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Chapter 9

Pathogenic Agents

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9.1. General Remarks

Recycling of biological wastes by aerobic or anaerobic biotechnological treatment is necessary to protect the environment and to save natural resources. The recycling process may be conducted either: (1) in large-scale plants operated mostly in urban industrial areas, or (2) in small plants operated primarily in the rural environment to improve the farmer’s income. Municipal solid wastes, sewage sludge, and other organic sludges may contain different kinds of pathogens that are infectious to several species of animals and plants, as well as to humans. The origin and nature of organic wastes and the different types of sludges always cause hygienic risks in storage, collection, handling, processing, and utilization. These risks exist if the organic wastes are collected and processed via source separation (biowastes), if they are collected mixed with other wastes from households or relevant processing industries, if they are generated in the treatment of industrial or municipal wastewater, or if the sludge results from industrial processing of
other organic materials. Generally, three main types of risks are associated with recycling of organic wastes:

1. occupational health risks;
2. environmental risks; and
3. risks associated with product safety.

Therefore, hygienic principles must be followed in the collection, transportation, processing, storage, and distribution of such materials.

Occupational health risks exist in small as well as in large-scale plants; however, this is not the subject of this book and more details on occupational health risks may be found in other publications (Hickey and Reist, 1975; Grüner, 1996; Böhm et al., 1998). Environmental risks and risks concerning epidemiological pathways closed via contaminated products, as well as measures that can be adopted against such risks, will be dealt with in this chapter, along with relations to the pathogens involved and to the different epidemiological situations that can be expected under the given conditions. The information in Table 9.1 summarizes the main epidemiological risks associated with recycling solid and liquid organic wastes.

The risks related to the different raw materials are mainly defined by the presence of certain organisms and their properties. This will be covered in more detail in other sections of this chapter. Generally, they can be divided into those causing phytohygienic risks and those related to human and animal health. The data in Table 9.2 provide a survey concerning the hygienic relevance of some organic wastes originating from households.
### Table 9.2. Hygienic relevance of different biological wastes originating from households

| Type of waste                                                                 | A   | B   |
|--------------------------------------------------------------------------------|-----|-----|
| **Meat-leftovers (raw or insufficiently heated)**                             |     |     |
| – meat cuttings, tendons, rinds, etc.                                         | +   | –   |
| **Food of animal origin**                                                     |     |     |
| – egg shells                                                                  | +   | –   |
| – several meat and dairy products                                            | +   | –   |
| – raw milk products                                                           | +   | –   |
| – leftovers from fish and shellfish                                          | +   | –   |
| **Other wastes (animal and man)**                                             |     |     |
| – dirty packing material for meat and products of animal origin               | +   | –   |
| – used litter and wastes from pets                                            | +   | +   |
| – used paper handkerchiefs and sanitary pads                                  | +   | –   |
| – diapers                                                                     | +   | –   |
| **Household wastes from**                                                     |     |     |
| – potatoes                                                                    | –   | +   |
| – carrots                                                                     | –   | +   |
| – onions                                                                      | –   | +   |
| – tomatoes                                                                    | –   | +   |
| – cucumbers                                                                   | –   | +   |
| – salad                                                                       | –   | +   |
| – cabbage                                                                     | –   | +   |
| – beans                                                                       | –   | +   |
| – cut flowers                                                                 | –   | +   |
| – balcony and indoor plants                                                  | –   | +   |
| **Garden wastes**                                                             |     |     |
| – boughs and plant material                                                   | –   | +   |
| – fruits                                                                      | –   | +   |
| – dead leaves and lawn trimmings (fecal contamination)                        | +   | +   |
| **Other wastes (plant origin)**                                               |     |     |
| – paper                                                                       | –   | –   |
| – paperboard                                                                  | –   | –   |
| – organic packing material (e.g., wood wool)                                  | –   | –   |

A — May contain pathogens of man and animals.
B — May contain plant pathogens and/or weed seeds.

The data in the table show that pathogens for humans and animals are not always limited to materials from warm-blooded individuals, but that they can originate from plant materials as well.

A relatively large number of pathogens are found in solid and liquid organic wastes; the most prevalent are bacteria, viruses, fungi, and parasites. A brief discussion of each type follows.
9.2. Bacteria

Bacteria may propagate in the raw materials during collection, transport, and storage and they are involved in the composting process itself. In the latter case, especially in thermophilic processes, the bacterial pathogens are more or less reduced in number. It has to be considered then that they often propagate in the raw materials before being processed. This increases the risk and leads to a general contamination of the collected materials during transport. If sludges from wastewater treatment (biosolids) are composted, nearly all gut-related pathogens may be found, while some materials of industrial origin are nearly sterile if they were heated during processing.

A compilation of bacterial pathogens of humans, animals, and plants that may be present in organic wastes is presented in Tables 9.3 and 9.4. Presence of bacterial pathogens alone has nothing to do with the resulting risk of infection. Transmission via the environment and the resulting route of infection must be a factor in the epidemiology of the resulting disease. The most important pathogens in this connection are *Salmonella* spp.; others, like *Listeria* or *Clostridia*, may also be present in the material, but they are also present in the soil and therefore are of secondary importance if the product is used as a soil conditioner.

9.3. Viruses

Several viruses of plant origin may be present in the raw materials (Table 9.5), as well as gut-related viruses of animal and human origin. Special risks are connected with viral

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### Table 9.3. A selection of obligatory and facultative pathogenic bacteria that can be isolated from biological and household wastes

| Obligatory pathogens                        | Facultative pathogens   |
|--------------------------------------------|-------------------------|
| *Salmonella* spp.                          | *Escherichia*           |
| *Shigella* spp.                            | *Klebsiella*            |
| *Escherichia coli* (enteropathogenic strains) | *Enterobacter*         |
| *Pseudomonas aeruginosa*                   | *Serratia*              |
| *Yersinia enterocolitica*                  | *Citrobacter*           |
| *Clostridium tetani*                       | *Bacillus cereus*       |
| *Clostridium perfringens*                  | *Proteus*               |
| *Clostridium botulinum*                    | *Providencia*           |
| *Bacillus anthracis*                       |                         |
| *Listeria monocytogenes*                   |                         |
| *Vibrio cholerae*                          |                         |
| *Mycobacterium* spp.                       |                         |
| *Leptospira* spp.                          |                         |
| *Campylobacter* spp.                       |                         |
| *Staphylococcus*                           |                         |
| *Streptococcus*                            |                         |
Table 9.4. A selection of plant pathogenic bacteria and viruses (adapted from Menke, 1992)*

| Pathogen                             | Susceptible plant                                                                 | Type |
|--------------------------------------|----------------------------------------------------------------------------------|------|
| *Xanthomonas campestris*             | White cabbage, turnip cabbage, Swede cauliflower                                 | B    |
| *Pseudomonas marginalis*             | Salad, endive                                                                    | B    |
| *Pseudomonas phaseolicola*           | Beans                                                                            | B    |
| *Pseudomonas lacrimans*              | Cucumber                                                                        | B    |
| *Pseudomonas tabaci*                 | Tobacco                                                                          | B    |
| *Corynebacterium michiganense*       | Tomatoes                                                                         | B    |
| *Corynebacterium sepedonicum*        | Potatoes                                                                         | B    |
| *Erwinia phytophthora*               | Potatoes, carrots                                                                | B    |
| *Erwinia amylovora*                  | Pomaceous fruits, flowers                                                        | B    |
| *Agrobacterium tumefaciens*          | Various hosts                                                                    | B    |
| Potato virus Y                       | Potatoes, tobacco, tomatoes                                                      | V    |
| Potato virus X                       | Potatoes, tomatoes, tobacco, paprika, eggplants                                  | V    |
| Aucuba virus                         | Potatoes, tobacco, tomatoes                                                      | V    |
| Tobacco ring spot virus              | Potatoes, tobacco, beans, cucumber                                               | V    |
| Rattle virus                         | Potatoes, tobacco                                                                | V    |
| Tobacco mosaic virus                 | Tobacco, tomatoes, paprika                                                       | V    |
| Tobacco necrosis virus               | Tobacco, beans                                                                   | V    |
| Horse bean mosaic virus              | Horse beans, peas                                                                | V    |
| Pea mosaic virus                     | Peas, horse beans                                                                | V    |
| Bean mosaic virus                    | Beans, runner beans                                                              | V    |
| Yellow bean mosaic virus             | Beans, peas                                                                      | V    |
| Cauliflower mosaic virus             | Several cabbage species                                                          | V    |
| Cucumber mosaic virus                | Cucumber, melon, pumpkin, spinach, peas, beans, salad, tomatoes, celery          | V    |
| Aucuba mosaic virus                  | Cucumber, melons                                                                 | V    |
| Cabbage ring spot virus              | Cauliflower, white cabbage, horseradish, spinach, tobacco, rhubarb, flowers      | V    |
| Lettuce mosaic virus                 | Salad, endive                                                                    | V    |
| Beet mosaic virus                    | Spinach, root beets, leaf beets, peas                                            | V    |
| Onion mosaic virus                   | Onions, leek                                                                     | V    |

B — Bacteria.
V — Viruses.

*More details about transmission and resistance may be taken from the original paper, which also contains information concerning parasitic nematodes and weed seeds.

causative agents of animal diseases such as Foot and Mouth Disease (FMD), which may be present in meat and meat products if the animals had been slaughtered before showing clinical signs of illness. In sludges from wastewater treatment or in other wastes with fecal contamination, hepatitis A virus, rotaviruses, and caliciviruses have to be taken into account as emerging pathogens.
Table 9.5. Selection of viral pathogens that may be present in biological wastes from households and from municipal sources

| Groups of viral pathogens of general importance | Viral pathogens of special veterinary importance |
|-----------------------------------------------|-----------------------------------------------|
| Enterovirus                                   | African Swine Fever (ASF) Virus               |
| Poliovirus                                    | Aujeszky Disease (AD) Virus                   |
| Coxsackievirus A                              | Classical Swine Fever (CSF) Virus             |
| Coxsackievirus B                              | Foot and Mouth Disease (FMD) Virus            |
| Echovirus                                     | Swine Vesicular Disease (SVD) Virus           |
| Adenovirus                                    | Newcastle Disease (ND) Virus                  |
| Reovirus                                      | Avian Influenza (AI) Virus                    |
| Hepatitis A virus                             |                                               |
| Rotavirus                                     |                                               |
| Astrovirus                                    |                                               |
| Calicivirus (Norwalk agent)                   |                                               |
| Coronavirus                                   |                                               |
| Parvovirus                                    |                                               |

9.4. Fungi

Fungi present in wastes and materials used for composting are mainly of interest from the point of view of occupational health and phytohygiene. From the species pathogenic to warm-blooded animals and humans in Europe, mainly *Candida albicans* and *Aspergillus fumigatus* have to be mentioned in this connection. For plants, a variety of species are important pathogens; well known in this framework is *Plasmodiophora brassicae*. A selection of relevant plant pathogenic fungi is given in Table 9.6.

9.5. Parasites

The presence of parasites or their infective stages in wastes or residues of plant, animal, or human origin depends on the nature of the wastes and the level of pretreatment. Parasites are of veterinary and medical importance if the raw materials used for composting are generated in wastewater treatment facilities or in slaughterhouses (e.g., contents of the digestive tract) and, in general, if the wastes are of fecal origin or contaminated with fecal matter. Some of the most important parasites of epidemiological relevance are listed in Table 9.7. From the protozoal ones, *Cryptosporidium parvum* seems to be the most relevant, while eggs of *Ascaris* species of humans and animals are the most important metazoic parasites. Parasites at large are not only important as pathogens, they may also be a risk factor as vectors in transmission of diseases from wastes to susceptible populations (e.g., by flies and cockroaches).
Table 9.6. A selection of plant pathogenic fungi (adapted from Menke, 1992)

| Pathogen                     | Susceptible plant |
|------------------------------|-------------------|
| Plasmodiophora brassicae     | Cabbage           |
| Phoma apiicola               | Cabbage           |
| Peronospora brassicae        | Celery            |
| Peronospora spinaciae        | Spinach           |
| Peronospora destructor       | Onions            |
| Marssonina panattoniana      | Salad             |
| Sclerotinia minor            | Salad             |
| Botrytis cinerea             | Salad             |
| Bremia lactucae              | Salad, endive     |
| Cercospora beticola          | Turnip            |
| Aphanomyces raphani          | Radish            |
| Alternaria porri             | Carrot            |
| Septoria apiii               | Celery            |
| Albugo tragoponis            | Scorzonera        |
| Albugo candida               | Horseradish       |
| Turburciniia cepulaceae      | Onions            |
| Sclerotium cepivorum         | Onions            |
| Alternaria porri             | Carrots           |
| Botrytis allii               | Onions            |
| Uromyces appendiculatus      | Beans             |
| Mycosphaerella pinodes       | Peas              |
| Ascochyta pinodella          | Peas              |
| Erysiphe polygoni            | Peas              |
| Cladosporium cucumerum       | Cucumber          |
| Sclerotinia sclerotiorum     | Cucumber          |
| Septoria lycopersici         | Tomatoes          |
| Alternaria solani            | Tomatoes          |
| Didymella lycopersici        | Tomatoes          |
| Rhizoctonia solani           | Potatoes          |
| Phytophthora infestans       | Potatoes, tomatoes|
| Synchytrium endobioticum     | Potatoes          |
| Verticillium albo-atrum      | Potatoes          |

Table 9.7. A selection of parasites that can be expected in organic wastes from humans and animals of fecal origin

| Protozoa                      | Cestodes          | Nematodes               |
|-------------------------------|-------------------|-------------------------|
| Cryptosporidium parvum        | Taenia saginata   | Ascaris lumbricoides    |
| Entamoeba histolytica         | Taenia solium     | Ancyllostoma duodenale  |
| Giardia lamblia               | Diphyllobothrium latum | Toxocara canis       |
| Toxoplasma gondii             |                   | Trichuris trichiura     |
| Sarcocystis spp.              |                   |                         |
Table 9.8. A selection of plant pathogenic nematodes (adapted from Menke, 1992)

| Species                | Host plant                                                                 | Survival data                        |
|------------------------|-----------------------------------------------------------------------------|--------------------------------------|
| Ditylenchus dipsaci    | Root beets, leaf beets, kohlrabi, carrots, potatoes, peas, beans, onions, celery, cucumber, salad, spinach, strawberries | Plant residuals and soil: up to 2 years |
| Ditylenchus destructor | Potatoes                                                                    | No data                              |
| Heterodera schachtii   | Root beets, leaf beets, kohlrabi, cabbage, celery, radish, spinach          | Soil: larvae up to 1 year            |
| Heterodera rostochiensis | Potatoes, tomatoes                                                          | Soil: cysts up to 6 years            |
| Heterodera goettingiana | Peas                                                                        | Soil: larvae up to 9 months          |
| Pratylenchus pratensis | Root beets, leaf beets, carrots, potatoes, tobacco, peas, onions, chives, cabbage, salad, horseradish, radish | Soil: cysts up to 7 years            |
| Aphelenchoides parietimes | Carrots, potatoes, onions                                                  | No data                              |
| Aphelenchoides ritzemabosi | Tobacco, onions                                                              | Soil: 4 months                       |
| Aphelenchoides avenae  | Carrots, onions                                                             | Soil: M. hapla over 6 months         |
| Meloidogyne spp.       | Root beets, carrots, potatoes, tobacco, peas, beans, onions, celery, cabbage, endive, cucumber, salad, horseradish, parsley, rhubarb, scorzonera, spinach, tomatoes | Soil: M. hapla over 6 months         |

Plant pathogenic parasites must also be considered, even if some of them are highly specialized on certain plants, which limit their epidemiological importance. Cyst-forming nematodes are the most relevant because these cysts may survive in the soil for several years. Roots and other soil-containing materials used in composting may contain such transmissible stages; more details can be obtained in the literature, such as in Menke (1992). A selection of relevant nematodes is listed in Table 9.8.

9.6. Hygienization

Most countries in the world, including most member states of the European Union, do not have legal regulations defining the hygienic requirements for finished compost. In most cases, indirect parameters, e.g., maturity of the product, frequency of turning of windrows, temperature–time relationships, have to be kept voluntary or in the framework of quality networks. Sometimes, only recommendations or legal demands are given by defining the particle size, minimum temperature, and exposure time, which are insufficient if they are not based on validation experiments or scientific investigations. A typical example
is the requirement for composting in a reactor requiring a 12 mm particle size combined
with an exposure time of at least 1 h and a temperature of at least 70°C, as set forth in
the European animal by-product regulations (EC, 2002), which is not covered by any
validation experiment.

There is no doubt that according to the state of science and technology, a composting
process if properly applied can inactivate relevant pathogens and weeds. Therefore, some-
times recommendations are given on how to operate the composting process in order to
achieve hygienic safety of the product. Such strict recommendations may be: "Composting
plants shall operate with the material at a moisture content of 45–50% and a pH of about
7. If the facility uses windrows, the exposure time shall be at least for a period of 2 weeks
at 55°C. If the facility uses in-vessel technology, the exposure time will be for a period of
1 week at 65°C." From a scientific point of view, other combinations of temperature and
exposure time will lead to sufficient inactivation of the target organisms, since those are
mainly vegetative bacteria, viruses, eggs of parasites, or similar transmission stages and
seeds. Even spores of Bacillus anthracis can be inactivated under certain circumstances,
as shown by Miersch (1975). Until now it is not definitely clear if transmissible spongi-
form encephalopathies (TSE) agents could be inactivated in composting; more research is
needed in order to confirm or disprove the findings of Brown and Gajdusek (1991), indi-
cating that the scrapie agent may survive for more than 3 years in the soil. However, their
experimental setup does not appear to be representative of the situation in composting.
Nevertheless, inadequate technical design and improper management of the composting
process may result in survival and transmission of the pathogens involved; therefore, only
treatment in a validated process under steady supervision will lead to a hygienically safe
product.

This means that the following strategies may be combined in order to assure
hygienically safe utilization of the processed materials:

- validation of treatment (disinfection by chemical, physical, or biological means),
- continuous recording of relevant process parameters (e.g., temperature, pH, exposure
time),
- microbiological monitoring of the final product (indicators), and
- restrictions on the utilization of the final product.

The capability of a process to inactivate pathogens causing risks that depend on the
raw material cannot be judged simply by analysis of presence or absence of indicators
(bacterial, viral, fungal, or parasitic) in the final product.

When only product monitoring is used in order to validate a process, it can provide
a false sense of security that the process is able to control the relevant hazards in the
final product. Absence of one or all of the mentioned pathogens or indicators in the final
product may be caused by several other reasons:

- they were not present in the raw material,
- they were present in the raw material, but in a low concentration (less than 5 log),
- due to ineffective enrichment procedures (e.g., bacteria), re-isolation was insufficient, or
- failure of isolation due to effects of the complicated matrix (e.g., viruses).
Therefore, the possibility of validating a process by input–output analysis of certain indicators is, in principle, possible but under practical conditions a rare event depending on the microbiological properties of the input materials processed and other strategies that must be followed, e.g., process validation with one or more representative test-organism. If either the thermophilic process itself or if an additional thermal treatment shall provide the inactivation of pathogens belonging to the indicated level of thermo- and chemo-resistance, representative test-organisms must be exposed in a similar matrix as that being treated in a suitable test-body in a defined validation experiment. The relevant process parameters must be recorded during the exposure in order to define the technical conditions to be kept for safe inactivation according to the results of the survival experiments. The validation of the treatment with respect to hygienic safety for animals, humans, and, if necessary, plants may be done in several ways. For example, the “German LAGA M 10, 1995” (LAGA-Länderarbeitsgemeinschaft Abfall, 1995) offered a relatively broad approach for solving this problem with respect to composting based on the “ATV” (German Association of Wastewater Experts) recommendations from 1988 for sewage sludge treatment (ATV-Abwassertechnische Vereinigung, 1988). Process safety concerning the inactivation of relevant transmissible agents for humans and animals is validated in two steps. The first step is the validation of the process as designed by the producer of the technical equipment in the basic procedure. The second step involves putting into service validation of a treatment process at the plant with the input material used under practical conditions. In both validation procedures, Salmonella senftenberg W775 (H2S negative) is used as a test organism exposed in specially designed test carriers. In such a validation procedure, testing is always done twice, in summertime and in wintertime. This is a very complete and safe system; if due to economic considerations the system should be simplified and only a one-step procedure should be the aim, it must be the one that deals with putting validation into service. A scheme of how this validation could be organized in this case, taking into account the annual throughput of material in the plants, is given in Table 9.9 from the “German Biowaste Ordinance” (1988).

The question of how validation should be performed under practical conditions and which test bodies can be applied is not easy to answer. In biogas plants, mainly two types of test bodies may be used, depending on the test organisms (Rapp, 1995; Böhm et al., 1997). Those test organisms like bacteria, fungi, and parasites type 1 test bodies that can be retained by a membrane filter in a test body filled with liquid, as shown in Fig. 9.1, could be used. Exposure of viruses to a process requires different test bodies. The virus material is adsorbed onto a special filter material and released after exposure by desorption, by washing with a special solution. Such a type 2 test body is shown in Fig. 9.2.

In composting, different approaches are described for bacteria, because a representative amount of raw material can be contaminated directly with the test strain and put into textile sacks protected from mechanical destruction by a perforated metal basket, as shown in Fig. 9.3. This system may also be used for phytopathogenic fungi and viruses (contained in plant tissue), as well as for seeds. Viruses not related to tissue may be exposed in the material, as described above in a type 2 test body.
Table 9.9. Example of a validation and supervision strategy for biogas and composting plants and the resulting products according to the German Biowastes Ordinance (1998)

| Investigated parameter | Direct validation of the process | Indirect process supervision | Supervision of the final product |
|------------------------|----------------------------------|-----------------------------|---------------------------------|
| Hygienic safety concerning risks for man, animals, and plants | – Newly constructed plants (within 12 months after opening of the plant)  
– Already validated plants if new technologies have been invented or if the process has been significantly modified (within 12 months after invention or modification)  
– Existing plants without validation within the last 5 years before this validation strategy was invented (within 18 months) | – Continuous registration of temperature at three representative locations in the process, responsible for the inactivation of the microorganisms and seeds  
– Recording of process data (e.g., turning of windrows, moisture of material, starting and finishing data) | Regular investigation of the final product for hygienic safety<sup>b,c</sup> |
| Number of test trials | Two test trials, at open air composting plants, at least one in winter | Continuous data recording to be filed for at least 5 years | Continuously all over the year at least:  
– semi-annually (plants with \( \leq 3000 \text{ tons/acre throughput} \))  
– quarterly (plants \( > 3000 \text{ tons/acre throughput} \)) |
| Number of test organisms | | | |
| Human and veterinary hygiene | One test organism (Salmonella senftenberg W775, H<sub>2</sub>S negative) | – | No Salmonella in 50 g product detectable |
| Phytohygiene | Three test organisms (Plasmodyphora brassicae, tobacco mosaic virus, tomato seeds) | – | Less than 2 seeds capable of germinating and/or reproducible parts of plants in 1 l of product |

(continued)
Table 9.9. Example of a validation and supervision strategy for biogas and composting plants and the resulting products according to the German Biowastes Ordinance (1998) — Cont’d

| Investigated parameter          | Direct validation of the process | Indirect process supervision | Supervision of the final product |
|---------------------------------|----------------------------------|-----------------------------|---------------------------------|
| Number of samples               |                                  |                             | Throughput of the plants in tons/acre: |
| Samples per test-trial:         |                                  |                             | 1. \( \leq 3000 \) (6 samples per year) |
| Human and veterinary hygiene    | 24\(^a\)                        |                             | 2. \( >3000-6500 \) (6 samples per year plus one more sample for every 1000 tons throughput) |
| Phytohygiene                    | 36\(^a\)                        |                             | 3. \( >6500 \) (12 samples per year plus one more sample for every 3000 tons) |

| Total                           | 60                               |                             |                                  |

\(^a\)At small plants half the number of samples (\( \leq 3000 \) tons/acre).

\(^b\)Every statement concerning the hygienic safety of the product is always based on the result of the supervision of the final product together with the result of the validation of the process.

\(^c\)Every sample is a “mixed sample” (about 3 kg) based on five single samples of the final product.
Pathogenic agents

Fig. 9.1. Type 1 test bodies for exposure of contaminated liquid substrates in biogas plants with bacterial test strains.

Fig. 9.2. Type 2 test bodies deemed to be used for the exposure of viruses in validation procedures (virus material is adsorbed onto the membrane between the two membrane filters).
The choice of test organisms and the techniques applied depend on the origin of the raw materials and the intended utilization of the product (Böhm, 2004). Several test organisms other than *Salmonella senftenberg*, W775 (H$_2$S negative) are in discussion for different purposes and treatment processes, especially in the Scandinavian countries (Christensen et al., 2001). Some of the most important are:

- *Enterococci faecalis*;
- *Escherichia coli*;
- *Campylobacter*;
- *ECBO virus*;
- *Bovine Parvovirus (BPV)*;
- *Coliphages*; and
- *Ascaris suum*.

*Enterococci*, better known as *Enterococcus faecalis*, may be especially used for validating thermal treatment, since their heat resistance covers most pathogens in this epidemiological context, providing a high safety margin. The application of *Enterococci* for the general purpose of validation of composting processes, on the other hand, has some disadvantages. First, the quantitative enrichment is less effective than in *Salmonella* if contaminant flora shall be excluded. If the carriers are exposed to a composting process, it may happen that the test material is contaminated with indigenous *Enterococci*. In this case, contamination from the substrate cannot be detected in an easy and reliable manner. Finally, it must be kept in mind that *Enterococci* are more chemo- and thermo-resistant than most relevant pathogens in this field, but do not have any epidemiological importance.
in this context. This means that the application of test organisms must be strictly related to the process to be judged. If only a process of thermal inactivation like a pasteurization unit is to be validated, *Enterococcus faecalis* is the proper organism. If a thermophilic aerobic or anaerobic process shall be validated, it is more realistic to use the above characterized strain of *Salmonella senftenberg* because *Enterococci* would be too hard a criterion for this purpose since their chemo-resistance is different from the relevant mostly Gram-negative pathogens. For practical considerations, the proposed serovar *Salmonella senftenberg* has the advantage that it is rarely present in the raw material and can be easily identified by a natural marker (H$_2$S negative) from accidental contaminants. *Escherichia coli* and *Campylobacter jejuni* are not as resistant as the above-mentioned *Salmonella* strain; the same applies to ECBO virus. It could be demonstrated that if dealing with a moderate epidemiological risk, e.g., given in composting source-separated biowastes, *Salmonella senftenberg W775* will cover the most relevant viral pathogens causing notifiable diseases in farm animals and which may be present in low concentrations in the raw material. The time necessary for the inactivation of viral pathogens is generally shorter or in the same range as for inactivating 5 log of *Salmonella senftenberg W775*, H$_2$S negative as has been demonstrated by Braumiller et al. (2000), as well as Moss and Haas (2000). The latter results are summarized in Table 9.10; the results demonstrate the range of time necessary for reducing the infectivity of the tested viral pathogens for 3–4 log 10 steps.

Since recently it was stated by Emmoth et al. (2004) that heating of animal by-products to 70°C for 60 min is not enough for the inactivation of Circoviruses and it had been demonstrated by Böhm et al. (2002) that the plant pathogenic tobacco mosaic virus withstands such treatment without any significant reduction, the consequence for the future will be to validate any kind of treatment if such viruses may be present in involved materials. It is obvious that the recommendations of regulation EC 1774 (2002) will not even lead to a safe inactivation of vegetative bacteria, as can be seen from Fig. 9.4, given the temperature curve measured in the reactor in relation to the survival of *Enterococci* in the substrate and on exposed carriers (Braumiller et al., 2000). As a consequence, concerning composting of animal by-products and catering wastes, a validation with a test organism covering the higher epidemiological risk is necessary. This means that there is a clear indication for using Bovine Parvovirus in such a situation.

| Pathogen                          | Initial titer$^a$ | Detection level$^a$ | Inactivation time |
|-----------------------------------|-------------------|---------------------|------------------|
| Swine Vesicular Disease Virus     | 6–8 log 10 KID$_{50}$ | 1–4 log KID$_{50}$ | 27–72h$^b$       |
| Foot and Mouth Disease Virus      | 6–7 log 10 PFU    | 1–2 log 10 PFU     | 12–144h          |
| Classical Swine Fever Virus       | 5–6 log 10 KID$_{50}$ | 1 log 10 KID$_{50}$ | 12–144h          |
| Aujeszky Disease Virus            | 5–6 log 10 KID$_{50}$ | 1 log 10 KID$_{50}$ | 20–192h          |
| African Swine Fever Virus         | 5–6 log 10 KID$_{50}$ | 1–2 log 10 KID$_{50}$ | 27–168h$^b$     |

$^a$ Reduced to full log steps.

$^b$ Insufficient inactivation within 27 h if not at least 55°C had been reached.
It must be kept in mind that validation with a representative test-organism alone ("direct process validation") has, as every solitary procedure, advantages and disadvantages, which can be summarized as follows:

**Advantages**
- Quickly provides the basic information regarding whether or not a technical process leads to a safe product.
- Validates that the producer or technical equipment is protecting the processing plant.
- Helps to define the technical requirement for a safe process.
- Gives a reproducible result which allows comparison of data.

**Disadvantages**
- High cost and labor intensive.
- It is a rare event.
- Cannot detect accidental disturbances of the process.

The validation with pathogens and seeds may be regarded as an “indirect process validation.” However, this validation must be accompanied by continuous recording of measurable process data like temperature, pH, humidity, etc. in order to detect deviations and disturbances of the process over the entire year, which may result in an insufficient bactericidal effect. The advantages and disadvantages of an indirect process control applied alone are as follows:

**Advantages**
- Easy to achieve and quick.
- Continuous control is possible.
- No special expertise is necessary and laboratory work is limited.
Disadvantages

- Limited representativity for the entire process (gradient formation in the material).
- Influences due to inhomogeneity of the material could not be detected.
- Valuable only in combination with the direct process validation.

The system of process validation and control has to be completed by continuous monitoring of the final product, at least twice a year. As mentioned above, the investigation of the final product to detect every pathogen that may be present in the material is extremely difficult. Therefore, representative indicator organisms have to be determined from the point of view of human and animal health, and if necessary for the purpose of safe plant breeding and production. Several strategies have been followed in different countries and in several normative approaches; an example is given in Table 9.9. Since the philosophies followed in this connection are very different, indicators will be discussed in another section. Additionally, sample collection, storage, and transport have to take into account the special properties and behavior of the biological agents; simple adaptation of methods and recommendations common for chemical analysis is misleading. The advantages and disadvantages of end product monitoring alone as a single strategy to achieve hygienic safety in composting are as follows:

Advantages

- Easy to achieve; limited expertise is necessary.
- Easy to administer and supervise.
- Inexpensive and independent from the site of production.

Disadvantages

- Representative sampling of bulky material is difficult.
- Occurrence of pathogens and indicators depends on the type, origin, and microbiological properties of the raw material.
- Microbiological analysis is influenced by the different products (e.g., growth inhibition).
- Valuable only if applied on products from validated and supervised processes.

Restriction in the use of compost resulting from insufficient treatment should prevent introduction of undesired chemical residuals by contaminated crops into the food chain or direct transmission of pathogens to susceptible animals via feedstuff. This had been practiced in the past, especially with sewage sludge. Such a strategy alone does not prevent the environmental risks or introduction of pathogens into vector populations, which will lead to indirect transmission cycles.

Several authors have given examples of how birds can become carriers of Salmonella (Hellmann, 1977). One of the sources of infection in seagulls has been found to be sewage treatment plants. Other ways of introduction of a certain lysotype of Salmonella enteritidis can be demonstrated by the work conducted by Köhler (1993). Köhler identified the waste delivered from West Berlin to a waste disposal site in the former German Democratic Republic and followed the introduction of this pathogen via birds into chicken populations and finally to humans via products containing eggs. Williams et al. (1977),
as well as several other authors such as Coulson et al. (1983) and Mayr (1983), described the importance of vectors in the transmission of *Salmonella* to farm animals and humans. Foster and Spector (1995) described specific molecular mechanisms responsible for the ability of *Salmonella* to survive the environmental stress, which is underlining the importance of this group of pathogens.

This means that even if the fertilizers containing pathogens are immediately ploughed into the soil, they may generate carriers by attraction of certain species (seagulls) or prolonged survival in subsurface soil layers. Thus, restrictions in use are a tool with limited effect from the point of view of epidemiology and should be avoided if possible and feasible. Moreover, concerning plant pathogens and seeds, this strategy is ineffective if the products are to be used in agriculture.

### 9.7. Regrowth Prevention

Regrowth mainly concerns bacterial pathogens, since viruses except bacteriophages cannot propagate due to the lack of host cells in this environment. Several bacterial pathogens like *Salmonella* can grow at temperatures between 4 and 44°C, as long as enough nutrients and moisture are available. This is not only a matter of material properties and storing conditions, but also of proper management of tools and equipment from a hygienic point of view. A strict division of the technical equipment involved must be provided. One type of equipment should be used only with the raw material and another one should be used only with the finished product (black and white principle). Otherwise, a reliable disinfection measure has to be carried out in between if the strict division cannot be assured. There is sufficient evidence from practical experiments that moist and contaminated fresh material adhering to the equipment will lead to recontamination of the product even if the material seems to be stable and dry. Since most bacteria will not propagate in a dry product even if nutrients are present, the product must be stored under dry conditions. The vapor pressure within the product should be in balance with a relative humidity of the air of at least 90%. Xerophilic fungi may still grow if this balance is above a relative humidity of about 65%. In general, most bacteria and fungi do not grow in the material if the water activity ($a_w$ value) is below 0.80. In order to achieve product safety by preventing regrowth, recontamination by living vectors like birds and rodents should be avoided by taking adequate measures.

### 9.8. Indicators

If a product must be evaluated in the framework of microbiological quality control, it is very difficult to investigate it for presence or absence of all pathogens that could be attended. Since financial and technical limitations are given, one of the most practical means to come to a conclusion concerning hygienic safety of a product with a reasonable effort and limited costs is the application of a suitable indicator concept in combination with process validation and indirect process supervision. The indicator concept is only effective if the raw materials are relatively homogenous in their microbiological properties.
or certain (mostly fecal) contaminants can either be totally excluded in the raw material or have to be always present. This, in general, is not the case here, neither for biowastes nor for industrial wastes or organic sludges. This means that any indicator concept within this framework must be a compromise and the selection of a microbial indicator is extremely difficult and mainly influenced by underlying philosophies. Nevertheless, those indicator organisms must fulfill several requirements:

- they have to be present with a high probability in the raw materials involved,
- the transmission via products must be a factor in epidemiology,
- if a biotechnological process is used, the indicator should not be involved in the process itself,
- the indicator should not be an organism that is generally present in soil and soil-related materials, and
- the method for isolation and identification must be simple, definitive, and reliable if applied to a substrate with a complex microbiological matrix such as compost, sludge, or related materials.

With respect to public health and veterinary requirements, several indicators and parameters are currently under discussion:

- *Salmonella*;
- *Enterococci* (*Streptococci* of group E);
- *Staphylococcus aureus*;
- *Enterobacteriaceae*;
- *Escherichia coli*;
- *Campylobacter*;
- *Yersinia* sp.;
- *Listeria* sp.;
- *Clostridium perfringens*;
- sulfite-reducing *Clostridia*;
- enteroviruses;
- rotavirus;
- eggs of nematodes; and
- larvae of nematodes.

Since compost and related products are coming out of a microbial degradation process and the knowledge about the microbiological ecology of such materials is very limited, one must be careful to use isolation and identification techniques common in clinical microbiology without careful validation in combination with the involved sample materials. The variety of species to be present in environment, samples, and materials resulting from aerobic or anaerobic biological treatment far exceeds the limited number of species to be taken into account in excreta, as well as in body fluids and the variability of species is high and not yet fully understood. Moreover, microbial parameters that are used in the field of water hygiene and food inspection are not applicable to substrates like manure, stabilized sludge, or compost because most of those indicators belong to the indigenous flora of agricultural soils (Böhlm, 1995). If the limited reliability and applicability of methods
used in clinical microbiology and in water inspection for the intended field of use are taken into account, as well as the fact that the exclusion of organisms that generally may be found in normal soils gives no sense for a substrate and fertilizer such as compost or sludge, the following microbial parameters are inappropriate: *Staphylococcus aureus*, *Enterobacteriaceae*, *Clostridium perfringens*, sulphite-reducing *Clostridia*, and *Listeria*. Especially *Enterobacteriaceae*, for which threshold values are given in the EC regulations 1774/2002, are an inappropriate parameter in the evaluation of products after aerobic or anaerobic biotechnological treatment. This is due to the fact that this parameter does not correlate with the presence or inactivation of pathogens as can be observed in Fig. 9.5. The data in Fig. 9.5 show the results of input and output analyses in validated and non-validated composting plants.

One parameter that seems to be very useful and reliable in this connection is the absence or presence of *Salmonella*. *Salmonella* are generally found in fresh biowastes or untreated sewage sludge with a high probability in various concentrations. Since it is known that the probability of identifying a positive sample is basically related to the amount of material investigated, a compromise between feasibility and reliability has to be found. It is proposed to investigate 50 or 100 g (2 × 50 g) of compost for the presence or absence of *Salmonella* with the method described in principle in the German Biowaste Ordinance using a pre-enrichment in buffered peptone water and an enrichment step (Rappaport et al., 1956; Edel and Kampelmacher, 1969; Vassiliadis, 1983), or other validated method that may be developed within the framework of CEN TC 308.

Some other parameters are still in discussion with respect to sewage sludge treatment and composting in the framework of EU directives. Enterococci, for example, cannot be used as an indicator in the examination of compost and compost-related products, but they are valuable for the thermophilic anaerobic treatment in biogas plants and for pure thermal treatment (Bendixen, 1999).

For *E. coli*, *Campylobacter*, and *Yersinia*, besides the lack of reliable re-isolation techniques, it must be stated that their thermo-resistance and, with minor exceptions, chemo-resistance are lower than those of *Salmonella*. This means that it will make no

Fig. 9.5. Distribution of enterobacterial count in composts containing *Salmonella* (Comp. +) and in composts coming out of validated processes being tested negative for *Salmonella* (Comp. −)
sense if they are used as additional microbial parameters for describing a hygienically safe product.

Enteroviruses are generally present in sludge of fecal origin but not regularly in sludges coming from other sources. In principle, enteroviruses may be used as additional indicators but the re-isolation procedures, as for all viruses from environmental samples, are labor intensive and costly. Their resistance in the involved treatment processes is not higher than that of *Salmonella*; this means that the additional information resulting from using these indicator organisms is also low. The same applies for rotavirus, even it is of special environmental importance, according to Metzler et al. (1996) and Pesaro et al. (1999). Recent investigations have shown that coliphages do not correlate high with gut-related pathogens or other comparable fecal indicators even in substrates of fecal origin, as can be seen from Fig. 9.6 (Samhan, 2005).

The question of whether or not nematodes or nematode eggs are useful indicators in this connection is not easy to answer. With respect to nematodes pathogenic to men and/or animals, experience shows that even eggs of *Ascaris suum* are less thermo-resistant than *Salmonella*, but behave differently in chemical treatment. This means that if *Salmonella* does not survive the composting process, *Ascaris* eggs and all other nematode eggs would not either. This does not apply for treatment with slaked lime or long-term storage, especially in connection with organic sludges. This means that *Ascaris* eggs will not be a necessary indicator in all processes in which the thermal effect is the predominant one, but they will give valuable additional information if used in the monitoring of all other treatment processes.

Finally, the problem of indicator organisms from the phytohygienic point of view must be discussed. No virus, fungus, or bacterium pathogenic to plants has been found thus far that is of comparable importance as *Salmonellae* are for the purpose mentioned above. The only indicator that is widely distributed in biological wastes from households is tomato seeds. Even knowing that this indicator will not totally cover all requirements, it seems to be reasonable and feasible to define the term “phytohygienic safety” of the

![Fig. 9.6. Correlation between the parameters “coliform bacteria” and “coliphages” in sewage samples (Samhan, 2005).](image-url)
product as follows: The final product should not contain more than two seeds capable to germinate and/or reproduce parts of plants in 1 L. A suitable test method is described by Bundesgütegemeinschaft Kompost (1994).

9.9. References

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