Phytosanitary risks of reuse of waste streams and treated wastes for agricultural purposes

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Table of contents

Summary

Possible risks related to reuse of biowastes and soil tares contaminated with phytopathogens (i.e., all organisms causing pests and diseases in plants) and weed seeds were investigated. Reuse of agricultural wastes, e.g., for bio-energy production and biofertilization of arable land has recently been gaining interest. Organic wastes of agricultural origin and soil tare (from potato tubers, sugar beets, chicory, carrots, flower bulbs, etc.) can be contaminated with phytopathogens and/ or weed seeds, and this aspect requires attention before reuse for agricultural applications. Processing of these waste streams, e.g., by composting and (co-) fermentation, should eliminate most, if not all phytopathogens and weed seeds from the final products. Data on survival of phytopathogens and weed seeds during composting can be found in literature, but information on survival in soil tares and during (co-) fermentation scarcely can be found. The purpose of this report is to assess potential risks associated with the reuse of waste streams from agriculture, to demonstrate knowledge gaps in our current understanding on phytopathogen and weed seed survival during processing of waste, like composting and (co-) fermentation, and to indicate which information will be required for safe production of end products from these processes (compost, digestate). Most phytopathogens and weed seeds do not survive composting and anaerobic fermentation, however, a few species can (e.g. _Clavibacter michiginensis_ ssp. _sepedonicus_, _Synchytrium endobioticum_ were shown to survive composting and _Plasmodiophora brassicae_ composting and mesophilic anaerobic fermentation). Because composting and anaerobic fermentation are complex processes, involving many different parameters affecting phytopathogen and weed seed survival, it is not possible to make generalized statements on their survival during these processes. Survival of phytopathogens and weed seeds in soil tares and eradication of these by (heat) treatments were scarcely found in literature. Further, data on phytopathogen and weed seed survival during thermophilic anaerobic fermentation was not found in literature. Biogas production via thermophilic co-digestion may be applied in the near future and additional information will be required to formulate guidelines for 'safe' (i.e. free of phytopathogens and weed seeds) production of biofertilizer from co-digestate.

1. Scope and delineation of the report

This literature survey was executed under the commission of the Food and Consumer Product Safety Authority (VWA), division Plant (former Plant Protection Service). The scope of this report was to make an inventory, based on published data, on all organisms causing pests and diseases in plants (in this report referred to as 'phytopathogens') that can be present in agricultural waste streams like soil tare, compost and digestate. Based on discussions with representatives of the former Plant Protection Service, Food and Consumer Product Safety Authority and the Dutch Ministry of Agriculture, Nature and Food Quality, it became clear that there are no regulations for reuse of soil tares and organic wastes processed by composting or anaerobic fermentation with respect to occurrences of phytopathogens and weed seeds. Especially, there are no regulations on the use of materials of plant origin for processing by (co-) fermentation, whereas these do exist for manure and animal-derived products. This can lead to phytopathogen dissemination due to the use of solid and liquid end-products as fertilizer for agricultural purposes. Therefore, the direction of this literature search is on the safe (re)use of waste streams (crop waste, soil tare, compost and digestate) for agricultural purposes.

The literature was screened for those phytopathogens that can survive in crop wastes and soils on land and in organic wastes that are processed by composting and anaerobic fermentation. Potential risks and gaps in our current understanding on phytopathogen survival under anaerobic fermentation conditions were identified. This led to recommendations for experimental research on the elimination of risks related to phytopathogen survival in anaerobic fermentors and infestations in arable crops.

2. Introduction

2.1 Waste streams

Wastes of plant origin and soil tares can be contaminated with viroids, viruses, bacteria, fungi, oomycetes, nematodes and arthropods that cause diseases in, or damage to crop plants, collectively called phytopathogens in this report. Further, plant-derived wastes and manure can contain viable weed seeds. These wastes originate from agriculture and horticulture, but also from industry, and may pose risks on plant production when reused, either in unprocessed or processed forms. In general, unprocessed plant wastes for reuse will be returned to the field of origin, although soil tares are also applied to other agricultural production fields. However, commercially processed plant wastes (compost and digestate) can be applied to other fields, and therefore can pose a much larger threat to plant production and export of agricultural products (e.g. seeds and seed potatoes). Therefore, the focus of this report will be on processing of plant wastes with special emphasis on (co) fermentation because of the renewed interest for bio energy production and the need to reduce local manure surplus.

2.2 Waste stream processing

Organic wastes are energy rich and can be used for the production of bio-energy. Processes used to obtain energy from waste streams are: torrefaction, a carbonization process at 200 to 400°C (mild form of pyrolysis), and incineration. Due to the high temperatures reached at torrefaction and incineration, it is not expected that biological contaminations play a role in end products. This is different from composting and (co-) fermentation where temperatures remain lower and where eventually biological contaminations still can occur in the end products.

Survival rates of phytopathogens and weed seeds in wastes are affected by the conditions prevailing during processing by composting or co-fermentation. The most important factor controlling phytopathogen and weed seed survival is temperature.

Composting is a process that occurs under aerobic conditions and to keep the composting pile well aerated, it is important to keep moisture content low (between 60 to 70% moisture at the start, and around 45% in the end product) (Ryckeboer *et al.*, 2003). At the start of composting, readily available nutrients will be consumed by saprophytic bacteria. Typically, the temperature will rise, and the microbial community in the waste material will change in activity and composition. The thermophilic stage will last approximately 15 days reaching peak temperatures between 70 and 75°C (Ryckeboer *et al.*, 2003). Then the heap will cool down and maturation of the final product, in the form of compost, will take place.

Co-fermentation is different from composting as it occurs under anaerobic conditions (Table 1). The process takes place in a slurry (high moisture content). The eldest form of fermentation is mesophilic fermentation (35 – 37°C, for 20 – 25 days) (Angelidaki & Ellegaard, 2003). Thermophilic plants were introduced later in time and in these plants, peak temperatures between 50 – 60°C were reached during 15 days. For veterinarian sanitation purposes, post-sanitation steps are integrated in the fermention processes where temperatures above 50°C are applied. The effects of these post-sanitation steps have not been evaluated yet on phytopathogens and weed seeds.

Table 1.   *Similarities and differences between composting and anaerobic fermentation.*

Composting	Anaerobic fermentation
Peak temperature: 60 – 70ºC	Mesophilic: 30 – 40ºC
	Thermophilic: 50 – 60ºC
Time at peak temperature: 15 – 20 days	Mesophilic: 30 days
	Thermophilic: 15 days
Aerobic	Anaerobic
Low moisture content (40 – 60%)	High moisture content (> 90%)
No or low fermentation	High production of fatty acids
No or low gas production	High gas production: CH_4, H_2, H_2S, NH_3, N_2O, CO_2

2.3   Fermentation and co-fermentation

Digestion (synonymous for anaerobic fermentation [AF]) of organic wastes for bioenergy production can be performed with 100% input of plant materials (e.g., [fodder] maize). The remains of this process, digested plant materials called 'digestate', can be used for fertilization of arable land because of its high mineral composition (Voća *et al.*, 2005). During fermentation, energy-rich compounds will be converted into gases like methane, hydrogen, hydrogensulfide, ammonia, nitrous oxide, and carbondioxide, and released from the waste fractions, whereas the mineral composition overall will remain unaffected. To derive energy (biogas) from manure, the addition of energy-rich materials is needed and this process is called co-fermentation. Manure itself does not contain enough energy rich compounds for biogas production (Angelidaki & Ellegard, 2003). The digestate derived from cofermentation can be used as fertilizer.

Energy-rich compounds (co-products) must be derived from organic wastes originating from agriculture, but also from industry (processed agricultural materials), food industry, household wastes, waste from gardens, wood clippings, and verge cuttings (Businessplan Boerderij Plus Rapportage, Van den Berg *et al.*, 2003, *in Dutch*). Input materials for co-fermentation should consist for at least 50% of manure, and for the rest of co-products, if digestate is to be used as fertilizer. End products derived from (co-) fermentations are regulated for the presence of minerals (N), for occurrences of human pathogens, (according to the Dutch legislation on manure application to arable land), and the presence of toxic compounds like heavy metals. Not all organic materials can be used for co-fermentation. There is a so called 'positive list' (website Dutch ministry of LNV, 'hetlnvloket' appendix Aa part IV, *in Dutch*) that prescribes which organic materials are legally allowed for use in co-fermentation in the Netherlands. There is thus legislation on the use of animal-derived materials for biogas production by co-fermentation, to prevent contamination of human pathogens in fermentation residues. However, there is no official regulation on the occurrence of phytopathogens or weed seeds in (co)-fermented end products. Nowadays, maize (fodder maize) is most commonly used for biogas production by co-fermentation in the Netherlands, because co-fermentation is still in an experimental stage at most occasions. However, this situation may change in the near future when other organic wastes can be used and control on the presence of phytopathogens and weed seeds in AF residues can become opportune.

2.4   Scope and outline of the report

Based on existing data in scientific literature, an overview was made on survival of phytopathogens and weed seeds in soil tares and in organic wastes that are treated by composting and co-fermentation. Predictions were made on phytosanitary risks related with soil tares and organic wastes with special emphasis on co fermentation. Due to the difference in oxygen levels and temperature present at composting and co-fermentation, major differences in chemical and microbiological composition can be expected between both waste processing types. The most important differences between composting and co-fermentation are summarized in Table 1. The impact of composting and co-digestion on phytopathogens and weed seed survival in waste streams thus will be different.

Key questions addressed in this report are:

1. Which phytopathogens and weed seeds were able to survive soil treatments by solarization and biological soil disinfestation and by pasteurization (steaming at a high temperatures)?
2. Which phytopathogens and weed seeds were demonstrated to survive compost processing?
3. Which phytopathogens and weed seeds were demonstrated to survive AF processing?
4. What are the knowledge gaps in our current understanding about phytopathogen and weed seed survival in waste processing, with special emphasis on recent developments in waste stream processing like AF?

3. Phytopathogens and weed seeds able to survive soil treatments

3.1 Crop wastes

Phytopathogens can be present in waste streams from agriculture or industry after processing of agricultural products (Breukers, 2002). The composition of phytopathogens in organic wastes will vary depending on the waste source. Examples are given to indicate that each type of organic waste can be contaminated with specific phytopathogens. Contaminated wastes can play important roles in the epidemiology of plant diseases.

Wastes from processed potato tubers (tuber peels and waste products from starch production) can be contaminated with *Pectobacterium* and *Dickeya* species (Czajkowski *et al.*, 2009), or with *Ralstonia solanacearum*, which is also a quarantine organism in the Netherlands, albeit present in some Dutch canals and streams (Wenneker *et al.*, 1999). Waste from agricultural production can cause infestations in recurrent crops on the same fields, and may play a central role in the epidemiology of plant diseases, which was demonstrated for two bacterial pathogens, *Pseudomonas syringae* pv. *pisi* (Hollaway & Bretag, 1997) and *Xanthomonas campestris* pv. *vitians* (Barak *et al.*, 2001). Recently, it was shown that waste from harvested and processed leek plants returned to leek fields was responsible for infection with *Pseudomonas syringae* pathovar *porri* in follow up leek crops on the same field (Van Overbeek *et al.*, 2010). Contamination of trunks, twigs, bark, and leaves of woody plants with *Phytophthora ramorum* is another example of organic waste materials that can pose a risk on plant production (Davidson *et al.*, 2008). Occasionally 'new' phytopathogens were found whose epidemiologies are mostly unknown, like *Pseudomonas syringae* pv. *aesculi* in horse chestnut trees (Schmidt *et al.*, 2008). More information is needed on epidemiological and ecological aspects for control of these phytopathogens in agricultural production.

3.2 Soils

Soils can become infested with phytopathogens and weeds that are difficult to eradicate. However, measures can be applied to reduce phytopathogens or weed seed numbers.

Addition of compost to soil, artificially contaminated with a *R. solanacearum* biovar 2 strain, in combination with solarization led to a stronger decrease in phytopathogen density in treated soil than in untreated (control) soils (Schönfeld *et al.*, 2003). Temperature was considered to be an important factor in the reduction in *R. solanacearum* survival in soil, as was also found elsewhere (Date *et al.*, 1993). Survival of vegetative sporangia and zoospores of *Olpidium brassicae* in soil were affected at temperatures of 40 to 45°C (for 10 minutes), whereas resting spores were not affected (Campbell & Lin, 1976). In another study, a temperature of 80°C was needed to eliminate sclerotia of *Sclerotinia sclerotiorum* in dry soil, whereas a temperature of 60°C was sufficient for total eradication of these structures in moist soils (Van Loenen *et al.*, 2002; Shlevin *et al.*, 2004). Sclerotia of *Sclerotinia minor* and *S. sclerotiorum* were more affected in their survival in soil when exposed to a temperature of 35°C than to temperatures of 25 or 15°C, and there was a clear effect of the soil water potential on survival (Wu *et al.*, 2008). At 35°C, survival rates were lowest at higher (-0.3 and -0.01 MPa), than at lower water potentials (< -0.01 MPa). Further, an effect of oxygen availability on sclerotia survival was found in the same study, and survival was lower at an 'ultralow' oxygen concentration of 0.1% than at concentrations of 1.0 and 21%. Germination of ascospores of *Gibberella zeae* was affected by the interaction of temperature and relative humidity (Gilbert *et al.*, 2008). Highest applied temperature (30°C) resulted in lowest germination rates in all cases, but rates were consistently lower at 60% than at 30 and 90% relative humidity. *Phytophthora nicotianae* chlamydospores had lowest survival in soil saturated with water, however, survival decreased at higher temperatures at all applied water matric potentials (0, -10, -30 kPa) (Coelho *et al.*, 2001). Next to temperature, other factors also contribute to phytopathogen declines in soils. Combined effects often result in highest reductions in phytopathogen densities in soils.

Soil solarization (combination of heat, low oxygen availability, and volatile accumulation) was successfully applied to reduce *Meloidogyne javanica* numbers (nematodes, infected plants and galls on roots) in soil (Candido *et al.*, 2004). However, soil solarization cannot be applied in regions in temperate climate zones, where biological soil disinfestation can be a better solution for control of phytopathogens and weed seeds. Biological soil disinfestation (combination of low oxygen availability, volatile accumulation, and possible shifts in soil microbial communities) aimed to reduce phytopathogen numbers in soils was shown to be successful for *F. oxysporum* f. sp. *asparagi, Rhizoctonia solani*, and *Verticillium dahlia* (Blok *et al.*, 2000), *Pratylenchus fallax* (Goud *et al.*, 2004), and *R. solanacearum* biovar 2 (Messiha *et al.*, 2007).

3.3   Soil tares

Soil tare is the soil adhering to harvested products like potato tubers and sugar beets. Due to the close contact with plants, these soil tares may contain phytopathogens, and once removed from crop products, these soils are waste products likely to be infested with phytopathogens. For instance, soil tares from potato tubers and sugar beets can be contaminated, respectively, with potato (*Globodera spp.*) and sugar beet (*Heterodera* spp.) cyst nematodes. High temperature treatments may be the best option to eradicate these phytopathogens in soil tares. Treatment with aerated steam at 50 or 60°C for three minutes resulted in complete elimination of survival structures of *V. dahliae, S. sclerotiorum, Sclerotium cepivorum, Pythium ultimum*, potato cyst nematodes of *Globodera rostochiensis,* and *G. pallida,* and seeds of *Chenopodium album,* and *Agropyron repens* in agricultural soil (Van Loenen *et al.*, 2003). Temperature treatments at minimally 70°C for at least 30 minutes were successfully applied by growers to eradicate most phytopathogens from soil tares in the Netherlands (Runia, 2000 and 2010). Different disinfection treatments meant to eradicate phytopathogens from soils were evaluated by Runia (2000 and 2010).

4. Phytopathogens and weed seeds demonstrated to survive compost processing

4.1 Microbial community shifts during composting

The microbial composition and activities in biowaste shift during composting. It was found that fungi and Streptomycetes species were most affected in their survival and activity at the thermophilic phases of composting (peak temperature of 55°C and above), whereas *Bacillus* and *Paenibacillus* species proliferated under these circumstances (Ryckeboer *et al.*, 2003). Fungi and Streptomycetes numbers reached detectable numbers during the cooling and maturation phases. Remarkable was the number of unidentifiable *Bacillus* species found during the thermophilic phase, indicating that still a lot can be learned about microbiological processes occurring at composting. However, the factors that affect micro-organisms indigenous to biowaste, used as input material for composting, also can affect resident (inoculant) phytopathogen populations.

4.2 Phytopathogen survival during composting

Peak temperatures between 64 and 70°C for 21 days appeared to be sufficient to reduce three out of nine studied viruses, all seven bacterial, 33 of 38 oomycete, and fungal, and all nine nematode phytopathogens to undetectable levels, as reviewed in Noble & Roberts (2004). Not all results shown in this study were derived from composting or anaerobic digestion systems, and some of the data were gathered from in vitro studies (e.g., on survival of *S. endobioticum* at 60°C in water). Although most phytopathogens will be eradicated in compost heaps, there is a potential risk that the eradicating temperatures will not be reached in cooler regions of the heap and in protected areas (so called 'pockets'). It was shown that phytopathogen survival was affected by location (as affected by differences in peak temperature) and incubation time in a static compost pile (Downer *et al.*, 2008). Also, differences in input materials influenced phytopathogen survival. Of all phytopathogens studied by Downer *et al.*, (*Armillaria mellea, Phytophthora cinnamomi, S. sclerotiorum, Tylenchulus semipenetrans*), the sclerotia-forming fungi (*S. sclerotiorum*) survived longest over all tested treatments during composting. It was therefore recommended to regularly turn composting heaps in order to eradicate most phytopathogens in the composting material (Noble & Roberts, 2004).

Validation of the compost process, by making use of 'tester organisms' (Tobacco mosaic virus, Enteric human pathogens like *Escherichia coli,* and *Salmonella enterica, P. brassicae,* and tomato seeds), and temperature logging inside the heap should warrant from phytosanitary risks of the end products (Noble *et al.*, 2009). However, the use of tester strains was not always reliable, and also there was not enough information on absolute eradication of quarantine phytopathogens like *Guignardia citricarpa, S. endobioticum, Tilletia indica, Phytophthora kernoviae, C. michiganensis* subspecies *michiganensis,* and potato spindle tuber viroid under composting conditions (Noble *et al.*, 2009).

Plasmodiophora brassicae, artificially-inoculated in Chinese cabbage, and naturally present in Brussels sprouts, was shown to survive composting in experimental small-scale set ups as well as in a large-scale windrow set up for seven days at peak temperature of 50°C or for one day at 60°C (Fayolle *et al.*, 2006). However, survival differed between two *P. brassicae* isolates, but for both isolates, the moisture content of the composting matrix influenced survival most (higher moisture content resulted in lower survival). In a study done by Bollen and coworkers (1989), it was shown that *P. brassicae* survived the heat phase of composting (2-3 weeks at 50-70°C), but not the entire composting process. In their discussion, Fayolle et al contributed the discrepancy found between observations made in their study and in that in Bollen et al to the differences in matric potential (measure for the moisture content in the composting matrix). The matric potential in the study of Bollen et al was lower than in that of Fayolle et al, illustrating

again that peak temperature alone during composting is not indicative for survival of plant pathogens. Under the same composting conditions, Bollen et al demonstrated that *O. brassicae* also survived the entire composting process, whereas 15 other phytopathogens (*Botrytis aclada, Colletotrichum coccodes*, four formae speciales of *Fusarium oxysporum, Phomopsis sclerotioides, Phytophthora cryptogea, P. infestans, Pyrenochaeta lycopersi, S. sclerotorium, S. cepivorum,* and *Stromatinia gladioli*) did not.

Uninucleate *Rhizoctonia* species (teleomorph *Ceratobasidium bicorne*), a root pathogen in forest nurseries, was shown to survive composting processes at temperatures of 37, 42, and 46°C, all exceeding the maximum temperature required to inhibit growth of the pathogen under laboratory conditions (35°C) (Veijalainen *et al.*, 2005). The authors explain their results by the protection that was provided to the inoculated pathogen in plant materials present in the composting matrix. This illustrates that extreme care should be taken with maximum temperatures that allows growth of phytopathogens measured under laboratory conditions. In the same study, uninucleate *Rhizoctonia* species were not demonstrated in baiting experiments with Norway spruce seedlings at a composting temperature of 60°C. Under about the same conditions (between 40 and 60°C), *P. cinnamomi, Pythium irregulare, R. solani, B. cinerea,* and *Dickeya chrysanthemi* were shown not to survive (Hoitink *et al.*, 1976). In their review, Noble & Roberts (2004) concluded that peak temperatures between 64 and 70°C during composting were sufficient to reduce numbers of viral, bacterial, fungal, oomycete, and nematode phytopathogens to below the limit of detection. Based on measurements in compost, the authors concluded that *P. brassicae, F. oxysporum* f.sp. *lycopersici, Macrophomina phaseolina,* and Tobacco mosaic virus (TMV) were more temperature tolerant (they all were shown to survive peak temperatures of between 62 and 74°C for at least 20 days).

Synchytrium endobioticum survived at 60°C for two hours, but survival during compost processing was not demonstrated for this pathogen at the time of publication of the review. However, in a later study possible viable spores of this phytopathogen were found to survive composting at a maximal temperature of 65°C for maximally 21 days (Steinmöller *et al.*, 2007). In the same study, *C. michiganensis* ssp. *sepedonicus* also was demonstrated to survive composting under the same conditions. Also, both phytopathogens were demonstrated to survive a succeeding 'sanitation' step, at 70°C for two hours. It can thus be concluded that composting, when reaching peak temperatures between 60 – 70°C, will eradicate most, but not all phytopathogens. Of the surviving ones, some even were shown to survive post-pasteurization steps. These phytopathogens are risky in the sense that they were shown to survive composting of host plant materials, and were present in the final compost product that can be used for agricultural applications (Table 2).

4.3   Weed seed survival during composting

Critical temperatures to prevent germination of seeds of 10 weeds (*Alopecurus myosuroides, Avena fatua, C. album, Cirsium arvense, Galium aparine, Polygonum persicaria, Rumex obtusifolius, Senecio vulgaris, Sonchus asper,* and *Veronica persica*) was in the range of 50 to 80°C (Thompson *et al.*, 1997). Within this temperature range, weed seed germination was completely inhibited upon composting, and this range was higher than previously assumed. Critical parameters influencing weed seed survival were: (i) buffering against high temperature by the surrounding matrix, (ii) water moisture content of the composting material, and (iii) possible occurrence of toxic compounds in compost that prevent weed seeds from germination. In another study, major differences in seed germination of six weed species (*Sonchus oleraceus, Echinochloa crus-galli, Solanum nigrum, Portulaca oleracea, Sisymbrium irio,* and *Amaranthus albus*) upon exposure to high temperatures were observed (Dahlquist *et al.*, 2007). Full mortality rates were reached after treatment at 50°C between 4 (*Sonchus oleraceus*) and 113 (*Amaranthus albus*) hours. Remarkably, seeds of all species were able to survive between 0.17 and 0.67 h upon treatment at 70°C, confirming the conclusion made by Thompson *et al.* (1997) that it can be difficult to eradicate weed seeds from compost even at high temperatures. Seeds of *Sorghum halepense*, A*maranthus* sp. (primarily *hybridus* and palmeri), *Kochia scoparia* L. Schrad, *Echinochloa crus-galli* L. Beauv), and *Sorghum bicolor* L. Moench were found not to survive composting at a peak temperature of 49°C for minimally three days, whereas seeds of *Convolvulus arvensis* could survive this condition (Wiese *et al.*, 1998). After exposure at a peak temperature of 83°C for seven days, all seeds, including the ones of *Convolvulus arvensis*, did not survive. Differences in survival between weed species occur during composting, and this makes it difficult to present a generalized statement on weed seed survival during composting.

Table 2.   Phytopathogens that were shown to survive and not to survive composting.

Pathogen	Reference
Pathogens that survive composting	
Plasmodiophora. brassicae	Fayoll *et al.*, 2006
Olpidium brassicae	Bollen *et al.*, 1989
Rhizoctonia spp.	Veijalainen *et al.*, 2005
Fusarium oxysporum fsp. *lycopersici*	Noble & Roberts, 2004
Macrophomina phaseolina	Noble & Roberts, 2004
Tobacco Mosaic Virus	Noble & Roberts, 2004
Clavibacter michiganensis ssp. *sepedonicus*	Steinmöller *et al.*, 2007
Synchytrium endobioticum	Steinmöller *et al.*, 2007
Pathogens that do not survive composting	
Armillaria mellea	Downer *et al.*, 2008
Phytophthora cinnamomi	Downer *et al.*, 2008
Sclerotinia sclerotiorum	Downer *et al.*, 2008
Tylenchulus semipenetrans	Downer *et al.*, 2008
Botrytis aclada	Bollen *et al.*, 1989
Colletotrichum coccodes	Bollen *et al.*, 1989
Fusarium oxysporum	Bollen *et al.*, 1989
Phomopsis sclerotioides	Bollen *et al.*, 1989
Phytophthora cryptogea	Bollen *et al.*, 1989
Phytophthora infestans	Bollen *et al.*, 1989
Pyrenochaeta lycopersi	Bollen *et al.*, 1989
Sclerotinia sclerotorium	Bollen *et al.*, 1989
Sclerotium cepivorum	Bollen *et al.*, 1989
Stromatinia gladioli	Bollen *et al.*, 1989
Dickeya chrysanthemi	Hoitink *et al.*, 1976
Pythium irregulare	Hoitink *et al.*, 1976
Rhizoctonia solani	Hoitink *et al.*, 1976
Botrytis cinerea	Hoitink *et al.*, 1976
Cucumber green virus	Noble & Roberts (2004)
Mottle mosaic virus	Noble & Roberts (2004)
Lettuce big vein virus	Noble & Roberts (2004)
Melon necrotic spot virus	Noble & Roberts (2004)
Pepper mild mottle virus	Noble & Roberts (2004)
Tobacco necrosis virus	Noble & Roberts (2004)
Tomato mosaic virus	Noble & Roberts (2004)
Tomato spotted wilt virus	Noble & Roberts (2004)
Erwinia amylovora	Noble & Roberts (2004)
Pseudomonas savastanoi pv. phaseolicola	Noble & Roberts (2004)
Ralstonia solanacearum	Noble & Roberts (2004)
Didymella lycopersici	Noble & Roberts (2004)
Pseudocercosporella herpotricoides	Noble & Roberts (2004)
Sclerotium rolfsii	Noble & Roberts (2004)
Thielaviopsis basicola	Noble & Roberts (2004)
Verticillium dahliae	Noble & Roberts (2004)
Phytophthora cryptogea	Noble & Roberts (2004)
Phytophthora ramorum	Noble & Roberts (2004)
Globodera rostochiensis	Noble & Roberts (2004)
Heterodera schachtii	Noble & Roberts (2004)
Meloidogyne chitwoodii	Noble & Roberts (2004)
Meloidogyne incognita	Noble & Roberts (2004)

4.4   Human pathogen survival during composting

Survival of human pathogens (*S. enterica* ssp. *enterica*) during composting has been described in literature and their dynamics during composting may serve as a models for phytopathogens. Total eradication of *S. enterica* ssp. *enterica* was achieved during composting at temperatures between 40 – 45°C after seven to 14 days, between 50 and 55°C after seven days, and at 60°C after ten hours (Ceustermans *et al.*, 2006). In this study it was also shown that moisture content of the composting matrix influenced survival, and the lower the matrix moisture content, the better was survival.

4.5   Concluding remarks on phytopathogen and weed seed survival during composting

It can be concluded that composting will have a 'sanitary' effect on infected plant materials and weeds seeds. Phytopathogen survival structures and seed numbers can strongly be reduced during composting. However, composting processes do not always result in total eradication of spore, cyst and seed numbers, and this is a risk for agricultural use of the final product. High peak temperatures (62 – 70°C) in combination with an incubation time of 20 days will result in eradication of most, but not all phytopathogens. Most risky are those phytopathogens that form resting spores and survival of these phytopathogens cannot be predicted on forehand by laboratory studies done at set temperatures and time periods outside the composting matrix. Further, circumstances favoring or counteracting survival of phytopathogens and weed seeds in compost heaps will differ between different composting types.

From composting the following lessons can be learned:

1.   Many phytopathogens could not survive composting (Table 2),
2.   Phytopathogens demonstrated to survive composting at higher (> 50°C) temperatures and additional pasteurization steps are: *P. brassicae, S. endobioticum* and *C. michiganensis*,
3.   Higher temperature is an important factor governing phytopathogen survival in compost heaps, but not the only one,
4.   Conditions affecting phytopathogen and weed seed survival are different in different composting systems,
5.   Attention must be paid for those phytopathogens that drop to undetectable levels during composting, but that still might be present in the final product.

5. Phytopathogens demonstrated to survive anaerobic fermentation processing

5.1 Differences between anaerobic fermentation and composting

Anaerobic fermentation (AF) differs from composting in the sense that digestion of organic substrates takes place under anaerobic conditions (Table 1). As a result, other compounds than methane and hydrogen sulfide are produced, such as ammonia, nitrous oxide, acetic acid, propionic acid, butyric acid, valeric acid, caprylic acid, heptanonic acid, and other fatty acids. Susceptibility of phytopathogens to these compounds will differ among different (microbial) groups, and the fate of phytopathogens present in plant-derived materials will depend on the presence of these volatiles. Also, the structure of the microbial communities resident in fermenting material will change overtime towards a thermophilic and anaerobic community as was shown in Ueno *et al.* (2006). As expected, microbial competition for available nutrients in fermenting plant materials will change. Further, the moisture content necessary for AF is higher than for composting. Phytopathogen survival rates in biowaste thus will differ between composting and AF.

5.2 Survival of human pathogens during anaerobic fermentation

In AF plants, survival rates of human pathogens are more often studied than of phytopathogens. The dynamics of human pathogens during anaerobic fermentation may serve as models for phytopathogens. The effects of fatty acids, typically produced during AF, on *Escherichia coli* survival were studied (Abdul & Lloyd, 1985). It was found that fatty acid toxicity depends on the applied concentration and on the chain length of the fatty acid molecules. Most enteric bacteria possess mechanisms that protect cells against toxic effects of fatty acids, however, enteric bacteria are adapted to fatty acid concentrations present in digestive track systems, and not to concentrations present in anaerobic digester systems, which are much higher. Differences in fatty acid composition and concentrations can be expected in different anaerobic reactor types, depending on waste input materials, and this can result in different effects on inoculant micro-organisms in AF plants. Differences among inoculant human pathogens were found in an anaerobic digesting plant, daily fed with substrate, and run at 28°C (Kearney *et al.*, 1993b). Decimal reduction times (T_{90} expressed in days) differed among the different studied human pathogenic bacteria: *E. coli, S, typhimurium* (*enterica*), *Yersinia enterocolitica, Listeria monocytogenes,* and *Campylobacter jejuni*. Inoculant *Y. enterica* had the shortest T_{90} (18.2), whereas *C. jejuni* had the longest (438.6). Although, all studied human pathogens are bacteria, their taxonomic identities are different. *E. coli, S. enterica,* and *Y. enterocolitica* are Gram negative bacteria belonging to the *Enterobacteriacea* (*Gammaproteobacteria*), *C. jejuni* also are Gram negative bacteria, but belonging to the *Epsilonproteobacteria*, and *L. monocytogenes* are Gram positive bacteria belonging to the *Firmicutes*. This illustrates that large differences in survival time in mesophilic AF substrates can be expected between taxonomic closely related species (T_{90} values of *Enterobacteriaceae* differed between 18.2 and 76.9 and of the different gram-negative bacteria between 18.2 and 438.6). However, T_{90} values of the same pathogen were different in different evaluated digestion types: semi-continuous and batch digestion (Kearney *et al.*, 1993a). *L. monocytogenes* had a higher mean T_{90} at semi-continuous (35.7) than at batch digestion (12.3). Higher solid matter content in AF substrate had a positive effect on *S. enterica* survival (Kumar *et al.*, 1999). An increase from 10 to 15% of solid matter content in AF substrate led to an increase of five days in *S. enterica* survival time (respectively from 20 to 25 days). These studies indicate that survival rates of phytopathogens during AF can vary between species under the same circumstances, and that different survival rates of the same species can be expected under different AF conditions.

5.3 Phytopathogen survival during anaerobic fermentation

Anaerobic fermentation was suggested to be a suitable method to reduce phytopathogen loads in waste materials that can serve as fertilizer on arable land (Turner *et al.*, 1983). In their study, Turner and coworkers showed that introduced populations of *F. oxysporum, C. michiganensis,* and *G. pallida* were strongly reduced during 10 days of AF at 35°C. *F. oxysporum* quickly declined, within one day, and repeated inoculation with fresh suspensions of *F. oxysporum* during the AF process also resulted in a fast decline of the introduced phytopathogen. *C. michiganensis* cell numbers declined five orders in magnitude in four days to undetectable cell levels during fermentation. Viable hatched *G. pallida* cysts were still observed after six days, and thereafter only dead larvae were found. Anaerobic fermentation can be regarded 'safe' in the sense that *F. oxysporum* and *G. pallida* phytopathogens were not demonstrated anymore in the final product, digestate. Based on linear extrapolation of the *C. michiganensis* decline curve the digestate was expected to be free of this pathogen within seven days. However, limitations of the detection technology limit reliable cell estimates of *C. michiganensis,* and bacterial decline curves tend to become non linear when reaching low detectable cell numbers. Cells thus might be present in the digestate for over a longer period in time than was estimated on the basis of linear extrapolations. and therefore care should be taken with this estimate. Considering the fact that *C. michiganensis* was difficult to eradicate from waste materials during composting, and that it is a quarantine phytopathogen in the Netherlands, digestate obtained by AF under mesophilic conditions should not directly be considered as 'safe' for production of propagation materials like seeds or seed potatoes.

F. oxysporum chlamydospores also were found to be sensitive to mesophilic AF by Termorshuizen *et al.* (2003). Three other phytopathogens (*R. solanacearum, P. brassicae,* and *S. cepivorum*) and one human pathogen (*S. typhimurium –S. enterica-*) were studied under the same circumstances. Introduced *S. enterica* and *R. solanacearum* were completely eradicated from the AF residues (digestate and filtrate). In their discussion the authors state that they did not check for the presence of viable but nonculturable (VBNC) cells in the AF residues. The presence of *R. solanacearum* VBNC cells in digestate may form a risk for agricultural production, although it is uncertain whether these cells are still virulent (Van Overbeek *et al.*, 2003). *P. brassicae* was eradicated in five of the six AF runs and diseased Chinese cabbage plants (serving as experimental bait plants) were found at one occasion, although the number of diseased plants brought into contact with digestate was lower than in the corresponding control experiments. *P. brassicae* was strongly reduced in number at the same (35°C, for 14 days) and higher (55°C, for 7 and 14 days) peak temperatures at AF, but not entirely eradicated from the end products, digestate (Engeli *et al.*, 1993). *P. brassicae* still can be a risk in digestate used as biofertilizer on arable land. *S. cepivorum* sclerotia were found to be present in all runs and introduced populations remained unaffected during mesophilic AF (Termorshuizen *et al.*, 2003). Clearly, these resting forms pose a risk to agricultural applications of digestate made under mesophilic conditions.

5.4 Thermophilic versus mesophilic fermentation types

Mesophilic fermentors are commonly used by small- and medium-scale biogas producers in the Netherlands. Thermophilic fermentors are less common, and therefore hardly any information is available on phytopathogen and weed seed survival in these fermentor types. Oocysts of the protozoan *Eimeria tenella* were visibly damaged and lost their virulence when applied to poultry manure and subjected to anaerobic digestion at 55°C (Lee & Shih, 1988). However, anaerobic digestion of the same inoculated material under mesophilic conditions (35°C) did not result in complete mortality and virulence loss of the oocysts. Treatment of oocysts suspended in saline and subjected to 35°C resulted in lower oocyst mortality and virulence than when under anaerobic digestion conditions, indicating that temperature is not the only factor relevant for survival of *E. tenella* oocysts under AF. Under thermophilic AF circumstances (peak temperature of 53°C), *L. monocytogenes, E. coli, S. enterica,* and *C. jejuni* survived for not more than 24 hours in the slurry, and all human pathogens were shown to be absent in the samples taken after seven days (Wagner *et al.*, 2008). The human pathogen shown to be most recalcitrant under mesophilic AF (*C. jejuni*) was present in one replicate sample after 24 h under thermophilic AF, whereas all other pathogens were eradicated from the AF substrates.

Reduction of plant seeds during AF (at 35 and 55°C) was studied in Engeli *et al.*, (1993) and it was found that *Solanum lycopersicum* (tomato) and *Rumex obtusifolius* seeds were completely destroyed during AF at both temperatures. This indicates that the 'sanitizing' effect of AF on biowastes under thermophilic circumstances is broader for different biological groups than under mesophilic circumstances.

5.5 Variation in phytopathogen and weed seed survival during anaerobic fermentation

There is much variation among AF types that are in use, and that means that there will be much variation in the conditions critical for phytopathogen and weed seed survival among the different fermentation types. A critical step for phytopathogen and weed seed survival during all fermentations is mixing of the liquid and solid phases. Without mixing, phytopathogen structures (cysts, spores) and weed seeds can float on the liquid fraction, resulting in a fast passage through the reactor and thus escaping from inactivating circumstances. However, mixing increases the risk on contamination of output streams with source materials. Another factor that will influence potential risks on phytopathogen and weed seed contaminations in digestate is the source of input material used for energy production during (co-)fermentations. The energy crop used for co-fermentation in the Netherlands nowadays is maize (http://www.senternovem.nl/). Fodder maize and rye grass were found to be suitable materials for production of hydrogen by direct fermentation (Kyazze *et al.*, 2008). If other materials will be used in the near future, than they must occur on the 'positive list' established by the Dutch agricultural ministry. On that list are waste products, out-of-date food products, and light alcoholic drinks, juices, ice, unpacked food, peels from fruits, potatoes, starch, vegetable oils, potato residues from starch production, waste products from vegetables, beets, grains, grass, etc. Because maize is already used as 'energy' crop for co-fermentation in the Netherlands, it is likely to assume that this crop will deliver most materials for co-fermentations in the near future (total production of maize in the Netherlands in 2009 was 270, 000 ha; source CBS). Grass cuttings can also serve as an input source for co-fermentation (see http://www.acrres.nl/), but it is not yet used at large scale levels for co-fermentation in the Netherlands (800,000 ha was agricultural permanent grassland in the Netherlands and that excludes grass from other sources like grass cuttings from nature, road verges, gardens, etc.). Potato (tubers) can become an important energy source for co-fermentation, and a number of potato waste products already are present on the 'positive list', although these products are not used for co-fermentation at large scale levels yet (total annual potato production area in the Netherlands is 72,100 ha). Other potential energy crops like grains (annual production area of 185,000 ha) and sugar beets (annual production area of 69,000 ha) also can be good candidates, although these crops are not used as input sources for co-fermentation (yet).

5.6 Concluding remarks on phytopathogen and weed seed survival during anaerobic fermentation

In spite of the fact that rather low temperatures were achieved during mesophilic AF processing in the studies done by Engeli *et al.* (1993), Turner *et al.* (1993), and Termorshuizen *et al.* (2003), most phytopathogens were strongly reduced in number in the final product, digestate, that can be used as biofertilizer of arable soils. As stated by Turner *et al.* (1993), it is true that AF is sanitizing towards phytopathogens, but not to all (Table 3). *P. brassicae, S. cepivorum,* and *C. michiganesis* were the phytopathogens found to survive AF. Special care should thus be taken with phytopathogens that produce resting forms, because these may survive AF processing and form a risk for agricultural production when present in digestate. Cross contamination with human pathogens, followed by their outgrowth in already processed substrates, may occur (Sahlström, 2003; Wagner *et al.*, 2008). Therefore, separation of source and output materials is of major concern for AF. Expectedly, thermophilic fermentations will lead to lower residue levels of phytopathogens and weed seeds in digestates. Because commercial applications of co-digestion are still explorative in the Netherlands, it is rather unpredictable how developments will proceed and what other phytosanitary risks will appear in the near future in digested products used for fertilization of arable land.

Table 3. *Phytopathogens shown to survive and not to survive mesophilic anaerobic fermentation.*

Pathogen	Reference
Pathogens that survive anaerobic fermentation	
Plasmodiophora brassicae	Termorshuizen *et al.*, 2003; Engeli *et al.*, 2003
Sclerotium cepivorum	Termorshuizen *et al.*, 2003
Pathogens that do not survive anaerobic fermentation	
Fusarium oxysporum	Turner *et al.*, 1983
Clavibacter michiganensis	Turner *et al.*, 1983
Globodera pallida	Turner *et al.*, 1983
Ralstonia solanacearum	Termorshuizen *et al.*, 2003

6.   Conclusions

Clear examples of phytopathogens that were proven to survive composting and/ or anaerobic fermentation are: *Clavibacter michiginensis* ssp. *sepedonicus*, *Plasmodiophora brassicae*, *Sclerotium cepivorum,* and *Synchytrium endobioticum* Generally speaking, most, if not all phytopathogens and weed seeds decline in number during composting or anaerobic fermentation. Phytopathogen and weed seed survival during composting and anaerobic fermentation is influenced by a combination of factors. Most important factors in order of relevance are:
1. highest (peak) temperatures reached during composting,
2. exposure time at this temperature level,
3. moisture content of the composting matrix,
4. other factors like microbial composition (most likely influenced by input materials) and production of toxic compounds like ammonia, and other volatiles like organic acids (Table 1).

Further, phytopathogen survival was affected by location in static compost piles, production of resting forms that resist severe environmental circumstances and the source of input materials used for fertilization. Anaerobic fermentation is still in an explorative state and it is rather unpredictable if, and to what extent new phytosanitary risks will emerge in the near future. Based on the total production areas in the Netherlands, grass, maize, and grains are expected to be used in the near future. However, risks associated with potato wastes (potato has a smaller total production area in the Netherlands than grass, maize, and grains) can be much larger. Thermophilic anaerobic fermentation is not commonly applied yet, and due to the higher peak temperatures reached at thermophilic anaerobic fermentation, it is expected that phytopathogens and weed seeds survival times will be more reduced under thermophilic than under mesophilic anaerobic fermentation.

7. Knowledge gaps in our current understanding on phytopathogen and weed seed survival in waste streams and future perspectives

There is a lack of information on the survival of phytopathogens and weed seeds during anaerobic fermentations (mesophilic and thermophilic). Eradication of phytopathogens and weed seeds from organic waste materials may lead to useful products for agriculture. Currently, fodder maize is used as energy crop for bio-energy production by anaerobic fermentation. Fodder maize is not a major reservoir for phytopathogens, and digestate derived from it must be considered as 'safe' for agricultural applications. As long as there are no incentives to use other organic waste materials for energy production, then there is no reason to presume that digestates are not safe from a phytosanitary perspective. However, this situation can change when other organic waste materials will be more frequently used for energy production.

Soil tares contaminated with phytopathogens cannot be used for agricultural applications. Phytopathogen survival studies in soil tares and measures aimed to reduce phytopathogen numbers in soil tares are not available in literature. However, at a practical level there is much information available on soil tare treatments aimed to reduce phytopathogens. This information may be not complete or experiments may give inconsistent results. Therefore, it is so important to show effects of soil tare treatments on survival of different categories of phytopathogens (e.g. nematodes, fungi, bacteria), and to evaluate results for practical applications. Optional treatments to reduce phytopathogens in soil tares are heat treatments (e.g. by steaming), inundation and biological soil disinfection (soil mixed with organic materials covered by plastic). The first approach may be more energy consuming than the second and third ones, but may be faster, and may lead to a more complete eradication of phytopathogens. Soil tare treatments are important to make valuable products out of this waste stream. Reliable procedures aimed to eradicate phytopathogens from soil tares thus are relevant for agriculture.

References

1. Abdul, P. & D. Lloyd, 1985.
   Pathogen survival during anaerobic digestion: fatty acids inhibit anaerobic growth of *Escherichia coli*. Biotech Lett **7:** 125-128.

2. Angelidaki, I. & L. Ellegaard, 2003.
   Codigestion of manure and organic wastes in centralized biogas plants. Appl Biochem Biotechnol. **109:** 96-105.

3. Barak, J.D., S.T. Koike & R.L. Gilbertson, 2001.
   Role of crop debris and weeds in the epidemiology of bacterial leaf spot of lettuce in California. Plant Dis **85:** 169-178.

4. Blok, W.J., J.G. Lamers, A.J. Termorshuizen & G.J. Bollen, 2000.
   Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology **90:** 253-259.

5. Bollen, G.J., D. Volker & A.P. Wijnen, 1989.
   Inactivation of soil-borne plant pathogens during small-scale composting of crop residues. Neth J Plant Pathol **S1:** 19-30.

6. Breukers, A., 2002.
   Verspreidingsrisisco's van quarantaineorganismen via afvalstromen. Rapport Wageningen Universiteit leerstoelgroep Agrarische Bedrijfseconomie (in Dutch).

7. Campbell, R.N. & M.T. Lin, 1976.
   Morphology and thermal death point of *Olpidium brassicae*. Amer J Bot **63:** 826-832.

8. Candido, V., V. Miccolis, M. Basile, T. Addabbo & G. Gatta, 2004.
   Soil solarization for the control of Meloidogyne javanica on eggplant in Southern Italy. ISHS Acta Horticulturae 698: VI International Symposium on chemical and non-chemical soil and substrate disinfection.

9. Ceustermans, A., D. De Clercq, A. Aertsen, C. Michiels, J. Coosemans & J. Ryckeboer, 2006.
   Inactivation of *Salmonella* Senftenberg strain W775 during composting of biowastes and garden wastes. J Appl Microbiol **103:** 53-64.

10. Coelho, L., D.J. Mitchell & D.O. Chellemi, 2001.
    The effect of soil moisture and cabbage amendment on the thermoinactivation of *Phytophthora nicotianae*. Eur J Plant Pathol. **107:** 883-894.

11. Czajkowski, R., G.J. Grabe & J.M. Van der Wolf, 2009.
    Distribution of *Dickeya* spp. and *Pectobacterium carotovorum* subsp. *carotovorum* in naturally infected seed potatoes. Eur. J. Plant Pathol. **125:** 263-275.

12. Dahlquist, R.M., T.S. Prather & J.J. Stapleton, 2007.
    Time and temperature requirements for weed seed thermal death. Weed Sci **55:** 619-625.

13. Date, H., H. Nasu & M. Hatamoto, 1993.
    Thermal death range of *Pseudomonas solanacearum* under various conditions. Ann Phytopathol Soc Japan **59:** 709-713.

14. Davidson, J.M., H.A. Patterson & D.M. Rizzo, 2008.
    Sources of inoculum for *Phytophthora ramorum* in a redwood forest. Phytopathol. **98:** 860-866.

15. Downer, A.J., D. Crohn, B. Faber, O. Daugovish, J.O. Becker, J.A. Menge & M.J. Mochizuki, 2008.
    Survival of plant pathogens in static piles of ground green waste. Phytopathol. **98:** 547-554.

16. Engeli, H., W. Edelmann, J. Fuchs & K. Rottermann, 1993.
    Survival of plant pathogens and weed seeds during anaerobic digestion Wat Sci Tech **27:** 69-76.

17. Fayolle, L., R. Noble, E. Coventry, S. Aime & C. Alabouvette, 2006.
    Eradication of *Plasmodiophora brassicae* during composting of wastes. Plant Pathol. **55:** 553-558.

18. Gilbert, J., S.M. Woods & U. Kromer, 2008.
    Germination of ascospores of *Gibberella zeae* after exposure to various levels of relative humidity and temperature. Phytopathol. **98:** 504-508.

19. Goud, J.K., A.J. Termorshuizen, W. Blok & A.H.C. Van Bruggen, 2004.
    Long-term effect of biological soil disinfestation on Verticillium wilt. Plant Dis **88:** 688-694.

20. Hoitink, H.A.J., L.J. Herr & A.F. Schmitthenner, 1976.
    Survival of some plant pathogens during composting of hardwood tree bark. Phytopathol **66:** 1369-1372.

21. Hollaway, G.J. & T.W. Bretag, 1997.
    Survival of *Pseudomonas syringae* pv. *pisi* in soil and on pea trash and their importance as a source of inoculum for a following field pea crop. Austr J Exp Agricult **37:** 369-375.

22. Kearney, T.E., M.J. Larkin & P.N. Levett, 1993a.
    The effect of slurry storage and anaerobic digestion on survival of pathogenic bacteria. J Appl Bacteriol **74:** 86-93.

23. Kearney, T.E., M.J. Larkin, J.P. Frost & P.N. Levett, 1993a.
    Survival of pathogenic bacteria during mesophilic anaerobic digestion of animal waste. J Appl Bacteriol **75:** 215-219.

24. Kumar, R., M.K. Gupta & S.S. Kanwar, 1999.
    Fate of bacterial pathogens in cattle dung slurry subjected to anaerobic digestion. World J Microbiol Biotechnol **15:** 335-338.

25. Kyazze, G., R. Dinsdale, F.R. Hawkes, A.J. Guwy, G.C. Premier & I.S. Donnison, 2008.
    Direct fermentation of fodder maize, chicory fructans and perennial ryegrass to hydrogen using mixed microflora. Bioresource Technol **99:** 8833-8839.

26. Lee, M-R. & J.C.H. Shih, 1988.
    Effect of anaerobic digestion on oocysts of the protozoan *Eimeria tenella*. Appl Environ Microbiol. **54:** 2335-2341.

27. Messiha, N., A.D. Van Diepeningen, M. Wenneker, A.R. Van Beuningen, J.D. Janse, G.C. Coenen, A.J. Termorshuizen, A.H.C. Van Bruggen & W.J. Blok, 2007.
    Biological soil disinfestation (BSD), a new control method for potato brown rot, caused by *Ralstonia solanacearum* race 3 biovar 2. Eur J Plant Pathol **117:** 403-415.

28. Noble, R., J.G. Elphinstone, C.E. Sansford, G.E. Budge & C.M. Henry, 2009.
    Management of plant health risks associated with processing of plant-based wastes: a review. Biosource Technol. **100:** 3431-3446.

29. Noble, R. & S.J. Roberts, 2004.
    Eradication of plant pathogens and nematodes during composting: a review. Plant Pathol. **53:** 548-568.

30. Runia, W.T. (2010).
    Soil disinfestation by soil heating; steam sterilization of soil. Book APS Press: Soil solarization: Theory and practice (In press).

31. Runia, W.T., 2000.
    Steaming methods for soils and substrates. Proceedings of the fifth international symposium on chemical and non-chemical soil and substrate disinfestation, Torino, Acta Horticulturae **532:** 115-123.

32. Ryckeboer, J., J. Mergaert, J. Coosemans, K. Deprins & J. Swings, 2003.
    Microbiological aspects of biowaste during composting in a monitored compost bin. J Appl Microbiol. **94:** 127-137.

33. Sahlström, L., 2003.
    A review of survival of pathogenic bacteria in organic waste used in biogas plants. Bioresource Technol **87:** 161-166.

34. Schmidt, O., D. Dujesiefken, H. Stobbe, U. Moreth, R. Kehr & T.H. Schröder, 2008.
    Pseudomonas syringae pv. *aesculi* associated with horse chestnut bleeding canker in Germany. For. Path. **38:** 124-128

35. Schönfeld, J., A. Gelsomino, L.S. Van Overbeek, A. Gorissen, K. Smalla & J.D. Van Elsas, 2003.
    Effects of compost addition and simulated solarisation on the fate of *Ralstonia solanacearum* biovar 2 and indigenous bacteria in soil. FEMS Microbiol Ecol **43:** 63-74.

36. Shlevin, E., Y. Mahrer & J. Katan, 2004.
    Effect of moisture on thermal inactivation of soilborne pathogens under structural solarization. Phytopathology **94:** 132-137.

37. Steinmöller, S., P. Müller & C. Büttner, 2007.
    Effect of composting and pasteurization on two important quarantine pests of potato. Comm Appl Biol Sci Ghent University 72/2: 341-351.

38. Termorshuizen, A.J., D. Volker, W.J. Blok, E. Ten Brummeler, B.J. Hartog, J.D. Janse, W. Knol & M. Wenneker, 2003.
    Survival of human and plant pathogens during anaerobic mesophilic digestion of vegetable, fruit and garden waste. Eur J Soil Biol **39:** 165-171.

39. Thompson, A.J., N.E. Jones & A.M. Blair, 1997.
    The effect of temperature on viability of imbibed weed seeds. Ann Appl Biol **130:** 123-134.

40. Turner, J., D.A. Stafford, D.E. Hughes & J. Clarkson, 1983.
    The reduction of three plant pathogens (*Fusarium, Corynebacterium* and *Globodera*) in anaerobic digesters. Agricult Wastes **6:** 1-11.

41. Ueno, Y., D. Sasaki, H. Kukui, S. Haruta, M. Ishii & Y. Igarashi, 2006.
    Changes in bacterial community during fermentative hydrogen and acid production from organic waste by thermophilic anaerobic microflora. J Appl Microbiol **101**: 331-343.

42. Van den Berg, S.A.M., P.O. Cohn & R.L. Cornelissen, 2003.
    Businessplan boerderij plus. Rapportage Cornelissen Consulting Services BV Enschede, The Netherlands, http://www.senternovem.nl/mmfiles/Businessplan%20Boerderij%20Plus_tcm24-240749.pdf.

43. Van Loenen, M.C.A., Y. Turbett, C.E. Mullins, N.E.H. Feilden, M.J. Wilson, C. Leifert & W.E. Seel, 2003.
    Low-temperature-short duration steaming of soil kills soil-borne pathogens, nematode pests and weeds. Eur J Plant Pathol. **109**: 993-1002.

44. Van Loenen, M.C.A., Y. Turbett, C.E. Mullins, M.J. Wilson, N. Feilden & W.E. Seel, 2002.
    Low temperature/short duration steaming as a sustainable method for soil disinfection. UK Organic Research, Proc. COR Conf. Aberystwyth: 211-214.

45. Van Overbeek, L.S., J.H.H. Bergervoet, F.H.H. Jacobs & J.D. Van Elsas, 2004.
    The low-temperature-induced viable-but-non-culturable state affects the virulence of *Ralstonia solanacearum* biovar 2. Phytopathol. **94**: 463-469.

46. Van Overbeek, L., E. Nijhuis, H. Koenraadt, J. Visser & G. Van Kruistum, 2010.
    The role of crop waste and soil in *Pseudomonas syringae* pathovar *porri* infection of leek (*Allium porrum*). Applied Soil Ecology **46:** 457-463.

47. Veijalainen, A-M., A. Lilja & M-L. Juntunen, 2005,
    Survival of uninucleate *Rhizoctonia* species during composting of forest nursery waste. Scand J For Res **20:** 206-212.

48. Voća, N., T. Krička, T. Ćosić, V. Rupić, Z. Jukić & S. Kalambura, 2005.
    Digested residue as a fertilizer after the mesophilic process of anaerobic digestion. Plant Soil Environ. **51:** 262-266.

49. Wagner, A.O., G. Gstraunthaler & P. Illmer, 2008.
    Survival of bacterial pathogens during the thermophilic anaerobic digestion of biowaste: laboratory experiments and *in situ* validation. Anaerobe **14:** 181-183.

50. Wenneker, M., M.S.W. Verdel, R.M.W. Groenveld, C. Kempenaar, A.R. Van Beuningen & J.D. Janse, 1999.
    Ralstonia (Pseudomonas) solanacearum race 3 (biovar 2) in surface water and natural weed hosts: First report on stinging nettle (*Urtica dioica*) Eur. J. Plant Pathol. **105:** 307-315.

51. Wiese, A.F., J.M. Sweeten, B.W. Bean, C.D. Salisbury & E.W. Chenault, 1998.
    High temperature composting of cattle feedlot manure kills weed seed. Appl Eng Agricult **14:** 377-380.

52. Wu, B.M. & K.V. Subbarao, 2008.
    Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. Phytopathol. **98:** 1144-1152.