# PM 3/66 (3) Guidelines for the management of plant health risks of biowaste of plant origin

**Specific scope:** This Standard describes requirements for the treatment of biowaste of plant origin to ensure its phytosanitary safety. It covers composting, anaerobic digestion and heat treatment.

**Specific approval and amendment:** First approved in 2005–09. Revisions approved in 2007–09 and 2022–09. Authors and contributors are given in the Acknowledgements section.

# 1 | INTRODUCTION

Organic waste ('biowaste') includes animal manure, crop residues, food processing waste, municipal biosolids and waste from households and some industries (see section 2). Possible uses of biowaste includes use as fertilizer and soil amendment in agriculture, energy recovery (heat, liquid fuels, electricity), and production of chemicals (volatile organic acids, ammonium products, alcohols).

One of the options for recycling of biowaste is decomposition. Decomposition, i.e. composting and/or anaerobic digestion, reduces the bulk of biowaste considerably (by approximately 65%) and stabilizes the organic matter in the resulting product for subsequent utilization. Because of its nutritional nature, the resulting treated biowaste (in the form of compost or sludge) is particularly suited for addition to soil, and most such material is used in agriculture, horticulture, amenity gardening and private gardens. In addition, it presents an environmental benefit compared to disposal methods such as landfill. Already, millions of tonnes of treated biowaste are produced and used each year in EPPO countries.

The present Standard deals with treatment of biowaste from plant material that may initially be infested with various kinds of pests (including quarantine pests and temperature tolerant pests) or residues from plants that are pests (such as invasive plants). Though the temperatures reached in composting should in principle eliminate most pests (including weeds, see section 2), there is clear published evidence (e.g. Noble & Roberts, 2004; Sansford, 2003; Wichuk et al., 2011) that some pests may survive treatment processes. This may be due to heat tolerance of the organism or failures in the treatment process. Therefore, there is a need to establish safety targets

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and related minimum processing conditions to avoid such failure.

This Standard describes:

- 1. Processing of biowaste.
- 2. Requirements for the treatment process to ensure phytosanitary safety of treated biowaste for most pests.
- 3. Specific requirements for biowaste that may contain quarantine pests or heat-tolerant pests.
- 4. Supervision, test procedures and validation methods to ensure that the treatment process and final product comply with plant health requirements.
- 5. Documentation and labelling requirements during production and exchange of treated biowaste.

This Standard also provides as supplementary information, a compilation of pest-specific studies which can be used as scientific evidence showing if treatment conditions are effective (or not) in eliminating a pest of concern.

Aspects other than plant health (e.g. health rules for animal by-products in mixed composts, tolerances for heavy metals, physical contaminants such as glass, plastic, metal or stones, physical characteristics such as pH, salt, organic matter or dry matter, amount of waste allowed to be applied to land and other limitations on end use) are not covered by this Standard. Treatment processes should comply with relevant national or international standards. Environmental risks related to residues of plant protection products are also not covered. NPPOs are encouraged to cooperate with the relevant governmental authorities responsible for composting and anaerobic digestion plants.

# 2 | EXPLANATION OF TERMS USED IN THE CONTEXT OF BIOWASTE IN THIS STANDARD

#### Biowaste

Any plant or animal waste destined for 'end-use' (as defined below) that can be degraded by microorganisms, soil organisms or enzymes. This Standard is concerned only with biowaste of plant origin which may be derived from the following sources: kitchen (household or catering) waste, forest or wood-processing waste (e.g. bark, wood, sawdust), garden and park waste, waste from food markets, waste from agricultural processes (e.g. husk and cereal dust, potato peelings and washings), waste feeding-stuffs, human food stored beyond its safety period, waste from industrial food processes (e.g. fruit pulp, spent hops, sludges). The European Waste Catalogue (EU, 2001) lists and indexes types of wastes. Plant residues generated on land used for agricultural, horticultural or silvicultural purposes, and remaining on that land, are not included in this Standard but may be regulated by other national or international standards.

#### End use

Spreading of biowaste on soil, or mixing biowaste with soil, on agricultural, horticultural or silvicultural land or in landscaping; or mixing biowaste with other ingredients in the preparation of various plant growing media.

#### Heat treatment as defined in ISPM 5 (FAO, 2021)

The process in which a commodity [in this Standard: 'biowaste'] is heated until it reaches a minimum temperature for a minimum period of time according to an official technical specification.

#### Mixture

Treated biowaste which has been mixed with one or more of the following: other treated biowaste, untreated biowaste, farm manure, licensed fertilizers, soil, peat, minerals.

#### Pest as defined in ISPM 5 (FAO, 2021)

Any species, strain or biotype of plant, animal or pathogenic agent, injurious to plants or plant products.

#### Sanitation

Any treatment that aims to minimize the risk of spreading pests when the treated biowaste is used.

#### Treated biowaste

Either: (a) biowaste that has been subjected to an aerobic treatment process (compost); (b) biowaste that has been subjected to an anaerobic treatment process (anaerobic digestion residues).

# **3** | **PROCESSING OF BIOWASTE**

### 3.1 | Compositing

Most treated biowaste derives from composting in which the material is maintained in heaps for several weeks to allow the decomposing organisms to break down the organic material. Composting is an aerobic and exothermic process in which the temperature rises above 50°C (usually between 50 and 70°C) for several weeks or months, before dropping back again below 40°C as the decomposition process slows down (the process is shorter in indoor composting facilities). The compost heaps are periodically re-mixed ('turned') during this period to ensure even decomposition, although systems without turning but with forced aeration also exist. In industrial plants, composting is most commonly performed in long heaps ('windrows') which are turned mechanically. The windrows may be maintained outdoors (preferably protected from rain) or kept indoors in vessels or halls. Other systems of composting have been developed.

# 3.2 | Anaerobic digestion

In addition to the aerobic composting process, anaerobic systems are also used for decomposition of organic waste. Anaerobic digestion is a four-step approach, consisting of hydrolysis, acidogenesis, acetogenesis as well as methanogenesis, facilitated by different groups of microorganisms. This process can take place under different temperature regimes, i.e., under mesophilic (35-37°C) or thermophilic (50-60°C) conditions, respectively. Organic matter can be fed into the process in batches or continuously, in smaller or larger vessels or moving lines. For an optimal process, external factor control, such as temperature, pH and the carbon to nitrogen ratio (C/N ratio) of the feedstock, is essential. The process time depends on system configurations, feedstock composition and moisture content (wet, dry) (VKM, 2021). Apart from nutrient-rich liquid and solid digestates, anaerobic digestion generates methane (biogas) which is collected and used for energy production by combustion. After digestion, the solid residues can be subjected to an additional heat treatment or a composting stage.

# 4 | TREATMENT PROCESSES

Before end-use in agriculture, horticulture, silviculture, or landscaping, biowaste should be treated by the methods described below in order to eliminate pests, and tested by the methods described below, to determine their compliance with the requirement for freedom from the majority of pests or, where known or suspected to be present, freedom from quarantine pests and heat-tolerant pests. If the treated biowaste is mixed with other materials, the mixture components should, as relevant, comply with these requirements or be shown to be free from pests.

The treatment process will differ depending upon whether the waste is high risk or low risk. High risk waste is waste containing or suspected to contain quarantine pests or heat-tolerant pests. Other waste is considered to be low risk. Where there is any doubt about the pest risk posed by the waste the NPPO should be consulted.

# 4.1 | Minimum requirements for the treatment process

Minimum requirements aimed at eliminating most pests are defined below. Additional specific requirements related to biowaste known or suspected to contain quarantine pests or heat-tolerant pests are presented in section 4.2.

In order to prevent potential contamination, all treated material should be stored in such a way as to avoid any contact with untreated materials (direct contact, or contact though run- off water, wind, machinery, tools, storage containers, etc.).

# 4.1.1 | Composting

The processes at composting facilities should be managed in such a way as to guarantee a thermophilic temperature range and a high level of biological activity over a period of time that is sufficient to eliminate pests. This should be achieved under appropriate conditions of humidity (e.g. water content of 40% w/w) and nutrients, as well as by optimum structure and air conduction. In the course of the composting process, the entire quantity of materials being treated should be exposed to temperatures of 60°C or higher for several days (usually 3 days) or a temperature of at least 55°C for longer periods (e.g. a continuous period of 2 weeks). A minimum number of turnings may be required to ensure that the whole mass is exposed to the required time-temperature combination. When appropriate, countries should take account of national and supranational (EU, 2019) requirements for the treatment of biowaste.

The time-temperature combinations for composting mentioned above will eliminate most pests. However, it has been shown that some heat-tolerant organisms such as Synchytrium endobioticum; heat-tolerant viruses, e.g. Tobacco mosaic virus and other tobamoviruses, and viroids e.g. Potato spindle tuber viroid (pospiviroidae) can survive these time-temperature combinations. Some heat-tolerant pests may still be eliminated during composting. For example, several experiments have shown inactivation of tobamoviruses during composting which may result from microbial decomposition of the virus particles (Aguilar et al., 2010; Avgelis & Manios, 1989; Noble & Roberts, 2004; Ryckeboer et al., 2002b; Suárez-Estrella et al., 2002). However, the time needed for inactivation of such organisms may vary strongly depending on composting conditions. Therefore, direct process validation is recommended for the verification of the sanitation effect of a composting process for heat tolerant organisms (see section 6).

# 4.1.2 | Anaerobic digestion

The efficacy of sanitation via anaerobic digestion relates strongly to the technical conditions of the treatment process (temperature and time during which the organic material is exposed to the temperature). Two temperature types of anaerobic digestion can be distinguished: thermophilic at 50–60°C and mesophilic at 35–37°C (Van Overbeek & Runia, 2011).

A thermophilic anaerobic digestion exposing all material to 55°C for 24 hours is sufficient to eliminate insects and mites. Studies are available showing that relatively low temperatures are also sufficient to eliminate pests such as Meloidogyne incognita (Gokte & Mathur, 1995; Wang & McSorley, 2008), Ralstonia solanacearum (Date et al., 1993; Termorshuizen, 2006) and Xylella fastidiosa (Goheen et al., 1973; Sanderlin & Melanson, 2008). For pests that can tolerate temperatures above 50°C for longer periods, elimination during anaerobic digestion is uncertain although inactivation may already occur at lower temperatures by effects of the process itself (Henry et al., 2013; Ryckeboer et al., 2002a; Van Overbeek & Runia, 2011). For these pests, the biowaste should be heat treated or the process should be validated before it can be accepted as a phytosanitary control treatment (section 6).

It should be noted that tobacco mosaic virus is not eliminated by thermophilic anaerobic digestion (Ryckeboer et al., 2002a; Termorshuizen, 2006).

The temperature reached during mesophilic anaerobic digestion will generally not be sufficient to eliminate pests.

In conclusion, anaerobic digestion should be combined with a subsequent heat treatment (section 4.1.3) or composting (section 3.1) to be used as a phytosanitary control treatment aimed at eliminating most pests.

# 4.1.3 | Heat treatment

Heat treatment of biowaste can be used to eliminate pests. The recommended time-temperature combination is 70°C for 1 h. Heat treatment with this time-temperature combination will eliminate most pests (Bollen, 1985; Noble & Roberts, 2004). However, some heat tolerant pests may survive this treatment. For those pests, pest-specific evidence is needed, or the process should be validated (section 4.2 and 6).

During the heat treatment, the moisture content of the biowaste should be sufficient (e.g. 40% w/w) to guarantee heat transfer between and inside the particles. The specified temperature should apply to the whole bulk of material over a continuous period of time. For the heat treatment to be effective, the particle size should be small (e.g. preferably not thicker than 12 mm) and the biowaste should be homogenized to avoid dry pockets.

# **4.2** | Specific requirements for biowaste containing quarantine pests or heat-tolerant pests

Biowaste of plant origin known or suspected to contain any heat-tolerant or quarantine pests should receive a heat treatment specific to the pests using moist heat, before further treatments or processing. Prior to the heat treatment, contact of biowaste suspected to contain heattolerant or quarantine pests with other types of biowaste or untreated material should be strictly avoided. If these conditions cannot be fulfilled, all materials and biowaste that enter in contact with the biowaste suspected to contain heat-tolerant or quarantine pests, need to be subjected to a heat treatment before or after processing. The heat treatment is not required if it can be concluded that the minimum requirements for the treatment process (section 3.1) are sufficient to eliminate the pest. This conclusion should be based on:

1. Pest-specific evidence (scientific evidence showing that the treatment process is effective in eliminating the pest of concern). Examples of the specific heat treatment conditions for a number of pests (including references) are available in the supplementary information,

or

2. Direct process validation (the treatment process has undergone direct process validation and given the characteristics of the pest it can be concluded that this treatment is effective in eliminating it).

For quarantine pests, it is the responsibility of the NPPO to consider that the minimum requirements for the treatment process are effective, or to require specific heat treatment conditions.

For some quarantine pests, such as *Synchytrium endobioticum*, there is evidence that composting or specific heat treatment will not eliminate them (Pietsch et al., 2015; Steinmöller et al., 2012). For other pests, e.g. *Clavibacter sepedonicus*, there is conflicting information available on heat tolerance (Steinmöller et al., 2013; Stevens et al., 2021).

Finally, the treatment process should be authorized and supervised by the NPPO, including confinement conditions to prevent escape of any quarantine pest, and, if appropriate, specification of a 'non-risk' outlet for the end-use.

# 5 | COMPLIANCE

In general, both 'indirect process supervision' and 'product analyses' (see below) are used to determine whether treated biowaste complies with requirements. Treated biowaste should not be released for end-use unless all requirements are satisfied. Products having failed to meet any of the requirements should be treated again or should not be used for the end use specified in this Standard. Direct process validation or pest-specific evidence is required if the untreated biowaste is known or suspected to be infested with a quarantine pest or a heat-tolerant pest (see above).

Heat treatment facilities should be verified before the first treatment and at regular intervals (preferably at least yearly) thereafter by a technical expert.

#### 5.1 | Indirect process supervision

Temperatures should be recorded at regular intervals throughout the processing of the biowaste. If possible, these measurements should be continuous. They should be recorded in at least three representative zones of the biowaste unless the technical equipment of the process prevents access to these zones. During the thermal inactivation, the temperature should be recorded at least once every working day.

If the biowaste is subjected to a specific heat treatment, the temperature should be monitored and recorded continuously and automatically for each treatment period.

# 5.2 | Product analysis

The analysis of the product after treating biowastes (final product controls) should include tests to ensure that the final product meets specified phytosanitary requirements. These tests mainly concern the detection of viable seeds or reproductive parts of plants (Appendix 1). If other validated test methods for the detection of specified pests in treated biowaste exist, the product may also be tested for these pests. Product analyses should be carried out on appropriate samples at regular intervals and number of samples should depend on the capacity of the facility. An example is given below.

#### Example of a sampling procedure (DE, 1998)

Samples are taken at least every 6 months (for facilities with a capacity of up to 3000 tonnes per year), or every 3 months (for facilities with a capacity over 3000 tonnes per year), at every composting facility. If the product is stored before sale or distribution, product analysis should be done at the end of the storage period. The number of samples to be tested per year varies according to the capacity of the facility according as follows: (1) for facilities with a capacity of up to 3000 tonnes per year, six samples; (2) for facilities with a capacity of 3000–6500 tonnes per year, six samples, plus an additional sample for every 1000 tonnes or part thereof over 3000; (3) for facilities with a capacity of more than 6500 tonnes per year, 12 samples, plus an additional sample for every 3000 tonnes or part thereof over 6500.

The number of samples can be reduced (by up to 50%) if an adequate level of indirect process supervision is maintained. If viable weed seeds or reproductive parts of plants are detected during product analysis, the number

of analyses should be returned to the recommended level for at least 1 year before it can be reduced again. No viable weed seeds or reproductive parts of plants should be detected during this period.

Samples of approximately 6 litres should be taken, each composed of at least five different sub-samples per batch of finished compost or anaerobic digestion residue. The test result is considered satisfactory if none of the samples contain viable seeds or reproductive parts of plants. The presence of any viable seeds or reproductive parts of plants is considered to be evidence of failure in the treatment process. See Appendix 1 for details of the test.

# 6 | DIRECT PROCESS VALIDATION

Direct process validation is a verification of a composting or anaerobic digestion process to determine its efficacy in eliminating pests in biowaste. It should be distinguished from quality control methods such as indirect process supervision or product analysis. It can be used to evaluate whether a treatment process is effective in eliminating a heat-tolerant pest or a quarantine pest (Section 4.1 and 4.2). It is carried out once for a treatment process. Indicator organisms are used to determine efficacy at all stages of the process. Indicator organisms used should have similar characteristics to the pest to be eliminated. These organisms are buried in representative decomposition zones or are introduced in the process stages responsible for their inactivation. After completion of the process, they are removed, and tested for survival or infectivity. Examples of procedures are provided in Appendix 2.

Each direct process validation should be carried out in two test series, separate in time. In the case of open-air facilities, one of these test series should take place in the winter months. For windrow composting, the indicator organism(s) is/are buried in three different zones (top, middle and bottom) as well as at four different points representing a total of 12 individual samples per organism. In the case of an anaerobic digestion process, it may be justified to reduce the number of samples because of the more homogeneous conditions.

For adequate monitoring, the biowaste treatment facilities should have openings for burying and removing indicator organisms. If this is not possible, the process cannot be validated by the proposed method. Other efficacy test methods proposed by suitable experts may be considered.

# 7 | DOCUMENTATION/ LABELLING

Verifiable records of temperature patterns and compost restacking times (composting) should be kept in the archives for a sufficient time to allow supervision by the authorities and presented to supervisory authorities upon request. If treated biowaste is to be sold, distributed or exported, it should be accompanied by a 'compost passport', which should include the following elements:

- a. Name and address of the producer of the waste
- b. Quantity
- c. Description of the treated biowaste or mixture based on the nature of the materials used in unmixed form, including the type of treatment.
- d. Batch number and date of treatment or an identification number allowing trace-back
- e. Assurance that the requirements of this Standard are satisfied.

# 8 | SUPPLEMENTARY INFORMATION

This Standard also provides a compilation of pestspecific studies which can be used as scientific evidence showing if treatment conditions are effective (or not) in eliminating a pest of concern. Data for the table was collected in spring 2019 as a part of a project entitled *Plant health risks associated with side streams from potato processing* funded by the Ministry of Agriculture and Forestry of Finland (decision 532/03.01.02/2018) and during the revision of the Standard. No critical analysis of the validity of these studies was carried out, the data should therefore be interpreted with caution. The supplementary information can be accessed in the html version of this Standard on the publishers website.

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# SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of the html version of this article.

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#### **APPENDIX 1 - PRODUCT ANALYSIS**

A process to determine the content of viable seeds and plant propagules in the treated biowaste is described in CEN/TS 16201 (CEN, 2013). The sample is passed through a 20mm mesh sieve; the volume of the obtained fraction should be at least 6 L. A minimum of 3 L of the original sample is used as a test sample. The electrical conductivity (EC) and pH of the sample are determined and if necessary, adjusted to an EC of  $<50 \text{ mS} \cdot \text{m}^{-1}$  and pH 5.5-8 with unfertilized sphagnum peat and/or limestone as required. The test sample is moistened to an optimum moisture content according to 'fist test',<sup>1</sup> spread in seed trays (plastic dishes with perforated bottoms, or equivalent containers lined with a water-absorbent mat, and a perforated plastic sheet to keep them clean) and gently compressed to an even layer of approximately 20 mm. The trays are kept moist in accordance with good horticultural practice at 18-30 °C; lightning intensity at least 2000 lux for 12 hours/day; without direct exposure to sunlight for 21 days. The number of plants germinating during the 21 days growth period is recorded. For further details, refer to CEN/TS 16201.

# APPENDIX 2 - DIRECT PROCESS VALIDATION (EXAMPLES OF PROCEDURES)

Of the variety of plant pathogens and seeds that may be present in input materials for biowaste treatment facilities, the following indicator organisms have been selected for use in direct process validation: *Tobacco mosaic virus* (*tobamovirus*) (TMV), *Plasmodiophora brassicae* (clubroot) and tomato seeds. However, TMV should not be used for the testing of an anaerobic digestion process. A judgement should be made whether these indicator organisms are representative for the pest(s) that need(s) to be eliminated. If, in the case of samples which have been passed through the direct process validation, any of these organisms is found to have survived as indicated by any detectable level of infectivity for TMV (only relevant for composting) *or* clubroot *or* germination of tomato seeds, the direct process validation is considered to have failed.

#### 1. TMV test method

Testing of the composting process is carried out using the method of Bruns et al. (1993), based on that of Knoll et al. (1980), and Ryckeboer (2001), Ryckeboer et al. (2002a, 2002b).

#### 1.1. Preparation of test samples

TMV is inoculated into *Nicotiana tabacum* cv. 'Samsun' which becomes systemically infected by the virus.

Tobacco plants are grown under normal glasshouse conditions up to the 5-leaf stage. For inoculation, extracts from TMV-infected tobacco plants are prepared in 0.05 M sodium phosphate buffer, pH 7, and rubbed with a brush, glass spatula or with a gauze wad onto two or three lower leaves which have been lightly dusted with an abrasive. Care should be taken to avoid cross contamination between samples. Two to three weeks after inoculation, systemically infected leaves showing mosaic-like discolorations are harvested and can be used directly for the test (or can be stored frozen at about  $-20^{\circ}$ C for later use). For every sample, 10 g of TMV-infected tobacco leaves are placed in non-decomposable gauze sacks (mesh size  $1 \times 1$  mm). These samples are buried in the biowaste. It should be ensured that no sample material escapes into the surrounding compost. Control samples are TMV-infected leaves kept in the freezer at a temperature of approximately -20°C.

#### 1.2. Testing samples for infectivity

The gauze sacks are removed from the composting facility and the 10 g samples of tobacco leaves are immediately taken out, chopped with a sterile pair of scissors, and ground in 30ml of sodium phosphate buffer (0.05 M, pH 7.0) using a pestle and mortar or a sterilized blender. The resulting pulp is squeezed through a nylon net (mesh 1 mm). Plant sap is extracted from the control samples in a similar manner. To determine residual infectivity, the extracts are applied to the test plants Nicotiana glutinosa or N. tabacum cv. 'Samsun NN' using a modified halfleaf method. Four fully developed leaves (about  $90 \text{ cm}^2$  in size) are detached from plants at the 6-8 leaf stage grown under constant conditions (Walkey, 1991) and used for inoculation of each sample. The leaves are lightly dusted with Celite (abrasive) and the extract is applied to one half of each of the four leaves, while the opposite leaf halves are treated with control extract. The extracts are uniformly rubbed onto the leaves with rotating movements using a glass spatula (Walkey, 1991). Soon after inoculation, the leaves are rinsed for 10 s with running tap water. The inoculated leaves are then placed on several layers of moistened filter paper in a Petri dish or similar vessel (e.g. flat styrofoam boxes about  $35 \times 50$  cm that can be covered with a glass plate) and incubated in a growth chamber under controlled conditions (22-24°C; at least 16h of lighting at approximately 4000 Lux). Since the test plants are resistant to TMV, they develop small necrotic local lesions (small, round spots with a necrotic centre). Local lesions are counted 6 days after inoculation. No lesions should be detected in the test samples, while the controls should show typical lesion development.

#### 2. Plasmodiophora brassicae testing method

Testing of the composting or anaerobic digestion process is carried out using the method of Bruns

<sup>&</sup>lt;sup>1</sup>Fist test: Take a handful of compost, squeeze it firmly and then open the fist. If the compost is too dry, the compost will then fall apart.

et al. (1993), based on that of Knoll et al. (1980). Isolates of *P. brassicae* should be selected for heat tolerance (resistant to incubation at 65°C for 1 day) (Idelmann et al., 1998).

#### 2.1. Buried samples

The test material is composed of galls from cabbage plants infected with *P. brassicae*. This material is deep-frozen at  $-25^{\circ}$ C until the beginning of the test. When testing a composting process, samples are made up from 30 g of gall material, 430 g of soil and 200 g of biowaste to be tested, thoroughly mixed, and packaged in non-de-composable bags (maximum mesh size  $1 \times 1$  mm). When testing an anaerobic digestion process, samples are made with no additional soil around the galls (i.e. in the non-decomposable bags). These samples are buried in the biowaste. No sample material should escape into the surrounding biowaste. For the control, sterile sand replaces the biowaste and the samples are stored in damp, sterilized sand at room temperature during the test period.

#### 2.2. Test of infectivity by means of biotests

After being recovered from the treatment process, all samples have any coarse wood and stones removed from them and thoroughly ground. 325ml of each sample is mixed with 275 ml of a sand/peat mixture (30:70 v/v; sand steamed for 5 h at 80°C). The pH, which has a strong influence on the infectivity of P. brassicae, should be about 6, and can if necessary be adjusted by increasing or reducing the proportion of peat. This mixture of sample, sand and peat is added to a container into which four seedlings of a susceptible cultivar of *Brassica juncea* are planted. As P and K concentrations in the compost are normally high, there is usually no need to add nutrients in the containers holding the test samples. Fertilizer should be added to the control samples (250 mgN, 100 mg P<sub>2</sub>O<sub>5</sub>, 300 mg K<sub>2</sub>O and 100 mg Mg per L). The biotest is carried out in a randomized split-plot design with a 16h day at 8000 Lux and at a temperature of 16-18°C in the first week, and 22°C from the second week onwards. The growing period for the biotest is 5 weeks, after which plants are examined for disease symptoms (root galling). No infection should be detected in the test samples, while the controls should show typical disease development.

#### 3. Testing method for tomato seeds

Testing of the composting or anaerobic digestion process is carried out according to the method of Pollmann and Steiner (1994).

#### 3.1. Buried sample

Seeds of tomato (*Solanum lycopersicum*, cv. Saint Pierre, [synonyms S. Pierre, San Pedro]), 1 g or 400 seeds, are poured into a small bag made of non-decomposable gauze (mesh size  $1 \times 1$  mm) and spread over the entire gauze surface as thinly as possible. The closed bag is put in a sample sack containing at least 5 L of fresh biowaste from the batch to be investigated. In the case of anaerobic digestion, a similar quantity of tomato seeds contained in a carrier with semi-permeable membranes is introduced into the process. The germination rate of the tomato seeds should be determined before testing and only seeds with a germination rate of at least 90% should be used.

#### 3.2. Germination test

The germination test should normally start immediately after removing the samples from the compost. If the samples have to be transported or stored, they should be kept cool in an airtight container (cool box, refrigerator). 200 tomato seeds are counted from each bag. The remaining seeds are dried under room conditions (approximately 20°C and 20–50% relative humidity) and then placed into an airtight container and kept in the refrigerator as a reserve. The counted seeds (washed, if necessary) are divided into 4 lots of 50 seeds and spread on four layers of moist filter paper in four covered 9 cm Petri dishes, maintained at 25°C and exposed to light in a suitable room or growth cabinet. Germinated tomato seeds are counted at 7-day intervals and removed from the Petri dishes. A seed is considered to have germinated if its root and/or shoot has visibly emerged. Counting continues until no more seeds have germinated. If no seeds have germinated after 21 days, the germinating capacity tests are concluded. The total number of germinated seeds should be recorded and expressed as a percentage of the seeds used in the tested aliquot (200 seeds). No germination should be detected in the test samples, while the controls should show normal seed germination.