGB) at 37 C for a period of 24 hours. 6 ml istransferred from the TGB bouillon to MYA plates; the supernatant is then pipetted off. TheMYA plates are incubated at 37 (± 1) °C. After the third day of incubation, the state of thecultures is assessed using a

spore stain (e.g. racket-like staining). Following that, the MYAplates are incubated again at 30 °C for 7 to 10 days. The colonies from the MYA plates arethen washed away with 3 ml of sterile distilled water (aqua dest).The spore suspension obtained is centrifuged (3,000 rpm for 10 minutes), the supernatant isdiscarded, and the pellet is resuspended with aqua dest.In order to determine the number of spores, the suspension is first heated for a period of 10minutes at 75 (± 1) °C, after which the number of spore per millilitre of suspension isdetermined using Koch's surface method.

4.1.1.2 Implementation of examination

The biological tracer is added to the fermenter once in the form of a spore suspension evenlyover a feeding interval. As many spore suspensions as possible are mixed into a feedingbatch so that there are at least 10^{6} spores per grams of fermenter content. The concentration of Bacillus globigii spores in the suspension must be monitored.

The sampling shall take place after the feeding of the spore suspension (single sample ofapproximately 1 kg) in the outlet until the tracer is first detected in a sample, and this mustbe at least

every hour up to and including the 24th hour,

subsequently every two hours up to and including the 36th hour,

subsequently every four hours up to and including the 48th hour,

subsequently every six hours.

4.1.1.3 Detection method

20 g worth is weighed out from the samples to be examined for pre-dilution in 180 ml of sodium chloride (0.9 % saline solution) and mixed for approximately 20 hours at 4°C in the shaker. After sufficient mixing, 1 ml of the sample in a geometric series up to dilution stage to 10^{-8} is then pipetted into 9 ml of NaCl solution. Following that, 0.1 ml of each dilution stage is pipetted into two parallel standard I agar plates and evenly distributed with an annealed spatula (incubation at 37 °C for 24 hours).

Only colonies that display a typical orange-red growth on the agar plates are counted.

4.1.1.4 Minimum residence time

The minimum residence time arises from the period between the addition of the Bacillus globigii spore suspension up until the last sample, which found no initial evidence of the biological tracer in the outlet of the fermenter.

4.1.2 Tracer study with lithium

4.1.2.1 Preparation

Determination of lithium background level in the waste slurry

Firstly, the natural lithium background level in the waste slurry must be determined. In order to do this, a representative sample of the outlet of the fermenter must be collected on a daily basis at least for a period of 5 days prior to the start of the inspection. Depending on the bio-waste composition, the background level of lithium is typically between 1 and 5 mg per kg of dry matter.

Materials required

Tracer: Lithium hydroxide monohydrate

4.1.2.2 Implementation of examination

The lithium concentration of 50 mg/kg of dry matter must be adjusted in relation to theentire fermenter contents (complete mixing) for the examination. The required amount oflithium is dependent on the effective volume of fermenters in anaerobic digestion plantsused for sanitation purposes that are to be inspected. The tracer is added in dissolved formto the fermenter evenly over a feeding interval.A reserve sample of this lithium suspension must be retained until the results becomeavailable.

The sampling shall take place after the tracer has been fed (single sample of approximately1 kg) in the outlet until the tracer is first detected in a sample (lithium concentration andbackground level), and this must be at least

a) every hour up to and including the 24th hour,
b) subsequently every two hours up to and including the 36th hour,
c) subsequently every four hours up to and including the 48th hour,
d) subsequently every six hours.

4.1.2.3 Detection method

The samples are analysed in accordance with DIN EN ISO 11885:2009 (Published by Beuth-Verlag GmbH, Berlin, and archived in a secure manner at the German Patent and Trademark Office in Munich) in order to determine the lithium concentration.

4.1.2.4 Minimum residence time

The minimum residence time arises from the period between the addition of the lithium tracer up until the last sample without any increase in concentration, which found no initial evidence of the tracer in the outlet of the fermenter. The tracer is detected when the detected concentration of lithium exceeds the background levels determined by twice the standard deviation, which is determined for samples taken in accordance with number 4.1.2.1.

4.2 Epidemic sanitation testing

4.2.1 Process inspection

4.2.1.1 Test organism and limit value

The audit inspection in epidemic sanitation is performed with the Salmonella senftenberg W775 (H2S negative) test organism.

The process inspection shall be deemed to have been successfully completed if no levels of salmonella can be detected in any samples in the two consecutive examination stages after the relevant procedural step for sanitation.

4.2.1.2 Deposit samples for aerobic sanitising procedures (thermophilic composting)

The Salmonella senftenberg W775 (H2S negative) test organism is incubated in standard I bouillon at 37 °C for between 18 and 24 hours. The bacterial suspension produced as a result should contain a microorganism concentration of at least 10^7 to 10^8 CFU/ml. The concentration is determined by comparison with a standard (e.g. McFarland) or with the surface method or with the MPN method (most probable number). When composting for sanitation purposes, approximately 225 g of fresh, homogenised, crushed bio-waste material from the plant to be inspected is soaked with 25 ml of this bacterial suspension for each sample and then packaged in sterile onion or plastic bags. The samples are deposited in the compost either in this manner or in coarse perforated stable sample containers that are suitable for the respective process. After the procedural step that is relevant for sanitation has been completed, the sample containers are removed again and 50 g of the homogenised contents of a sample bag are slowly shaken in 450 ml of buffered peptone water with novobiocin for 30 minutes at 4 °C (150 rpm) and then incubated for approximately 22 (± 2) hours at 36 (± 2) °C. The suspension solution obtained as a result is then used to identify any salmonella.

4.2.1.3 Deposit samples for anaerobic sanitising procedures (thermophilic anaerobic digestion)

The bacterial suspension with the Salmonella senftenberg W775 (H2S negative) test organism is prepared as described in number 4.2.1.2, paragraph (1).

In anaerobic digestion plants used for sanitation purposes, 1 ml of the bacterial suspension of Salmonella senftenberg W775 (H2S negative) is infiltrated into the process with diffusion germ carriers[4]. The diffusion germ carriers are filled out with 9 ml of digestate in addition to 1 ml of the bacterial suspension and are introduced in the process sections or plants that are relevant for thermal inactivation for the established minimum residence time (see number 4.1) and sanitising temperature. After the procedure is completed, the respective total content of the diffusion germ carrier (10 ml) is added in 90 ml of buffered peptone water with novobiocin (pre-enrichment), briefly shaken (150 rpm), and incubated for 22 (± 2) hours at 36 (± 2)°C. The suspension solution obtained as a result is then used to identify any salmonella.

Method according to Schwarz, Michael, Vergleichende seuchenhygienisch-mikrobiologische Untersuchungen an horizontal und vertikal beschickten, bewachsenen Bodenfiltern mit vorgeschalteter Mehrkammerausfaulgrube bzw. einem als Grobstoff -Fang dienenden Rottebehälter (Rottefilter) (Comparative epidemic sanitation and microbiological examinations on horizontally and vertically fed and vegetated soil filters with upstream outlet pits with several chambers and a rotting container (rotting filter) used for large particles/catches), p. 45, veterinary dissertation, FU Berlin, 2003; archived in a secure manner at the German National Library in Leipzig.

4.2.1.4 Detection method

Existing salmonella are identified using the suspension solutions that have been produced in accordance with the methods described above (see numbers 4.2.1.2 and 4.2.1.3). For this purpose, 0.1 ml from the well-mixed pre-enrichment is incubated in 10 ml of enrichment bouillon according to Rappaport at 36 (± 2) °C and at 42 (± 1) °C for 22 (± 2) hours. Parallel smears are then applied on xylose lysine deoxycholate agar (XLD) and on another salmonella differential culture medium able to document other biochemical properties than XLD agar. Any suspect salmonella colonies are inoculated on nutrient agar and incubated at 36 (± 2) °C for 22 (± 2) hours. The biochemical or serological identification is carried out based on the body and flagella antigens (O and H antigens).

In order to control the viability (tenacity) of the test strain, four control samples are prepared parallel to the process inspection. These control samples are not incorporated into the sanitation procedure, but rather they are stored during the inspection period in moist sand (e.g. buckets with quartz sand, moistened with deionised water) at room temperature (20 to 25 °C) and are then processed following the discontinuation of the process inspection. At least three of the four control samples should provide positive salmonella findings; if not, then the tenacity of the test strain shall not be deemed sufficient.

4.2.2 Examinations of sanitised bio-waste

In order to examine the sanitised bio-waste in epidemic sanitation, 50 g of the respective material from a well-mixed sample (approximately 3 kg) is examined for the presence of salmonella in accordance with the method described above (see number 4.2.1.2). The composite mixed sample is made up of at least five different sub-samples of a batch of material subjected to sanitising treatment which is to be examined in accordance with number 3.3.

The examination of sanitised bio-waste in epidemic sanitation shall be deemed to have been successfully completed if no levels of salmonella can be detected in 50 g of the respective composite samples.

4.3 Phytohygienic tests

4.3.1 Process review

4.3.1.1 Test organisms and limits

From the variety of plant pathogens and plant seeds that appear in the starting material ofbiological waste treatment plants, the following control or indicator organisms are used inphytohygiene process tests:

- Plasmodiophora b rassicae (clubroot) with a o ne-week heat tolerance of 5 0 °C,Limit in the bioassay: Infestation in dex ≤ 0.5 per test area,

- Tomato seeds, Limit in the bioassay: ≤ 2 % of viable seeds per test area,

- In a ddition to aerobic sanitising treatment (thermophilic composting) in a ccordance with number 2.2.2: Tobacco mosaic virus (TMV), Limit in the bioassay: ≤ 4 % residual infectivity (relative value to the positive control) for each t est area.

The process inspection is completed successfully in phytohygiene if, in the two consecutive tests after each process step relevant for the sanitising process, the samples in each test area do not exceed the stipulated limits

– for the parameters Plasmodiophora brassicae and tomato seeds, as well as

– for the parameter tobacco mosaic virus by more than 30 %.

4.3.1.2 Plasmodiophora brassicae test organism

The process inspection in phytohygiene with the Plasmodiophora brassicae test organism is performed using the method described below.

4.3.1.2.1 Production of core samples for aerobic sanitising treatment (thermophilic composting)

The bilious material (infection material with the pathogen Plasmodiophora brassicae) is frozen until core samples are produced at -25 °C. The demonstrably infectious, heat-tolerant bilious material with the Plasmodiophora brassicae pathogen must be used from infested cabbage plants. The heat tolerance is proven when the bilious material demonstrates a high level of infectivity (infection level ≥ 2) when incubated at 50 °C for 7 days.

Each of the samples used in the composting process for sanitising contains 30 g bilious material, 430 g soil and 200 g of each compost raw material. This represents a ratio of approximately 5 % bilious material to 65 % soil and 30 % compost. The individual sample components are thoroughly mixed and filled in rot-resistant bags (maximum mesh size 1 x 1 mm); it is necessary to ensure that nothing is discharged from the sample into the surrounding compost.

The control samples prepared in this way are stored during the test period in moist, sterilised sand at room temperature.

4.3.1.2.2 Production of core samples for anaerobic sanitising treatment processes (thermophilic anaerobic digestion)

The bilious material (infection material with the Plasmodiophora brassicae pathogen) used is subject to number 4.3.1.2.1., paragraph (1) accordingly.

In anaerobic digestion plants used for sanitisation, 30 g bilious material contained in a gauze bag (mesh size max. 1 x 1 mm) is introduced into process sections or parts relevant for thermal inactivation.

The control samples prepared in this way are stored during the test period in moist, sterilised sand at room temperature.

4.3.1.2.3 Evidence of infectivity by bioassay

Any residual infection of Plasmodiophora brassicae is identified in the core samples using the test described below.

Equipment needed

– Mixing tub,

– Measuring cups (1,000 ml),

– Plastic pots (13 x 13 x 13 cm, approximately 1 l), matching stands,

– Certified seed of Sarepta mustard (Brassica juncea),

– Substrate damper,

– Sand, grain size 0.8 – 1.2 mm (e.g. variegated sandstone with good buffering capacity, pH approximately 6.5),

– Light peat (pH approximately 3.5),

– pH meter,

– Disposable gloves (one pair for each sample),

– Water-soluble fertiliser (solid or liquid).

Sample preparation

Once recovered from the tested sanitisation process, the core samples are carefully crushed with the Plasmodiophora brassicae pathogen and then filled to a volume of 1,000 ml and homogenised with a sand-peat mix (attenuated for 5 hours at 80 °C).

As the pH exerts a strong influence on the infectivity of Plasmodiophora brassicae (optimum: pH 6.0 ± 0.2), the pH of the prepared substrate mixture must be checked and corrected by increasing the peat content where appropriate.

Bioassay

13 x 13 x 13 cm wide plastic pots are used as test vessels. For each reisolated pathogen sample which has been filled up to 1,000 ml with the sand-peat mixture, a vessel is prepared with 16 control Sarepta mustard (Brassica juncea) plants; early seedlings (first true leaf formation) are pricked out into each vessel. The bioassay is set up as a randomised trial in the greenhouse or in a climate chamber at 6,000 – 9,000 lux and a minimum temperature of 20 °C. The plants are fed with fertiliser once a week starting from the third week. The vegetation period of the bioassay is 4 to 5 weeks until the control plants are assessed.

At the end of the bioassay, the number of infected plants is counted and the root bile formation is assessed on a scale from 0 to 3: **Infestation class**	**Description of Symptomes**
0	No visible Symptomes
1	Slight bile formation on the main and secondary roots
2	Average bile formation on the main and secondary roots
3	Strong bile formation on the entire root system

Annex 2

Tabelle : Overview about the exiszing pathogens in untreated waste, manure and digestat

	Pathogen	Nachweismethode	Referenzen
Bakterien	*Salmonella* spp.	PCR Kultur, quantitativ,	Brooks et al., 2014; McLaughlin et al., 2009; Gantzer et al., 2001; Guzman et al., 2007
	Pathogene *E. coli* und ESBL-*E. coli*	qPCR[1] Kultur, quantitativ	Klein et al., 2010; Haack et al., 2015 Schauss et al., 2015
	Enterococcus spp.	qPCR Kultur, quantitativ	Klein et al., 2010 McLaughlin et al., 2009; Gantzer et al., 2001
	Erysipelothrix rhusiopathiae	16S rDNA PCR und Sequenzierung	Han et al., 2011
	Staphylococcus aureus	16S rDNA PCR und Sequenzierung	Han et al., 2011
	Listeria monocytogenes	16S rDNA PCR und Sequenzierung qPCR	Han et al., 2011; Klein et al., 2010
	thermophile *Campylobacter* spp.	PCR und Sequenzierung qPCR	Brooks et al., 2014; Han et al., 2011; Klein et al., 2010
	Clostridium perfringens	Kultur, quantitativ 16S rDNA PCR und Sequenzierung	McLaughlin et al., 2009; Bagge et al., 2010
	Clostridium botulinum	16S rDNA PCR und Sequenzierung	Bagge et al., 2010
	Mycobacterium avium ssp. *paratuberculosis*	qPCR	Slana et al., 2011
	Coxiella burnetti Europäisches Schweinepestvirus	PCR Zellkultur, qPCR Zellkultur, ELISA Zellkultur	Roest et al., 2012 Weesendorp et al., 2008; Bøtner und Belsham, 2012 Turner et al., 2000

	Zellkultur, ELISA	Bøtner und Belsham, 2012
	Zellkultur	Turner et al., 2000
Afrikanische Schweinepestvirus	Zellkultur	Turner et al., 1999
Vesikuläre Schweinekrankheit-Virus	Zellkultur	Turner et al., 1999
Aujeszky Virus	Zellkultur	Turner et al., 2000
Adenoviren	qPCR	Hundesa et al., 2009; Fongaro et al., 2013; Haack et al., 2015
Circovirus	Zellkultur, qPCR	Viancelli et al., 2012b
	qPCR	Viancelli et al., 2012b
PRRS-Virus	Zellkultur	Linhares et al., 2012
Parvovirus	qPCR	Viancelli et al., 2013
Enterovirus	qPCR	Haack et al., 2015
Hepatitis E-Virus	qPCR	Haack et al., 2015; Gentry-Shields et al., 2015; Garcia et al., 2013
	Zellkultur, PCR	Yugo &Meng, 2013
MKS-Virus	Zellkultur	Turner et al., 2000
	Zellkultur, ELISA	Bøtner und Belsham, 2012
BVD-Virus	Zellkultur, ELISA	Bøtner und Belsham, 2012
Influenzaviren	Zellkultur, ELISA	Bøtner und Belsham, 2012
	Zellkultur	Lu et al., 2003

Parasiten	*Giardia* spp.	qPCR	Klein et al., 2010
	Cryptosporidium spp.	qPCR direkte Immunfluoreszenz	Klein et al., 2010 Guzman et al., 2007
	Nematoden-Eier	Flotation	McLaughlin et al., 2009
	Helminthen-Eier	Flotation	Guzman et al., 2007

[1] quantitative PCR

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